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Effect of *Lactobacillus* spp. strains on the microbiological, biochemical, and organoleptic properties of Moroccan goat's cheese during ripening

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Abstract

The aim of the present work was to investigate the effect of adding autochthonous lactic acid bacteria strains (LAB) to Moroccan goat's milk cheese, focusing on its microbiological and biochemical evolution during the ripening process, as well as on the product's sensorial properties. We prepared three types of cheese: (i) a control cheese (CNT) made by adding a starter culture composed of a strain of *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* and a strain of *Lactococcus lactis* subsp. *lactis*; (ii) a cheese ("LP") made with the same lactococcal starter culture, and adding a strain of *Lactobacillus plantarum* as an adjunct culture, and (iii) a cheese ("LPC") made with the same lactococcal starter culture, and adding *Lactobacillus paracasei* as an adjunct culture. The counts of most bacterial groups decreased as the cheeses matured, except for lactic acid bacteria, which increased during ripening. Throughout the ripening period, LP cheese showed the highest values for total nitrogen, soluble nitrogen, and soluble nitrogen in 12% TCA. On the 60th day of ripening, the highest contents of diacetyl and acetoin were recorded in LPC cheese. A significant difference in FFA was observed among the LP, LPC, and CNT cheeses, thus indicating a change in lipolysis extension (C4:0-C18:3 FFA) that might be influenced by the starter employed. These suggested that both adjunct strains could produce high-quality Moroccan goat cheese. However, the addition of *Lactobacillus paracasei* culture had the particular advantage of improving aroma intensity and overall cheese quality. Moreover, the lipolysis process increased continually until the end of the ripening period, thereby confirming that the ripening phase is the main factor that affects these cheeses' sensory properties. The cheeses made with adjunct cultures had the typical taste of Moroccan goat cheese, presented an acceptable flavour, and fulfilled the usual sensory requirements.

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Introduction

In 2008, the estimated goat population in Morocco was 5.1 million heads; since then, the number of heads has increased to ca. 5.7 million. This number remains unfortunately inferior to the total count of sheep (MAPMDREF, 2020), a predominance mainly attributed to the strong demand for sheep (Boulanouar and Paquay, 2008), and to the voluntary abandonment of goat farming due to its low efficiency. Consumers are currently more aware of the importance of nutrition, and are increasingly seeking to purchase healthy foods, hence the importance of encouraging and promoting local products.

The Moroccan cheese market grew by 7.6% in 2018, with a higher consumption of fresh cheeses, and a preference for goat cheese (Corvaisier, 2020). White fresh cheese, also known as "*Jben el Beldi*," is a well-known type of Moroccan artisanal cheese characterised by soft texture and acidic flavour. Such traditional cheeses are generally made from cow or

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goat milk, or mixtures thereof. Coagulation is conducted by adding rennet to the raw milk without requiring any starter, then the resulting cheese is wrapped in palm leaves and sold at local markets.

A few specific industrial, *viz.* semi-industrial units, are available in Morocco for the production of ripened cheeses made from goat, cow, and camel milks. Although a wide variety of cheeses are already being produced, consumer interest and demand for new varieties remain strong. To ensure high organoleptic quality, ripened cheeses should be made from raw or pasteurised milk inoculated with commercial or autochthonous strains.

Cheese ripening consists a set of biochemical reactions, set in motion, and maintained by a population of bacteria that either indigenously form part of the milk or stem from adjunct cultures. After the conversion of lactose into lactate by starter lactic acid bacteria (SLAB), cheese undergoes multiple changes that are essentially due to proteolysis and lipolysis. These two reactions are mediated by the presence of adjunct bacteria, yeasts, or moulds, together commonly known as non-starter lactic acid bacteria (NSLAB).

Authors have shown that adjuncts boost the proteolysis process and improve cheese's sensorial qualities; they also shorten the ripening period, thereby reducing expenses (Gürsoy and Türkmen, 2018). Several studies have shown that goat dairy products are rich in microorganisms that have a series of technologically useful properties; autochthonous adjuncts are being increasingly used in cheese production due to their ability to improve flavour and texture, as well as a wide range of biochemical parameters. For instance, it has been demonstrated that *Lactobacillus plantarum* produces exopolysaccharides (EPS), and that it possesses probiotic and antimicrobial properties (Da Silva *et al*., 2019). Furthermore, strains of *Lactobacillus paracasei* from goat milk have a proteolytic and acidifying effect, along with the ability to use citrate to produce acetoin (Pisano *et al*., 2019).

The majority of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* strains isolated from goat dairy products displayed proteolytic activity and produced acetoin (Marroki and Bousmaha-Marroki, 2014). Other strains of *Lactobacillus* spp. isolated from semi-hard goat cheese have been shown to be capable of exerting proteolytic activity and producing EPS and acetoin. Taboada *et al.* (2017) demonstrated that *Lactobacillus* spp. strains were involved in citrate metabolism; they also performed esterase activity and displayed a strong production of diacetyl and acetoin.

The present work thus aimed to evaluate the impact of the addition of selected *Lactobacillus* strains isolated from goat's cheese, on the microbiological and biochemical properties of goat cheese ripened over a period of 60 days. We also discussed those strains' effects on sensorial properties. To the best of our knowledge, this was the first study ever conducted in northern Morocco.

Materials and methods

Starter cultures

The microorganisms used in the present work were isolated from cheeses made from raw goat milk, and have already been screened for their phenotypic and genotypic characteristics, as described previously (El Galiou *et al*., 2015). The starter culture for all three cheeses (CNT, LP, and LPC) was composed of two lactococcal strains: *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* (LC10) and *Lactococcus lactis* subsp. *lactis* (LC12), both possessing the capacity to produce acid and diacetyl. Experimental cheeses were supplemented with two adjunct cultures, namely *Lactobacillus plantarum* (LB9) for the "LP" batch and *Lactobacillus paracasei* (LB2) for the "LPC" batch. *Lactobacillus plantarum* presented proteolytic activity, while *Lactobacillus paracasei* produced both diacetyl and acetoin. *Lactobacillus* spp. strains were grown for 16 h at 30°C in 500 mL of reconstituted skim milk (10% w/v) previously heated for 10 min at 114°C. *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* and *Lactococcus lactis* subsp. *lactis* strains were activated *via* incubation for 12 h at 30°C in reconstituted skim milk.

Cheese making

The three cheese batches were made on the basis of data stemming from a survey conducted in northern Morocco (El Galiou *et al*., 2015), using the process described by El Galiou *et al*. (2014).

The milk used in the present work came from a goat breed indigenous to northern Morocco. The whole milk was first pasteurised (74°C for 15 s), then distributed into three vats containing 50 L of milk each.

To make a control batch (called "CNT"), the first vat was inoculated with two lactococcal starters: *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* and *Lactococcus lactis* subsp. *lactis* (at 0.5%). The two

remaining vats were supplemented with 0.5% with *Lactobacillus plantarum* ("LP" batch) and *Lactobacillus paracasei* ("LPC" batch), in addition to the lactococcal strains. Once pH had reached 6.0, calcium chloride (0.01 g/L) was added. After 20 min, milk was renneted (0.25 mL/L; F = 1:10.000). Forty-five minutes later, the curd was milled into small pieces, salted with dry salt (6.5 g/L), and pressed (1st round, 9.81 × 104 Pa for 1 h at 20°C; and 2nd round, 9.81 × 104 Pa for 1 h at 20°C). Cheeses ripened for 60 d at 90% RH and 5°C. Analyses were performed in triplicate on the 1st, 15th, 30th, 45th, and 60th days of the ripening process.

Microbiological analyses

Sample preparation and serial dilutions were performed following the guidelines established by the International Dairy Federation Standard (IDF, 1996): briefly, 10 g of the curd or cheese were mixed with 90 mL of sterile sodium citrate solution (2%) which had previously been heated to 45°C; a stomacher was used to homogenise the mixture for 1 min at room temperature. Mesophilic aerobic bacteria, lactic acid bacteria, yeasts and moulds, enterococci, and total coliforms were measured in duplicate at each sampling time, following the protocol described in El Galiou *et al*. (2015).

Physicochemical analyses and nitrogen fractions

pH, titratable acidity, protein, fat, dry extract, ash, water activity, and lactose assays were conducted following the guidelines of the Association of Official Analytical Chemists (AOAC, 2012).

The Kjeldahl method was used to determine total nitrogen (TN). Following the guidelines of Neviani *et al*. (1982), proteolysis was assessed by detecting the two main nitrogen fractions: soluble nitrogen at pH 4.6 (SN), and soluble nitrogen in 12% trichloroacetic acid (TCASN). Experiments were performed in triplicate.

Determination of diacetyl and acetoin contents

The colorimetric method was used to determine total diacetyl and acetoin contents (IDF, 1997). Experiments were performed in triplicate.

Free fatty acid analysis

For gas chromatographic analysis of the samples, we followed the method previously described by Poveda and Cabezas (2006). The acidified cheese slurry (10 g) was used as a base for lipid extraction through diethyl ether. Fatty acid methylation was performed with 20% trimethylanilinium hydroxide (TMAH) in methanol. Before injection, TMAH soaps in the lower layer were neutralised and subjected to pyrolysis to produce methyl esters in the chromatograph injector. Free fatty acids (FFA) were detected using gas chromatography *via* the flame ionisation detection (FID) method, as described by Fuente *et al*. (1993) with minor modifications. Experiments were implemented with the Varian model 3800 instrument (Varian Inc., Palo Alto, CA, USA) equipped with an automatic sampler (CP Wax 52CB, Varian) and a programmable temperature vaporiser (PTV) injector. Free fatty acids were separated on a fused-silica capillary column (DB-FFAP; 30 m × 0.25 mm, i.d. × 0.25 µm; Agilent Technologies, Wilmington, DE, USA) with helium as carrier gas for 1 mL/min (split/splitless ratio, 1:20). Oven temperature was kept at 60°C for 2 min, then raised to 180°C at a rate of 5°C/min, and held at 180°C for 60 min. The PTV program parameters were adjusted to an initial temperature of 60°C, then 300°C for 0.05 min; finally, the temperature was kept at 25°C for 4 min until the end of the program. Detector temperature was 250°C. Internal standards namely pentanoic, nonanoic, and heptadecanoic acids added to the cheese samples were used for quantification of FFA during extraction. Experiments were performed in triplicate.

Sensory evaluation

Sensory evaluation was performed after 15, 30, and 60 d from cheese manufacturing day, on 2 cm cubes, by a panel of 10 professional tasters (IDF, 1987) experienced in the tasting of traditional goat cheese. Appearance, texture, flavour, residual intensity flavour, aroma intensity, and general acceptance were rated on a scale ranging from 1 to 7 (1, low; 7, high).

Statistical analyses

Chemical, microbiological, and sensorial data from the three cheese groups were compared using analysis of variance (ANOVA). Differences among the groups were determined using Duncan's test. $p < 0.05$ was regarded as significant. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Microbiological analyses

Table 1 shows the modifications that occurred in the microbial groups along the entire cheese ripening period (1 to 60 days). On the first day of ripening, the counts of total aerobic mesophilic bacteria (TAMB) were in the order of 10.12, 9.98, and 10.25 log CFU/g for CNT, LP, and LPC batches, respectively. The counts decreased gradually until the end of ripening with values at 8.97, 8.74, and 9.01 log CFU/g for CNT, LP, and LPC batches, respectively.

On the first day, lactic acid bacterial count means were significantly ($p < 0.05$) higher in LP and LPC batches (9.22 ± 0.12 and 9.51 ± 0.06 log CFU/g, respectively); that significant difference between adjunct batches and control batches was maintained throughout the entire ripening process. From the first to the 60th day of ripening, lactic acid bacteria increased from 8.77 to 9.42, 9.22 to 9.97, and 9.51 to 10.12 log CFU/g in the CNT, LP, and LPC batches, respectively. The highest enterococcal count was observed on the 60th day. On day 1, all batches presented the maximum total coliform counts, which subsequently became undetectable. It is interesting to note that no significant differences were observed among cheeses in terms of yeast and mould counts.

Table 1. Microbial counts (log_{10} units/g) during ripening of the Moroccan goat cheeses manufactured with added lactobacilli (mean ± SE).

Parameter	Day 1	Day 15	Day 30	Day 45	Day 60
Mesophilic aerobic bacteria					
CNT	10.12 ± 0.15^{a}	9.87 ± 0.33^{a}	9.51 ± 0.25^{ab}	9.21 ± 0.12^{a}	8.97 ± 0.09^{a}
Batch LP	9.98 ± 0.11^{a}	9.68 ± 0.19^{a}	9.37 ± 0.13^{a}	9.08 ± 0.57^{a}	8.74 ± 0.26^{a}
Batch LPC	10.25 ± 0.20^{a}	9.92 ± 0.11^{a}	9.62 ± 0.08^{b}	9.33 ± 0.17^{a}	9.01 ± 0.18^{a}
Lactic acid bacteria					
CNT	8.77 ± 0.13^{a}	9.17 ± 0.09^{a}	9.28 ± 0.28^{a}	9.37 ± 0.25^{a}	9.42 ± 0.24^{a}
Batch LP	9.22 ± 0.12^{b}	9.61 ± 0.19^{b}	9.82 ± 0.16^{b}	9.91 ± 0.06^{b}	9.97 ± 0.10^{b}
Batch LPC	9.51 ± 0.06^{c}	9.72 ± 0.33^{b}	9.89 ± 0.23^{b}	10.01 ± 0.31^{b}	10.12 ± 0.33^{b}
Totals coliforms					
CNT	1.71 ± 0.22^{a}	nd	nd	nd	nd
Batch LP	1.77 ± 0.28^{a}	nd	nd	nd	nd
Batch LPC	1.81 ± 0.06^{a}	nd	nd	nd	nd
Enterococci					
CNT	5.25 ± 0.12^{a}	5.85 ± 0.26^{a}	5.98 ± 0.30^{a}	6.08 ± 0.15^{a}	6.20 ± 0.19^{a}
Batch LP	5.12 ± 0.14^{a}	5.72 ± 0.18^{a}	5.87 ± 0.16^{a}	5.98 ± 0.26^{a}	6.08 ± 0.36^{a}
Batch LPC	5.14 ± 0.21^{a}	5.74 ± 0.21^{a}	5.91 ± 0.14^{a}	6.10 ± 0.25^{a}	6.18 ± 0.25^{a}
Yeasts and moulds					
CNT	4.28 ± 0.56^{ab}	3.45 ± 0.19^{a}	3.21 ± 0.26^{a}	3.75 ± 0.21^{a}	3.81 ± 0.10^{a}
Batch LP	3.86 ± 0.13^{a}	3.15 ± 0.05^{b}	3.02 ± 0.13^{a}	3.57 ± 0.16^{a}	3.79 ± 0.11^{a}
Batch LPC	4.25 ± 0.11^{b}	3.38 ± 0.22^{ab}	3.11 ± 0.15^{a}	3.67 ± 0.30^{a}	3.89 ± 0.27^{a}

Means in the same column with different lowercase superscripts differ significantly ($p < 0.05$). nd: not detected.

Physicochemical analyses

pH, acidification, and diacetyl and acetoin

Figure 1 shows the pH, titratable acidity, and diacetyl/acetoin levels in goat milk cheeses during ripening (60 days): we noted an increase in acidity and a decrease in pH values along the entire ripening process. A considerable pH drop was also observed between day 45 and 60 for all batches, likely as a result of the increase in lactic acid bacteria. Cheese produced without adjunct cultures presented the lowest pH and the highest acidity on day 60. No relevant differences ($p > 0.05$) in terms of pH or acidity were detected among batches on any of the sampling days.

However, diacetyl and acetoin quantification revealed different profiles for each batch, and the

differences among them were significant ($p < 0.05$). Particularly in LPC batch, diacetyl and acetoin production followed an ascending curve from the onset to the end of the ripening process and recorded the strongest amount (291 mg/kg) on the 60th day as compared to the other batches. The levels in LP batch remained stable between days 1 and 15, then gradually increased until reaching 91 mg/kg on the last day of ripening. In CNT batch, a drop in diacetyl and acetoin was observed between day 1 and 15, followed by a gradual increase until a peak value of 195.63 mg/kg on the 60th day was reached.

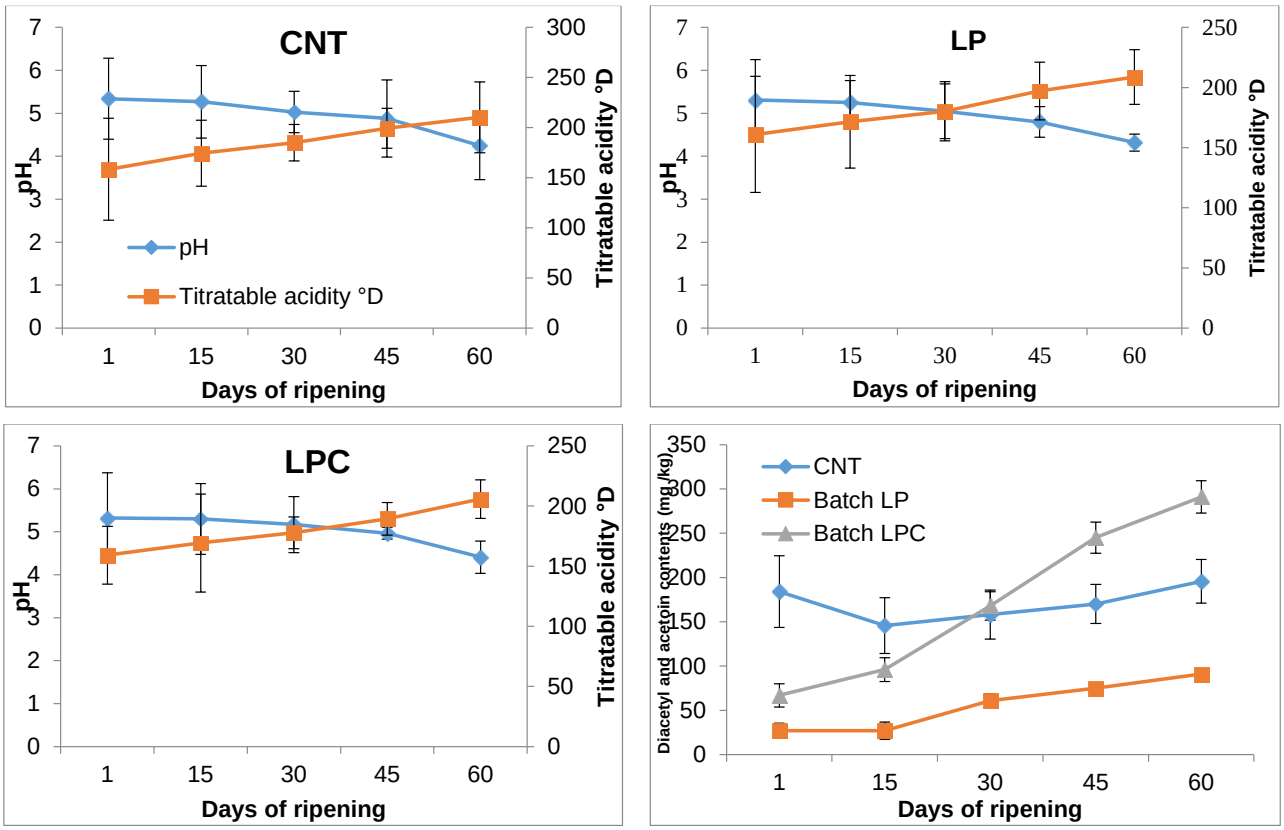

Figure 1. Changes in pH, titratable acidity, and diacetyl and acetoin content (mg diacetyl and acetoin/kg) values during ripening (60 days) of goat milk cheeses manufactured with added lactobacilli (mean ± SE). CNT: control batch; LP: batch with added *Lactobacillus plantarum*; and LPC: batch with added *Lactobacillus paracasei*.

Proteins, fat, dry extract, ash, lactose, and proteolysis evolution

Results of our chemical experiments are summed up in Table 2. No significant difference ($p > 0.05$) in terms of water activity was noticed between the three batches. In fact, water activity decreased during the ripening period until it reached 0.90, 0.92, and 0.93 in the CNT, LP, and LPC batches, respectively. In the two experimental adjunct batches, recorded water activity values decreased progressively during ripening, whereas in CNT, they remained relatively constant until day 45, when a sharp fall was noted, reaching a value of 0.91.

Protein and fat percentages increased along ripening as compared to dry matter percentages, thereby indicating a positive correlation among protein and fat. On the 60th day, no significant difference ($p > 0.05$) was found between *Lactobacillus* spp. batches regarding dry extract (34.41 and 34.02% for LP and LPC batches, respectively) and proteins (14.55 and 14.24% for LP and LPC batches, respectively); however, a significant difference ($p < 0.05$) was observed for both parameters between control and experimental cheeses, where 17.05, 17.85, and 18.10% represented the fat results for CNT, LP, and LPC batches on the 60th day, and differences among them were significant ($p < 0.05$).

A sharp increase in protein and dry extract contents was observed between day 1 and 15 in the LP and LPC batches. A marked rise in fat content was detected between day 45 and 60, whereas in the control cheese, these parameters gradually increased throughout the entire ripening period.

Table 2. Chemical parameters and evolution of nitrogenous fractions during ripening of Moroccan goat cheeses manufactured with added lactobacilli (mean ± SE).

Parameter	Day 1	Day 15	Day 30	Day 45	Day 60
Aw					
CNT	0.98 ± 0.03^{a}	0.97 ± 0.02^{a}	0.97 ± 0.02^{a}	0.91 ± 0.04^{a}	0.90 ± 0.05^{a}
Batch LP	0.97 ± 0.03^{a}	0.96 ± 0.01^{a}	0.95 ± 0.01^{a}	0.94 ± 0.04^{a}	0.92 ± 0.03^{a}
Batch LPC	0.91 ± 0.05^{a}	0.97 ± 0.01^{a}	0.96 ± 0.03^{a}	0.95 ± 0.03^{a}	0.93 ± 0.03^{a}
Proteins (g/100 g)					
CNT	12.70 ± 0.03^{a}	13.1 ± 0.14^{a}	13.31 ± 0.15^{a}	13.61 ± 0.26^{a}	13.84 ± 0.11^{a}
Batch LP	12.55 ± 0.04^{b}	13.75 ± 0.09^{bc}	13.91 ± 0.44^{b}	14.44 ± 0.35^{b}	14.55 ± 0.29^{b}
Batch LPC	12.81 ± 0.06^{c}	13.41 ± 0.22^{c}	13.57 ± 0.23^{b}	13.98 ± 0.17^{a}	14.24 ± 0.18^{b}
Fat content (g/100 g)					
CNT	16.30 ± 0.25^{a}	16.51 ± 0.22^{a}	16.88 ± 0.19^{a}	16.90 ± 0.05^{a}	17.05 ± 0.31^{a}
Batch LP	16.57 ± 0.13^{ab}	16.88 ± 0.36^{ab}	17.04 ± 0.04^{b}	17.12 ± 0.16^{b}	17.85 ± 0.27^{b}
Batch LPC	16.81 ± 0.16^{b}	17.07 ± 0.18^{b}	17.21 ± 0.09^{c}	17.55 ± 0.2^{c}	18.10 ± 0.19^{c}
Ash (g/100 g)					
CNT	1.85 ± 0.32^{a}	1.62 ± 0.11^{a}	1.51 ± 0.09^{a}	1.44 ± 0.11^{a}	1.28 ± 0.08^{a}
Batch LP	1.78 ± 0.49^{a}	1.64 ± 0.15^{a}	1.59 ± 0.17a	1.51 ± 0.07^{a}	1.31 ± 0.05^{a}
Batch LPC	1.70 ± 0.50^{a}	1.62 ± 0.33^{a}	1.54 ± 0.15^{a}	1.47 ± 0.22^{a}	1.12 ± 0.06^{b}
Dry extract (g/100 g)					
CNT	32.20 ± 0.25^{a}	32.48 ± 0.25^{a}	32.71 ± 0.21^{a}	32.84 ± 0.40^{a}	32.80 ± 0.66^{a}
Batch LP	32.18 ± 0.33^{a}	33.32 ± 0.16^{b}	33.45 ± 0.13^{b}	33.92 ± 0.19^{b}	34.41 ± 0.28^{b}
Batch LPC	32.64 ± 0.12^{a}	33.22 ± 0.22^{b}	33.26 ± 0.11^{c}	33.75 ± 0.10^{c}	34.02 ± 0.09^{b}
Lactose (g/100 g)					
CNT	1.35 ± 0.25^{a}	1.25 ± 0.30^{a}	1.01 ± 0.19^{a}	0.88 ± 0.12^{a}	0.63 ± 0.08^{a}
Batch LP	1.28 ± 0.22^{a}	1.05 ± 0.14^{a}	0.91 ± 0.11^{a}	0.85 ± 0.18^{a}	0.69 ± 0.10^{a}
Batch LPC	1.32 ± 0.15^{a}	1.12 ± 0.18^{a}	0.94 ± 0.09^{a}	0.75 ± 0.13^{a}	0.59 ± 0.10^{a}
TN (%)					
CNT	1.99 ± 0.25^{a}	2.05 ± 0.26^{a}	2.09 ± 0.11^{a}	2.13 ± 0.19^{a}	2.17 ± 0.11^{a}
Batch LP	1.97 ± 0.35^{b}	2.16 ± 0.33^{b}	2.18 ± 0.15^{b}	2.26 ± 0.31^{b}	2.28 ± 0.38^{b}
Batch LPC	2.01 ± 0.27^{b}	2.10 ± 0.13^{b}	2.13 ± 0.05^{c}	2.19 ± 0.33^{ab}	2.23 ± 0.23^{c}
SN/TN (%)					
CNT	10.17 ± 0.07^{a}	11.13 ± 0.90^{a}	11.25 ± 0.28^{a}	11.37 ± 0.56^{a}	11.41 ± 0.39^{a}
Batch LP	12.70 ± 0.25^{b}	14.25 ± 0.77^{b}	14.74 ± 0.62^{b}	14.90 ± 1.03^{b}	15.05 ± 0.22^{b}
Batch LPC	12.10 ± 0.32^{b}	13.37 ± 0.47^{c}	13.50 ± 0.11^{c}	13.65 ± 0.61^{b}	13.64 ± 0.33^{c}
TCASN/TN (%)					
CNT	3.45 ± 0.33^{a}	4.20 ± 0.14^{a}	4.29 ± 0.55^{a}	4.35 ± 0.05^{a}	4.41 ± 0.12^{a}
Batch LP	4.80 ± 0.66^{b}	6.57 ± 0.85^{b}	6.91 ± 0.22^{b}	7.05 ± 1.66^{b}	7.25 ± 0.86^{b}
Batch LPC	4.66 ± 0.26^{b}	5.05 ± 0.36^{c}	5.25 ± 0.17^{c}	5.74 ± 0.31^{b}	5.97 ± 0.63^{b}

Means in the same column with different lowercase superscripts differ significantly ($p < 0.05$). SN: soluble nitrogen; TCA-SN: nitrogen soluble in 12% TCA; and TN: total nitrogen.

Ash and lactose contents declined progressively in all batches along ripening. For ash content, a significant difference ($p < 0.05$) was only measured among LPC, LP, and control batches (1.12, 1.31, and 1.28, respectively) on day 60. Regarding lactose content, the final amounts were 0.63, 0.69, and 0.59 g/100 g of total solids for control, LP, and LPC batches, respectively, with no significant difference detected ($p > 0.05$).

The evolution of proteolysis in the three goat cheeses during ripening (0 - 60 days) is summarised in Table 2. We were able to assess the extent of proteolysis by monitoring water soluble nitrogen (WSN) and trichloroacetic acid soluble nitrogen (TCASN). All nitrogenous fractions increased gradually from the onset until the end of ripening. A considerable increase in WSN and TCASN percentages could be observed between day 1 and 15. LP batch displayed the highest values of TN, WSN, and TCASN on all sampling days. Moreover, on the 60th day, a significant difference ($p < 0.05$) among the three batches could be observed in terms of WSN. The highest WSN percentage was recorded in the LP batch at 15.05%, and the lowest was detected in the CNT batch at 11.41%. On day 60, the TCASN values for the LP and LPC batches were statistically different from CNT ($p < 0.05$); in contrast, no significant difference was specifically found between the LP and LPC batches. On that final day, the highest TCASN was observed in LP batch, followed by LPC and then CNT with 7.25, 5.97, and 4.41%, respectively.

Free fatty acid evolution

The changes observed in FFA amounts during cheese ripening are shown in Table 3. The lipolysis process was found to be more active during ripening ($p < 0.05$).

At the onset of ripening, FFA levels were 3507.10, 3560.20, and 3558.71 mg kg/L for CNT, LP, and LPC batches, respectively, and no significant difference was detected ($p > 0.05$). On the 60th day, FFA content significantly differed among the three batches ($p < 0.05$), with the following results: 13677.21 mg kg/L (CNT batch), 14338.11 mg kg/L (LP batch), and 15787.25 mg kg/L (LPC batch). To summarise, FFA concentrations were higher in *Lactobacillus* spp. batches than in control. The most abundant FFA were oleic (C18:1), palmitic (C16:0), stearic (C18:0), myristic (C14:0), and capric (C10:0) acids, representing about 80% of total free fatty acid content. The most common short-chain FFA was caprylic acid (3.5% of total FFAs), and palmitic acid was the prevalent medium-chain FFA (22% of total FFAs). From the 15th day of ripening, lactobacilli-based cheeses started to show higher FFA levels than control cheeses, regardless of whether there was a statistical difference or not. An exception could be noted: within the last two sampling days, stearic acid production was significantly greater in control than in *Lactobacillus* spp. cheeses. Significant differences ($p < 0.05$) in specific FFA, namely butyric, caproic, caprylic, capric, myristic, palmitic, and oleic acids, were observed between the LP and LPC batches on different sampling days.

All short-chain FFA detected in the *Lactobacillus paracasei* batch (LPC) showed highly significant differences on the 15th day, and on the 60th and 30th days for caproic and caprylic acids, respectively. Similarly, to short-chain FFAs, all medium-chain FFAs (except lauric acid) showed significant levels on day 15, but also on the 30th and 45th days for palmitic acid, the 60th day for myristic acid, and the 30th day for capric acid in the LPC batch. Oleic acid content was high in LPC batch from the 15th to the last ripening day. There were no significant differences in lauric, stearic, linoleic, and linolenic acids between the LPC and LP batches throughout the entire ripening period.

Organoleptic evaluation

Table 4 shows the organoleptic evaluation of goat cheeses. Regarding appearance and texture, no significant difference ($p > 0.05$) was observed between the CNT, LP, and LPC batches along the entire ripening period. In terms of aroma and flavour, the values given by the judges for LP and LPC batches were significantly higher ($p < 0.05$) than those for CNT batch. In comparison with the LP batch, the LPC batch had the highest aroma intensity, as well as a residual intensity flavour ($p < 0.05$) at the end of ripening.

Discussion

Microbiological analyses

On the last day of ripening, the counts of *Enterococcus* spp. in the three batches ranged between 6.08 and 6.18 log CFU/g. This result might be explained by this bacterium's ability to perform proteolytic and lipolytic activities. Previous studies have demonstrated that enterococci play a crucial role

Table 3. Free fatty acids (FFA) content during ripening of Moroccan goat cheeses manufactured with added lactobacilli (mean ± SE).

Free fatty acid (mg/kg)	Day 1			Day 15			Day 30			Day 45			Day 60		
	CNT	Batch LP	Batch LPC	CNT	Batch LP	Batch LPC	CNT	Batch LP	Batch LPC	CNT	Batch LP	Batch LPC	CNT	Batch LP	Batch LPC
Butyric acid (C4)	123.1 ± 14.02^{a}	135.2 ± 10.50^{a}	127.5 ± 15.30^{a}	245.00 ± 12.23^{a}	262.25 ± 13.40^{b}	298.32 ± 14.70^{c}	270.22 ± 11.90^{a}	278.45 ± 10.30^{a}	307.50 ± 12.01^{b}	317.00 ± 13.30^{a}	333.45 ± 9.70^{b}	350.78 ± 16.33^{b}	375.25 ± 10.33^{a}	398.0 ± 13.50^{b}	401.22 ± 11.20^{b}
Caproic acid (C6)	154.7 ± 11.1^{a}	160.70 ± 10.30^{a}	155.8 ± 12.33^{a}	310.00 ± 20.89^{a}	355.25 ± 19.92^{b}	391.09 ± 11.63^{c}	351.28 ± 21.66^{a}	395.25 ± 13.34^{b}	421.21 ± 24.67^{b}	402.23 ± 21.01^{a}	430.17 ± 14.56a^{b}	455.12 ± 26.22^{b}	414.10 ± 16.16^{a}	454.22 ± 25.66^{b}	495.13 ± 16.99^{c}
Caprylic acid (C8)	72.10 ± 5.15^{a}	71.20 ± 7.22^{a}	76.50 ± 9.66^{a}	208.00 ± 33.43^{a}	251.20 ± 20.07^{b}	290.56 ± 15.85^{c}	300.25 ± 33.26^{a}	355.14 ± 24.37^{b}	394.89 ± 13.62^{c}	428.02 ± 17.28^{a}	471.85 ± 21.88^{b}	495.50 ± 12.18^{b}	440.58 ± 30.33^{a}	500.12 ± 27.67^{b}	504.25 ± 19.36^{b}
Total C4:0–C8:0	349.90	367.10	359.80	763.00	868.70	979.97	921.75	1028.84	1123.60	1147.25	1235.47	1301.40	1229.93	1352.34	1400.60
Capric acid (C10)	370.40 ± 10.05^{a}	372.55 ± 18.20^{a}	372.40 ± 16.5^{a}	545.00 ± 34.00^{a}	625.00 ± 26.10^{b}	691.00 ± 29.30^{c}	610.20 ± 56.70^{a}	645.44 ± 32.99^{a}	725.16 ± 36.88^{b}	740.25 ± 22.10^{a}	851.14 ± 58.00^{b}	889.19 ± 54.05^{b}	820.25 ± 30.18^{a}	877.18 ± 54.42^{ab}	910.50 ± 36.10^{b}
Lauric acid (C12)	81.60 ± 7.00^{a}	85.77 ± 6.13^{a}	81.70 ± 4.33^{a}	250.65 ± 42.10^{a}	357.85 ± 33.33^{b}	410.50 ± 23.84^{b}	285.15 ± 44.14^{a}	405.50 ± 61.03^{b}	445.23 ± 33.56^{b}	340.15 ± 31.5^{a}	477.17 ± 40.33^{b}	500.16 ± 28.67^{b}	360.25 ± 44.55^{a}	497.14 ± 79.11^{b}	525.41 ± 87.06^{b}
Myristic acid (C14)	477.90 ± 28.66^{a}	481.55 ± 43.16^{a}	501.20 ± 38.22^{a}	767.02 ± 31.5^{a}	900.40 ± 27.21^{b}	967.45 ± 31.14^{c}	840.15 ± 85.16^{a}	1000.24 ± 51.30^{b}	1070.00 ± 52.4^{b}	1090.12 ± 46.20^{a}	1157.24 ± 50.62^{ab}	1188.25 ± 38.11^{b}	1189.28 ± 38.88^{a}	1260.77 ± 45.73^{b}	1378.24 ± 52.13^{c}
Palmitic acid (C16)	560.20 ± 16.34^{a}	577.85 ± 22.67^{a}	561.27 ± 35.12^{a}	1020.00 ± 52.4^{a}	1560.00 ± 66.13^{b}	1746.00 ± 83.00^{c}	1722.14 ± 55.74^{a}	2112.55 ± 159.30^{b}	2485.26 ± 135.20^{c}	2020.46 ± 52.19^{a}	2577.80 ± 161.34^{b}	2812.14 ± 187.66^{c}	3125.12 ± 99.68^{a}	3205.45 ± 173.64^{ab}	3441.15 ± 168.03^{b}
Total C10:0–C14:0	1490.10	1517.72	1516.57	2582.67	3443.25	3814.95	3457.64	4163.73	4725.65	4190.98	5063.35	5389.74	5494.90	5840.54	6255.30
Stearic acid (C18)	691.20 ± 33.33^{a}	690.85 ± 12.16^{a}	692.50 ± 24.06^{a}	935.00 ± 66.11^{a}	1098.00 ± 71.88^{b}	1115.00 ± 56.11^{b}	1171.80 ± 37.65^{a}	1199.20 ± 35.58^{ab}	1251.25 ± 33.99^{b}	1366.67 ± 25.10^{a}	1301.15 ± 16.77^{b}	1325.12 ± 33.11^{ab}	1394.20 ± 21.80^{a}	1340.78 ± 27.10^{b}	1369.40 ± 39.05^{ab}
Oleic acid (C18:1)	737.00 ± 26.65^{a}	745.25 ± 19.86^{a}	750.45 ± 55.73^{a}	3050.00 ± 91.60^{a}	3250.00 ± 135.87^{b}	4137.00 ± 227.89^{c}	3550.80 ± 188.11^{a}	3674.50 ± 109.76^{b}	4361.60 ± 219.66^{c}	4120.25 ± 50.66^{a}	4301.8 ± 100.66^{b}	5120.70 ± 155.71^{c}	4651.55 ± 62.77^{a}	4905.17 ± 102.14^{b}	5610.25 ± 260.54^{c}
Linoleic acid (C18:2)	169.90 ± 17.10^{a}	170.51 ± 20.52^{a}	169.50 ± 18.50^{a}	433.00 ± 40.76^{a}	525.00 ± 41.31^{b}	585.00 ± 32.98^{b}	505.81 ± 31.66^{a}	570.25 ± 32.88^{b}	621.25 ± 60.55^{b}	580.70 ± 37.02^{a}	626.66 ± 42.99^{ab}	655.81 ± 15.73^{b}	574.44 ± 42.00^{a}	680.17 ± 50.99^{b}	761.19 ± 43.75^{b}
Linolenic acid (C18:3)	69.00 ± 6.70^{a}	68.77 ± 7.11^{a}	69.89 ± 9.33^{a}	164.00 ± 38.23^{a}	253.00 ± 28.66^{b}	260.00 ± 41.22^{b}	214.22 ± 11.89^{a}	274.10 ± 22.86^{b}	296.74 ± 34.92^{b}	274.00 ± 35.10^{a}	343.13 ± 40.50^{ab}	377.45 ± 41.00^{b}	302.19 ± 20.25^{a}	399.11 ± 38.90^{b}	410.51 ± 26.05^{b}
Total C16:0–C18:3	1667.10	1675.38	1682.34	4582.00	5126.00	6097.00	5442.63	5718.05	6530.84	6341.62	6572.74	7479.08	6922.38	7325.23	8151.35
Total FFA	3.507.1	3.560.2	3.558.7	8.287.7	9.587.9	10.891.9	9.852.0	11.050.6	12.280.1	11.719.8	12.861.6	14.140.2	13.677.2	14.338.1	15.787.2

Means in the same row with different lowercase superscripts differ significantly ($p < 0.05$).

Table 4. Organoleptic characteristics during ripening of Moroccan goat cheeses manufactured with added lactobacilli (mean ± SE).

Organoleptic characteristic	CNT	Batch LP	Batch LPC
Appearance			
15	4.70 ± 0.75[a]	4.55 ± 0.67[a]	4.61 ± 0.25[a]
30	4.75 ± 0.62[a]	4.6 ± 0.13[a]	4.55 ± 0.16[a]
60	3.26 ± 0.55[a]	4.01 ± 0.26[a]	4.1 ± 0.16[a]
Texture			
15	2.80 ± 0.33[a]	2.45 ± 0.19[a]	2.53 ± 0.24[a]
30	2.75 ± 0.45[a]	2.25 ± 0.11[a]	2.35 ± 0.26[a]
60	2.10 ± 0.09[a]	2.11 ± 0.10[a]	2.15 ± 0.14[a]
Flavour			
15	4.10 ± 0.21[a]	5.20 ± 0.13[b]	5.75 ± 0.33[c]
30	4.15 ± 0.17[a]	5.35 ± 0.24[b]	5.86 ± 0.20[c]
60	4.19 ± 0.50[a]	5.74 ± 0.26[b]	6.02 ± 0.13[b]
Residual intensity flavour			
15	3.55 ± 0.34[a]	4.35 ± 0.28[b]	4.75 ± 0.13[c]
30	3.40 ± 0.36[a]	4.61 ± 0.37[b]	4.89 ± 0.66[b]
60	3.25 ± 0.21[a]	4.72 ± 0.14[b]	5.02 ± 0.09[c]
Aroma intensity			
15	2.56 ± 0.14[a]	3.75 ± 0.51[b]	4.44 ± 0.33[b]
30	2.74 ± 0.23[a]	3.86 ± 0.56[b]	4.69 ± 0.36[c]
60	2.79 ± 0.35[a]	4.01 ± 0.37[b]	4.81 ± 0.28[c]
General acceptance			
15	2.47 ± 0.35[a]	3.12 ± 0.16[b]	3.75 ± 0.27[c]
30	2.69 ± 0.27[a]	3.25 ± 0.28[b]	3.85 ± 0.30[c]
60	2.84 ± 0.33[a]	3.57 ± 0.30[b]	3.97 ± 0.19[b]

Means in the same row with different lowercase superscripts differ significantly ($p < 0.05$).

in the development of the aroma and flavour of cheese. It is now well established that they are a major ingredient in raw or pasteurised artisanal cheeses (Tabet *et al.*, 2016).

At the onset of ripening, the lactic acid bacterial count was 8.77, 9.22, and 9.51 log CFU/g in CNT, LP, and LPC cheeses, respectively, but these increased rapidly during the ripening stage, reaching 9.42, 9.97, and 10.12 CFU/g in CNT, LP, and LPC batches, respectively. Gurses and Erdogan (2006) reported that during the ripening period of Tulum cheese, several of its LAB isolates increased. These findings suggested that our LAB strains remained viable throughout the maturation process. No significant difference in terms of mesophilic aerobic bacteria was detected among the three batches. This was similar to those obtained by Karagozlu *et al.* (2009) who found that the average total mesophilic bacterial count was 8.5 log CFU/g.

The differences in terms of microbiological properties observed among the cheeses included in the present work could be attributed to factors such as strain interactions, chemical composition, varying time, and storage conditions, and the wide variety of production methods employed.

Chemical properties

We observed a simultaneous decrease in lactose levels in parallel with pH depletion, a finding that helped to explain the role played by glycolysis in pH decrease during the ripening period. Kocak *et al.* (2020) and Wang *et al.* (2019) have reported similar results. Furthermore, these findings showed that starter viability were maintained until the final days

of cheese aging. These were slightly different from those of Gursoy and Kinik (2010) who observed that *Lactobacillus paracasei* batches were more acidic than cheeses made without adjuncts. Several authors, however, have confirmed that adding *Lactobacillus plantarum* or *Lactobacillus paracasei* did not have any influence on pH values (Stefanovic *et al*., 2018), which was consistent with our findings.

Water activity decrease could be induced by a variety of factors, including salt, which can affect moisture and water activity levels when it penetrates the cheese matrix (Wemmenhove *et al*., 2016). Proteolysis is also involved, since protein breakdown produces ionic groups that adhere to water molecules, thereby limiting the amount of free water (Bettera *et al*., 2020).

The increase in amounts of protein and fat we observed during the ripening period could be explained by a decrease in moisture percentages, a finding that has been reported in previous studies (Taboada *et al*., 2017; Randazzo *et al*., 2021).

Ash content decrease may be a result of calcium solubilisation, a mechanism observed in cheeses with lower pH values. Milci *et al.* (2005) observed a similar phenomenon in their study of the chemical characteristics of Halloumi cheese; this data did not match the findings of Randazzo *et al*. (2021).

Lactose decrease is an obvious consequence of its consumption and conversion to lactate *via* lactic acid bacteria. In the present work, we observed that lactose levels dropped by 53, 46, and 55% from the first to the last day of ripening in CNT, LP, and LPC batches, respectively; this evolution is in agreement with previous a study (Moreira *et al*., 2019).

The ability of *Lactobacillus paracasei* to produce diacetyl and acetoin in a cheese model has been demonstrated: as mentioned earlier, the highest amounts of diacetyl and acetoin were detected in the LPC batch. Similar results were obtained in a previous study (Bancalari *et al*., 2020), in which a *Lactobacillus paracasei* strain was used as the adjunct strain in Caciotta cheese, and was found to improve its flavour.

Our data also suggested that the addition of bacterial adjuncts, namely *Lactobacillus plantarum* and *Lactobacillus paracasei*, contributed noticeably to primary and secondary proteolysis. These findings were consistent with those of Duan *et al.* (2019) who evaluated the proteolytic activity of *Lactobacillus plantarum* in Cheddar cheese.

The proteolysis-enhancing effect of *Lactobacillus plantarum* has also been observed in low-fat Cheddar cheese (Wang *et al*., 2019). Jia *et al*. (2015) demonstrated that *Lactobacillus casei* LC2W used as an adjunct caused significant differences in concentrations of soluble nitrogen.

The effect of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* on proteolysis in Edam cheese has also been investigated (Aljewicz *et al*., 2014); these authors found that those two bacterial adjuncts, particularly the *Lactobacillus rhamnosus* strain, significantly affected water, trichloroacetic acid, and soluble nitrogen.

However, our results were inconsistent with those obtained by Stefanovic *et al.* (2018) who investigated the proteolysis potential of *Lactobacillus paracasei* in short-lived Cheddar cheese; in their study, no statistical difference in terms of primary proteolysis was found among cheeses made with or without adjunct strains of *Lactobacillus paracasei*.

Bielecka and Cichosz (2017) made similar observations when using *Lactobacillus plantarum* in cheddar and *Lactobacillus paracasei* in Dutch-type cheeses, respectively. Their findings confirm that proteolysis enhancement in cheeses occurs in a strain-dependent manner: different adjunct strains may show varying potential for proteolysis.

From the total free fatty acid content observed in all three batches along 60 days of ripening, we deduced that Moroccan goat cheeses underwent intense lipolysis due to strong lipolytic enzyme activity.

The high degree of lipolysis, expressed here as the total FFA content of Moroccan goat cheeses, could be plausibly explained by the following parameters: (i) indigenous milk lipase, which might have been present in both cheeses because the cheese milk was heated below 78°C for 10 s, an essential step for complete inactivation of milk lipase (Georgala *et al*., 2005). (ii) the lipolytic activity of *Lactobacillus* strains.

Total fatty acid content increased during cheese ripening due to the lipolysis process, a result which confirmed the findings reported by Bontinis *et al*. (2012) and Georgala *et al*. (2005).

Short and medium-chain FFA content has a significant impact on the development of the flavour of Moroccan goat cheese (Tavaria *et al*., 2004).

It is interesting to note that FFA percentages of C2:0–C8:0 and C10:0–C14:0 cited in previous

research were superior to the percentages in our data (Georgala *et al*., 2005).

Extra fatty acid levels can result in a rancid flavour. Despite their presence in our cheeses, they did not generate any acrid taste, and flavour remained pleasant. At all stages, the LPC batch showed significantly higher total FFA levels than the two other batches; it is well known that the autolytic and/or lipolytic activities of inoculated strains exert an influence on the lipolysis process in cheese. Therefore, the significant differences observed in certain free fatty acids (C4:0, C6:0, C8:0, C10:0, C14:0, C16:0, and C18:1) among the three batches were certainly brought about by the introduced strains (Table 3).

Sensorial analysis

Mature cheese flavour is the result of a series of biochemical changes that take place in the curd during ripening, caused by an interaction between the starter bacteria, enzymes from the milk, enzymes from the rennet, concomitant lipases, and secondary microflora (Urbach, 1997).

The results of our organoleptic test revealed no significant differences ($p > 0.05$) in terms of appearance and texture between the CNT, LP, and LPC batches.

Regarding aroma and flavour, the values given by the panel members for the LP and LPC cheeses were significantly higher ($p < 0.05$) than those for CNT; apparently reflecting the contribution of total short chain (C2:0–C8:0) and medium-chain (C10:0 and C14:0) FFA. FFA were significantly higher ($p < 0.05$) in LP and LPC batches when compared with CNT. From the 15th day of ripening, lipid hydrolysis led to the production of FFA, which could directly enhance cheese flavour, and serve as a substrate for further reactions leading to the release of highly flavoured catabolic end products (Collins *et al.*, 2003).

On the last day of ripening, the CNT batch presented the lowest scores in almost all sensorial parameters ($p < 0.05$); this underscored the important role of adjuncts in the enhancement of sensorial qualities of cheese.

Cheese's quality is a top priority for consumers, and mainly based on its texture (Aday and Karagul Yuceer, 2014). In the present work, the use of adjuncts had no effect on cheese texture ($p > 0.05$). After 60 days of ripening, LPC batch received the highest scores for aroma intensity (4.81 ± 0.28). These results were similar to those reported by Celik and Tarakci (2017).

Conclusion

The present work demonstrated that the inclusion of mesophilic homofermentative lactobacilli exerted a positive influence on the microbiological and physicochemical parameters of Moroccan goat cheese, namely proteins, dry extract, and the production of diacetyl and acetoin. Moreover, the extent of cheese lipolysis (C4:0–C18:3 FFA) was affected by the particular strain that was being used in each batch. Differences in FFA detected among the LP, LPC, and CNT cheeses were significant. The addition of autochthonous adjuncts also had an impact on sensory properties, thus giving the cheeses more flavour. The cheese prepared with *Lactobacillus paracasei* strain (LPC batch) had significant strong flavour intensity, and the highest significant general acceptance, superior to that expressed for cheese made with *Lactobacillus plantarum* strain (LP batch). From these results, we deduce that a high-quality Moroccan goat cheese could be achieved on a larger scale by implementing any of the two adjunct cultures featured herein (*Lactobacillus plantarum* or *Lactobacillus paracasei*) in the production process.

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