Journal of Functional Foods 18 (2015) 106–116

Available online at www.sciencedirect.com

ScienceDirect

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

Evaluation of Toma Piemontese PDO cheese as a carrier of putative probiotics from table olive fermentations

Cristian Botta, Marta Bertolino, Giuseppe Zeppa, Luca Cocolin *

Department of Agriculture, Forestry and Food Sciences, University of Torino, Italy

ARTICLE INFO

Article history:
Received 17 January 2015
Received in revised form 30 June 2015
Accepted 30 June 2015
Available online 20 July 2015

Keywords:
Cheese
Probiotics
Functional food
Lactic acid bacteria

ABSTRACT

Two strains of *Lactobacillus plantarum* (Lp S11T3E and Lp S2T10D) and one of *Lactobacillus pentosus* (Lps S3T60C), originally isolated from table olive fermentations, and previously characterized for their probiotic properties, have been added as adjunct cultures to Toma Piemontese Protected Denomination of Origin (PDO) cheese, in order to develop a functional dairy product. The cheeses were sampled throughout their manufacturing process and during the two months of ripening. Moreover, they were also subjected to *in vitro* human digestion, followed by microbiological analysis. Monitoring of the inoculated strains was performed through molecular identification and biotyping. The organic acid and sugar contents of the cheeses were determined at each sampling point, while the organoleptic features were assessed by sensory evaluation. All isolated lactobacilli, during ripening and after digestion of the cheeses, were recognized as putative probiotics, and demonstrated to be well adapted to the food matrix investigated. The organic acid composition of the cheeses with the adjunct culture differed from the control, but this difference did not negatively affect the organoleptic profiles of the final product. Therefore, all the strains tested were to be usable as adjunct cultures in Toma Piemontese PDO production to develop a new functional food.

1. Introduction

The development of functional foods with beneficial features has attracted the interest of the scientific community for many years. In this context, the probiotic effect of milk-based products has been documented since the times of Metchnikoff, as they represent an optimal matrix for the growth of beneficial lactic acid bacteria (LAB) or *Bifidobacterium* (Awaisheh, 2012). In this class of food, cheeses are advantageous systems for the delivery of probiotics, due to their higher pH, longer shelf life, solid consistency and higher fat content compared to milk or fermented products such as yogurt (da Cruz, Alonso Buriti, Batista de Souza, Fonseca Faria, & Isay Saad, 2009; Plessas, Bosnea, Alexopoulos, & Bezirtzoglou, 2012; Sharp, McMahon, & Broadbent, 2008). However, their suitability as probiotic carriers is mainly due to the protection of bacterial cells during gastric digestion. Cheese is able to neutralize the hydrochloric acid present in the stomach and this capability is correlated to the age of the cheese, since the buffering capacity increases with ripening, due to proteolysis and the release of amino acids (Pitino et al., 2012; Ricciardi, Blaiotta, Di Cerbo, Succi, & Aponte, 2014; Sumeri, Adamberg, Uusna, Sarand, & Paalme, 2012).

* *Corresponding author.* Largo Paolo Braccini 2, 10095, Grugliasco, Torino, Italy. Tel.: +39 011 670 8553; fax: +39 011 670 8549.
E-mail address: lucasimone.cocolin@unito.it (L. Cocolin).

Chemical components: Lactose (PubChem CID: 84571); Glucose (PubChem CID: 53782692); Galactose (PubChem CID: 439357); Lactic acid (PubChem CID: 61503); Citric acid (PubChem CID: 311); Butyric acid (PubChem CID: 264); Acetic acid (PubChem CID: 176).

http://dx.doi.org/10.1016/j.jff.2015.06.063
1756-4646/

Moreover, the functionalization of fresh or ripened cheeses may represent an added value to a product that already offers several benefits, due to basic natural composition. This strategy has successfully been introduced into several kinds of traditional cheeses made with cow's, goat's and/or sheep's milk (dos Santos et al., 2012; Gomes et al., 2011; Songisepp et al., 2012). In addition, Ortakci, Broadbent, McManus, and McMahon (2012) evaluated microencapsulation as a method to supply probiotics to cheese, and this has improved their viability when the cheesemaking and storage conditions are extremely stressful, as in the case of mozzarella cheese. However, this approach is not capable of increasing the viability of probiotic bacteria in traditional cheeses, where very hostile conditions are present (Kailasapathy & Masondole, 2005). The latter consideration suggests dedicating greater efforts to the identification of the best coupling between probiotics and type of cheese.

Regardless of the cheese type or of the probiotic supplementation strategies used, it is necessary, during the development of a new functional cheese, to understand whether the microorganisms add an impact or not to the original sensory characteristics. This is particularly important when probiotic *Lactobacillus* spp. are incorporated in cheeses that have to be ripened. Their enzymes, together with those of non-starter LAB (NSLAB), could be involved in the secondary proteolysis and lipolysis pathways which contribute to the final cheese flavour (Gomes da Cruz, Alonso Buriti, Batista de Souza, Fonseca Faria, & Isay Saad, 2009). Safeguarding the organoleptic characteristics of traditional cheeses is a fundamental step in the functional development of the product (Karimi, Sohrabvandi, & Mortazavian, 2012), and this is even more important in the case of Protected Denomination of Origin (PDO) products whose final features are imposed by strict regulations.

Toma Piemontese PDO is described as a cylindrical, semi-hard and semi-cooked cheese made with raw or pasteurized cow's milk. Its can range in size from 2 to 8 kg and have a ripening period of one to two months, respectively (Bertolino, Zeppa, Gerbi, & McSweeney, 2008). Moreover, it is one of the most common cheeses produced throughout the Piedmont region (Northwest Italy), and its functionalization may represent a nutraceutical improvement and an economical advantage.

The aim of this paper was to use putative probiotic lactobacilli, isolated from fermented table olives, as adjunct cultures in Toma Piemontese PDO cheese and to evaluate their survival in the cheese at the end of the ripening and after a simulated digestion process. Their effect on the sensory characteristics of the cheese has also been evaluated.

2. Materials and method

2.1. Adjunct cultures

The adjunct lactobacillus cultures were the putative probiotics *L. plantarum* S2T10D (LpS2T10D), *L. plantarum* S11T3E (LpS11T3E) and *L. pentosus* S3T60C (LpsS3T60C), derived from a collection of LAB isolated during green table olive fermentations (Cocolin et al., 2013). The selected LAB were studied through a comprehensive *in vitro* approach, as previously described by Botta, Langerholc, Cencič, and Cocolin (2014).

The starters that were to be used in the pilot-scale cheesemaking of Toma Piemontese PDO cheese had been individually lyophilized to a known concentration (Probiotical group, Novara, Italy) and kept at –20 °C until their use.

2.2. Pilot-scale cheese manufacturing

Raw bovine milk was pasteurized in a local dairy plant (Caseificio Valle Josina, Cuneo, Italy) at 72.5 °C for 19 s, cooled to 37 °C and then transferred in vats for the cheesemaking. Overall four batches of cheesemaking were produced in duplicate (500 L each duplicate), starting from four different milkings. Three batches were inoculated with a mixed commercial starter culture (Lyofast Y 0.82 B; Clerici-Sacco Group, Como, Italy), and each lyophilized putative probiotic as adjunct culture. One batch was only inoculated with the commercial culture and was used as control (Fig. 1). The commercial culture, composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (count ratio 100:1), and the adjunct cultures were both inoculated at 10^6 colony forming units (CFU) mL^{-1}. After 30 minutes of incubation, rennet (Clerici-Sacco Group, Como, Italy) was added to the milk at a strength of 1:10,000 (75% chymosin, 25% pepsin). The milk was left to rest for 30 minutes, and then the curd was cut, stirred for 10 minutes, drained from the whey and placed into moulds. The obtained cheeses (forms of 2 kg) were kept in a conditioned room for 4 h (30 °C, 90% relative humidity, RH) and then brine salted (21% NaCl, at 6 °C for 6 h). Cheese ripening was carried out for 60 days at 6 °C and 75% RH.

The samplings were performed on pasteurized milk before the inoculation, 30 min after the inoculation, and 90 min after the inoculation on drained curd and on cheeses at 1, 3, 7, 30 and 60 days. Samples were also collected after 15 days of ripening for chemical analysis. At each sampling point, one entire cheese was used for analysis.

2.3. Microbiological analysis

Solid samples were 1:10 diluted in a sterile isotonic solution (Ringer's solution; Oxoid, Basingstoke, Hampshire, UK), and homogenized using a Stomacher® 400 Circulator (Seward Ltd, Worthing, UK). The milk samples were analysed directly. Serial dilutions were plated onto Rogosa agar (Lab M, Heywood, Lancashire, UK) and incubated anaerobically at 30 °C for 72 h, for the enumeration of the lactobacilli. Cocci LAB were counted by plating on M17 agar (Lab M), and incubated aerobically at 37 °C for 48 h.

2.4. Simulation of upper gastrointestinal digestion

At the end of the ripening period, the three cheeses inoculated adjunct culture LpS2T10D, LpS11T3E and LpsS3T60C were subjected to an *in vitro* simulation of human digestion, in which gastric and intestinal transits were performed sequentially, as described by Ortakci et al. (2012).

Sterile filtered gastric juice (GJ), prepared as described by Mainville, Arcand, and Farnworth (2005), containing 3 g L^{-1} of pepsin, 0.14 M of NaCl, 2.7 mM of KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4 was prepared and buffered at pH 2.0 with HCl 1 M (Sigma-Aldrich, St. Louis, MO, USA). A portion of 40 g of cheese was aseptically ground (0.2-0.5 cm diameter) in a sterile bag,

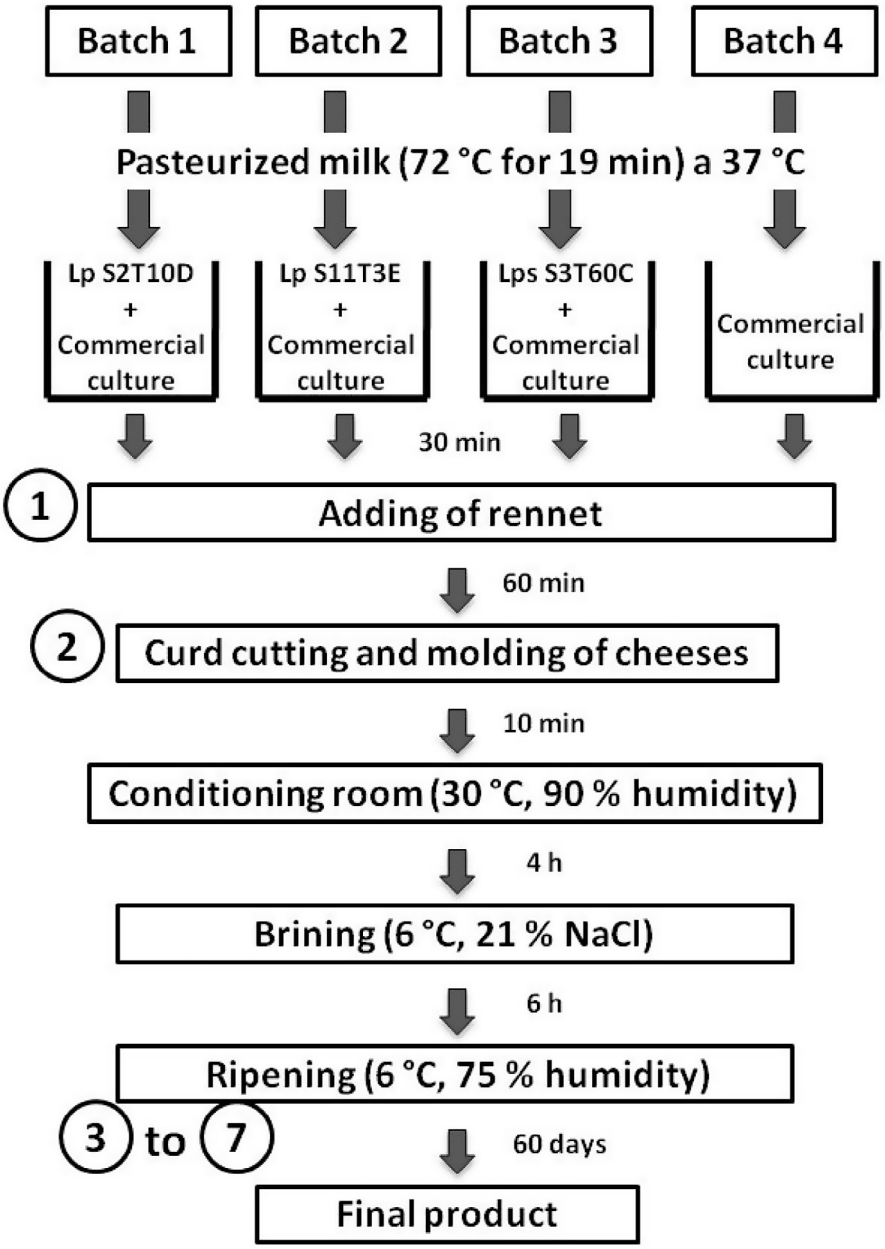

Fig. 1 – Flowchart of Toma Piemontese PDO pilot scale manufacturing carried out in this study. Sampling points made are shown in circles: (1) cheesemilk; (2) cut drained curd; (from 3 to 7) cheeses at different ripening stages, respectively 24 h, 3, 7, 30 and 60 days.

diluted in 120 mL (initial ratio of 1:4) of GJ and incubated at 37 °C for 2 h with periodical shaking. Sterile HCl 1M was periodically added (approximately 10 mL) to the GJ–cheese mixture, in order to maintain a pH <3.0.

After this gastric step, the pH of the mixture was increased to 8.0 with filtered NaOH 1M (approximately 18 mL) and 1 g L^{-1} of pancreatin (Sigma-Aldrich), after which 4.5 g L^{-1} of bile salts (Oxoid Basingstoke) was added to simulate the duodenal juice (DJ), as described by Huang and Adams (2004). The DJ–cheese mixture was then incubated for 4 h at 37 °C with periodic shaking.

Samples of the cheeses were collected before the simulation of digestion (T0), as well as after the gastric and the duodenal steps. In the latter cases, 20 g samples of cheese were collected and drained from the GJ and DJ, thus obtaining fractions of the cheese after the gastric (T1) and duodenal steps (T2). All the samples (T0, T1, T2) were subjected to microbiological analysis using Rogosa Agar as described above.

2.5. *Discrimination of adjunct cultures from indigenous lactobacilli*

A total of 10 lactobacillus colonies were randomly isolated from the highest dilution plate at each sampling point during the cheese manufacturing and ripening, and during the

simulated gastric and duodenal digestion (T0, T1, T2). All the isolates were purified by streaking and checked, through Gram staining and catalase activity. DNA was then extracted, as described by Cocolin et al. (2004), and normalized at 100 ng μL^{-1}. The lactobacillus isolates were identified by means of multiplex PCR analysis of the *rec*A gene with species-specific primers for *L. pentosus*, *L. plantarum* and *Lactobacillus paraplantarum*, according to the protocol described by Torriani, Felis, and Dellaglio (2001). Subsequently, Rep-PCR was performed on DNA extracted from *L. plantarum* and *L. pentosus* isolates with the single oligonucleotide primer $(GTG)_5$ (5′-GTGGTGGTGGTGGTG-3′) (Versalovic, Schneider, de Bruijn, & Lupski, 1994), according to the procedure described by Dal Bello et al. (2010).

The Rep-PCR profiles were visualized under ultraviolet light, and this was followed by digital image capturing using CCD UVI pro Platinum 1.1 (Eppendorf, Hamburg, Germany). The resulting digitized profiles were analysed by means of the BioNumerics 4.6 software package (Applied Maths, Sint-Martens-Latem, Belgium). Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) hierarchical clustering was performed using Pearson's Correlation Index to quantify the similarity (%). The DNA of Lps S3T60C, Lp S11T3E and Lp S2T10D was amplified in triplicate in order to calculate the Rep-PCR interstrain variability (%). These values were used as the limit to define any isolated lactobacilli from the cheeses equal to the inoculum (Mancini, Lazzi, Bernini, Neviani, & Gatti, 2012).

2.6. Organic acid profile, sugar content and pH

The organic acids (citric, pyruvic, lactic, acetic, butyric and propionic) and sugars (lactose, glucose and galactose) were determined by high performance liquid chromatography, according to the method described by Bertolino, Dolci, Giordano, Rolle, and Zeppa (2011), with some minor modifications. Briefly, milk samples (5 mL) were added to 20 mL of 0.0065 M H_2SO_4 (mobile phase) and mixed for 30 min with a horizontal shaker (Asal, Milan, Italy) at 100 oscillation/min. Curd and cheeses (5 g) were added to 25 mL of 0.0065 N H_2SO_4 (mobile phase) and homogenized for 10 min with a Stomacher® 400 Circulator (Seward Ltd, Worthing, UK). The slurry was subsequently centrifuged for 30 min at 5000 × *g* and 10 °C, and the supernatant was filtered through a 0.45 μm polypropylene membrane filter (VWR, Milan, Italy).

The HPLC system (Thermo Finnigan Spectra System, San Jose, CA, USA) was equipped with an isocratic pump (P4000), a multiple autosampler (AS3000) fitted with a 20 μL loop, a UV detector (UV100) set at 210 and 290 nm and a refractive index detector RI-150. The analyses were performed isocratically, at 0.8 ml min^{-1} and 65 °C, with a 300 × 7.8 mm i.d. cation exchange column (Aminex HPX-87H) equipped with a Cation H + Microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA). Three replicates were analysed for each sample. The data treatments were carried out using the Chrom Quest™ chromatography data system (Thermo Finnigan Spectra System). Analytical grade reagents were used as standards (Sigma-Aldrich).

The pH of the milk, curd and cheeses was measured using a pH meter (Crison, Modena, Italy).

2.7. Liking test

In order to evaluate the sensory characteristics of the cheeses, 40 consumers (19 males, 21 females) were recruited to conduct an acceptance test. The participants were regular cheese consumers and suffered from no food allergies. The test was performed inside a heated/air conditioned meeting room with white light, and the cheeses were analysed in two sessions at the end of ripening (60 days). The samples (30 g) were offered to the consumers in a completely randomized order. The consumers then rated the appearance, odour, taste, flavour, texture, and overall acceptance on a nine-point hedonic scale ranging from 'dislike very much' (1) to 'like very much' (9). A 5 min gap was enforced between each sample and the consumers were required to rinse their mouth with still water between each tasting. Paper score-sheets were used for data collection (Lavelli, Sri Harsha, Torri, & Zeppa, 2014), the ratings were treated as non-parametric data and the median values were calculated for each sensory parameter.

2.8. Statistical analysis

Microbiological counts were converted to Log_{10} CFU g^{-1} or mL^{-1} for statistical analysis. Moreover, in order to compare the survival of the lactobacillus population after the simulated digestion, the percentage ratio to the initial count (CFU g^{-1}) was calculated in each cheese with the added adjunct culture. The data from the microbiological and chemical analyses were expressed as the means of two independent experiments and standard deviation. In order to assess the overall variation and differences between the multiple groups, the numerical values were analysed by ANOVA (One way-Analysis of Variance) and using Tukey's post-hoc test. The non-parametric data obtained from the sensory evaluation were analysed with the Kruskal–Wallis test, in order to highlight any significant differences between the organoleptic profiles of the cheeses. Statistical analyses were performed with Statistica, ver. 7.0, (StatSoft Inc., Tulsa, OK, USA). The reference level of significance was 0.05 in all the assays.

3. Results

3.1. Survival of putative probiotics during cheesemaking and ripening

As far as the viable cell counts of starter LAB (SLAB), represented by the initially inoculated *Streptococcus thermophilus* starter, are concerned, no significant differences ($p > 0.05$) were observed between the three cheeses with the added adjunct cultures and the control, with the exception of the first sampling point at 30 min (Fig. 2A). On the other hand, significant differences ($p < 0.05$) were observed for the lactobacilli (Fig. 2B) between the putative probiotic cheeses and the control, from cheese manufacturing (inoculated cheesemilk) until day 30, with the highest viable counts being detected in the cheeses made with Lp S11T3E and Lp S2T10D. However, at the end of the ripening period, no differences were observed among the four experimental theses, with final lactobacillus values of over 7 Log_{10} CFU g^{-1} in all the cheeses.

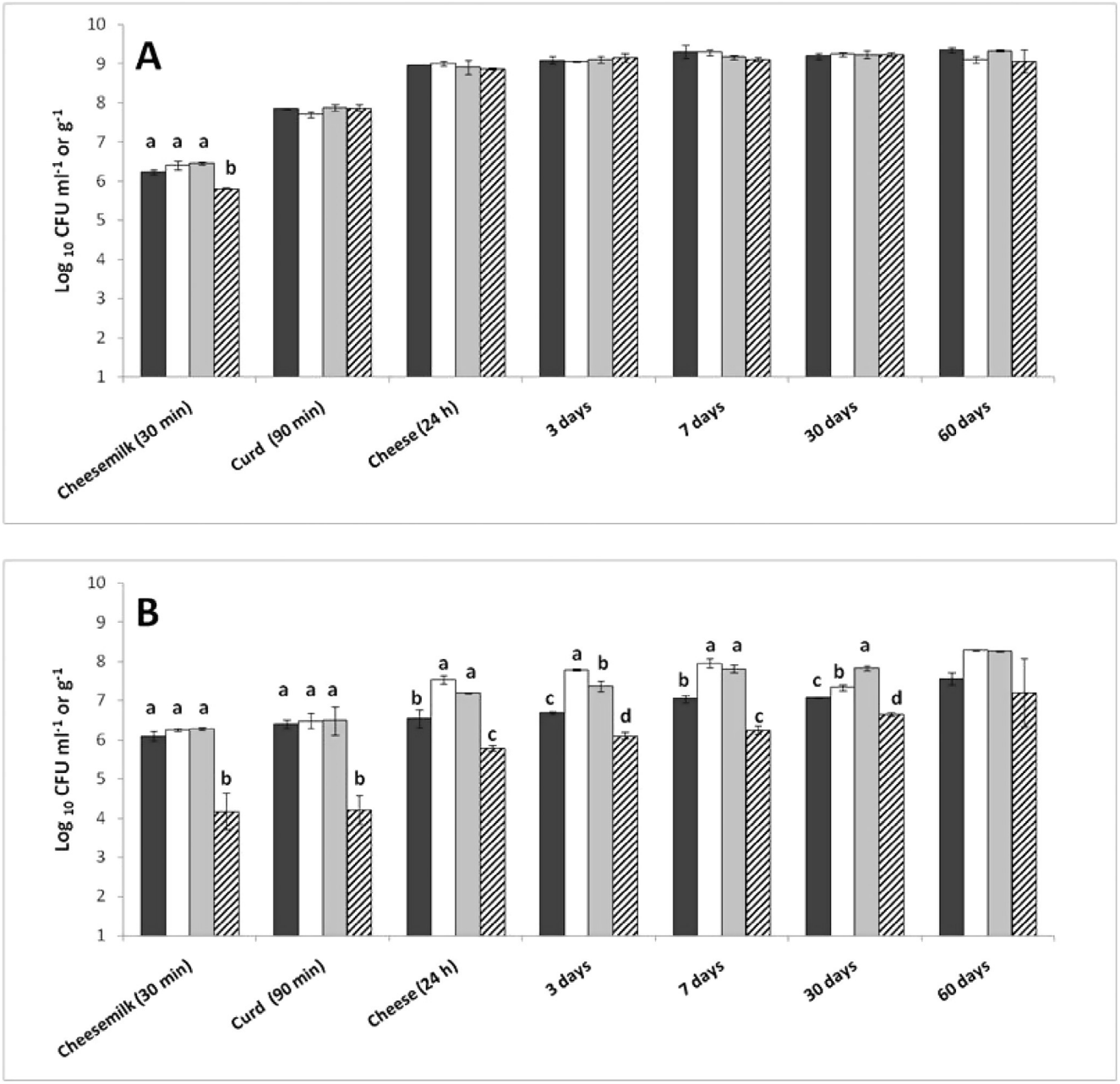

Fig. 2 – Evolution of SLAB (A) and lactobacillus (B) populations during the manufacturing and ripening of cheeses supplemented with (■) LpsS3T60C, (□) LpS11T3E, and (▒) LpS2T10D strains, and of the (▨) control cheeses made without adjunct cultures. The data are the mean values of two independent trials (±SD; n = 2) and different letters within the same sampling point (a, b, c, d) indicate significant differences among the values at $p < 0.05$ (ANOVA with Tukey's post-hoc test).

A total of 70 colonies of lactobacilli were isolated from each cheese with the added adjunct culture and tested by means of multiplex-PCR for identification purposes. All the colonies recovered from the cheeses made with LpsS3T60C were identified as *L. pentosus*, whereas in the cheeses made with Lp S11T3E and Lp S2T10D, all the isolates belonged to the *L. plantarum* species. On the other hand, no *L. pentosus* or *L. plantarum* strains were identified among the lactobacilli randomly isolated from the control trial (Table 1). The subsequent Rep-PCR and cluster analysis of the isolate profiles made it possible to establish the effective strain presence. Cluster analysis of the adjunct culture DNA replicates highlighted an Rep-PCR intrastrain variability (Pearson's correlation index) of 63.67%, 74.34% and 90.10%, for Lps S3T60C, Lp S11T3E and Lp S2T10D, respectively. Using these values in dendrograms, in which the isolates from the cheeses made with adjunct cultures were compared, it was possible to assess that all the colonies recovered during the cheese manufacturing and ripening clustered above the respective intrastrain variability percentage (data not shown).

Table 1 – Evaluation of the presence of the adjunct cultures among the lactobacilli isolated during the cheese manufacturing and ripening processes. The relative presence of each strain (Lps S3T60C, Lp S11T3E, Lp S2T10D) is reported in the last column and it was calculated considering the number of isolates recognized as the adjunct culture divided by the total number of isolated lactobacilli.

Cheeses	No.	No. of *L. plantarum*[a]	No. of *L. pentosus*[a]	Strain presence (%)[b]
Lps S3T60C	70	0	70	100
Lp S11T3E	70	70	0	100
Lp S2T10D	70	70	0	100
Control	20*	0	0	ND

ND: not detected.
* Isolation performed only at the first and last sampling points.
[a] Identification by means of *rec*A gene-based Multiplex-PCR.
[b] By means of cluster analysis (Rep-PCR profiles) of the isolates recognized at a species level.

3.2. *Survival of putative probiotics in simulated digestion*

The percentage survival of the lactobacillus populations during the simulated digestion and the effective presence of the three adjunct cultures are summarized in Table 2. The upper gastrointestinal transit determined a greater reduction in the relative lactobacillus presence for the cheeses inoculated with Lps S3T60C, in which a survival of 39.1% was observed at the end of digestion, a value that was significantly lower ($p < 0.05$) than that observed in the cheeses made with Lp S11T3E (90%). Furthermore, the first step of digestion for all three cheeses, which involved a homogenization in the SGJ at pH 2.0 for 2 hours, provoked a high reduction in vitality and the lowest percentage of survival.

As for the presence of the putative probiotic lactobacilli, for Lps S3T60C, a total of 29 of the 30 lactobacilli isolated from the cheeses before digestion and after the gastric and duodenal steps were recognized as *L. pentosus*, whereas in the other two cheeses (Lp S11T3E and Lp S2T10D), all the isolates were identified as *L. plantarum*. The subsequent cluster analysis of the Rep-PCR profiles grouped all the isolates together with the inoculated ones (data not shown).

3.3. *Organic acid and sugar profiles, acidity and sensory evaluation*

The organic acid and sugar contents, together with the pH changes, are reported in Table 3. As far as the initial milk composition is concerned, a higher concentration of lactose was detected in the two batches where Lps S3T60C and Lp S11T3E were added, than in the control or in the milk inoculated with Lp S2T10D ($p < 0.05$). After the first 24 hours, no significant differences ($p > 0.05$) were observed between the four trials, and lactose was detected in traces until the 30th day in all the trials and until the end of ripening in the cheeses with the added adjunct cultures. As far as the monomers derived from lactose hydrolysis are considered, glucose was not detected in the milk or cheese samples, whereas galactose was detected in all the cheeses, starting from the 90 min sampling point. The cheeses made with Lps S3T60C showed significantly higher ($p < 0.05$) concentrations of galactose than the other cheeses, including the control, with a maximum value of 17.76 ± 1.59 mg g^{-1} recorded after 7 days of ripening.

During the ripening, lactic acid represented the main organic acid produced by SLAB and NSLAB, with the greatest concentrations being found for all the cheeses at 60 days, and the highest amount detected in the cheese with Lps S3T60C (36.68 ± 1.59 mg g^{-1}). The citric acid concentration increased until the 7th day of ripening, and reached a maximum value of 2.47 ± 0.06 mg g^{-1} in the cheese made with Lps S3T60C. A constant decrease in the citric acid was then observed until the end of the ripening, with a complete absence only being recorded in the cheeses inoculated with Lps S3T60C. No pyruvic or propionic acids were detected in the samples, while the butyric acid concentrations increased during the ripening period in all the cheeses. The highest final concentration was found in the cheese inoculated with Lps S3T60C (3.25 ± 0.06 mg g^{-1}), and this was followed by the other two cheeses made with Lp S11T3E and Lp S2T10D (2.46 ± 0.21 and 2.02 ± 0.17 mg g^{-1}, respectively). In all the cheeses with the added adjunct cultures, its concentration at the end of ripening was significantly higher ($p < 0.05$) than the control cheese. Acetic acid was only present in the cheese samples with the added putative probiotic lactobacilli at 30 and 60 days of ripening. As already observed for the butyric acid, the cheese supplemented with the Lps

Table 2 – Percentage survival (%) and evaluation of the presence of adjunct cultures among the isolated lactobacilli (n = 2). Different letters within the same column (a, b) represent significant differences in lactobacillus survival (%) in the three cheeses ($p < 0.05$; ANOVA with Tukey's post-hoc test). The relative presence of each strain (Lps S3T60C, Lp S11T3E, Lp S2T10D) is reported in the last column and was calculated from the number of isolates recognized as belonging to the adjunct culture divided by the total number of isolated lactobacilli.

Cheeses	Gastric survival (%)*	Duodenal survival (%)*	*Lactobacillus* spp. isolated (T0, T1, T2)			
			No.	No. of *L. plantarum*[a]	No. of *L. pentosus*[a]	Strain presence (%)[b]
Lps S3T60C	23.8	39.1[b]	30	0	29	96
Lp S11T3E	57.1	90.9[a]	30	30	0	100
Lp S2T10D	56.8	72.6[a,b]	30	30	0	100

* $(CFUg^{-1})_{T1 \text{ or } T2}/(CFUg^{-1})_{T0} \times 100$.
[a] Identification by means of *rec*A gene-based Multiplex-PCR.
[b] By means of cluster analysis (Rep-PCR profiles) of isolates recognized at a species level.

Table 3 – Organic acid and sugar contents (mg g^{-1}) and pH values (mean ± SD; n = 6) of Toma Piemontese PDO cheeses produced with the Lps S3T60C, Lp S11T3E, Lp S2T10D adjunct cultures and the control, during their manufacturing and ripening. The data presented are the means of the two cheese productions.

		Cheesemaking			Cheese ripening					
		Pasteurized milk	Cheesemilk (30 min)	Curd (90 min)	24 h	3 days	7 days	15 days	30 days	60 days
Lactose	Lps S3T60C	64.32 ± 4.94[a]	66.11 ± 1.38[a]	38.09 ± 3.50[a]	4.27 ± 2.79	3.65 ± 2.82	2.11 ± 0.65	0.61 ± 0.03	1.33 ± 0.57	0.50 ± 0.11[a]
	Lp S11T3E	58.06 ± 1.67[a]	49.96 ± 14.2[a,b]	42.28 ± 4.09[a]	2.19 ± 1.63	0.97 ± 0.67	0.88 ± 0.85	0.55 ± 0.17	0.15 ± 0.05	0.16 ± 0.03[b]
	Lp S2T10D	38.47 ± 2.79[b]	36.36 ± 4.25[a,b]	16.48 ± 0.99[b]	4.65 ± 0.86	2.98 ± 0.16	0.97 ± 0.00	1.07 ± 0.62	0.62 ± 0.19	0.21 ± 0.05[b]
	Control	30.31 ± 0.42[b]	29.51 ± 1.79[b]	19.53 ± 1.73[b]	0.59 ± 0.03	2.61 ± 0.01	0.53 ± 0.37	0.22 ± 0.01	0.21 ± 0.07	0.05 ± 0.04[b]
Significance		***	*	**	ns	ns	ns	ns	ns	**
Galactose	Lps S3T60C	ND	ND	ND	16.88 ± 0.49	17.11 ± 1.27[a]	17.76 ± 0.49[a]	14.66 ± 2.46[a]	15.33 ± 0.39[a]	9.33 ± 0.45[a]
	Lp S11T3E	ND	ND	ND	13.15 ± 4.65	11.13 ± 0.49[b]	10.31 ± 1.84[b]	9.52 ± 1.37[a,b]	5.64 ± 0.85[b]	5.07 ± 0.16[b,c]
	Lp S2T10D	ND	ND	6.79 ± 0.55	9.63 ± 0.00	9.57 ± 0.13[b]	8.77 ± 0.06[b]	8.58 ± 0.46[b]	7.22 ± 0.56[b]	3.68 ± 0.97[c]
	Control	ND	ND	4.85 ± 1.58	7.96 ± 0.11	8.75 ± 0.29[b]	8.73 ± 0.24[b]	8.76 ± 0.12[b]	7.91 ± 0.94[b]	6.39 ± 0.78[b]
Significance				ns	ns	***	**	*	***	**
Lactic acid	Lps S3T60C	ND	ND	4.73 ± 0.58[a,b]	26.33 ± 4.44	28.19 ± 0.64[a]	32.21 ± 0.67[a]	27.65 ± 5.43	36.43 ± 0.84[a]	36.68 ± 1.59[a]
	Lp S11T3E	ND	ND	3.49 ± 0.30[b]	24.48 ± 8.51	22.06 ± 4.02[a,b]	21.47 ± 0.86[b]	26.82 ± 5.11	26.84 ± 4.07[b]	27.94 ± 0.37[b]
	Lp S2T10D	ND	ND	8.23 ± 1.09[a]	16.30 ± 0.16	17.19 ± 0.03[b]	18.51 ± 0.50[c]	20.16 ± 0.91	21.15 ± 0.00[b,c]	19.17 ± 1.05[c]
	Control	ND	ND	4.73 ± 1.68[a,b]	13.64 ± 0.26	14.17 ± 0.08[b]	14.89 ± 0.38[d]	15.07 ± 0.06	15.34 ± 0.68[c]	15.31 ± 0.27[c]
Significance				*	ns	**	***	ns	**	***
Citric acid	Lps S3T60C	0.67 ± 0.05[a]	0.72 ± 0.05[a]	0.88 ± 0.02[a]	2.21 ± 0.01	2.26 ± 0.02[a]	2.47 ± 0.06[a]	2.00 ± 0.54	1.33 ± 0.05[a]	ND
	Lp S11T3E	0.61 ± 0.01[a]	0.52 ± 0.14[a,b]	0.67 ± 0.02[a,b]	1.75 ± 0.56	1.58 ± 0.22[b]	1.44 ± 0.01[b]	1.67 ± 0.34	0.91 ± 0.11[a,b]	0.46 ± 0.13[a,b]
	Lp S2T10D	0.34 ± 0.01[b]	0.34 ± 0.02[b]	0.56 ± 0.13[b]	1.18 ± 0.04	1.21 ± 0.05[b]	1.29 ± 0.00[b]	1.18 ± 0.02	0.83 ± 0.07[b]	0.16 ± 0.03[b]
	Control	0.37 ± 0.00[b]	0.37 ± 0.02[b]	0.50 ± 0.04[b]	1.16 ± 0.03	1.13 ± 0.01[b]	1.23 ± 0.15[b]	1.23 ± 0.06	1.02 ± 0.20[a,b]	0.75 ± 0.10[a]
Significance		***	*	*	ns	**	***	ns	*	**
Butyric acid	Lps S3T60C	ND	ND	ND	1.22 ± 0.41	1.54 ± 0.23	1.95 ± 0.10[a]	1.76 ± 0.39	2.36 ± 0.05[a]	3.25 ± 0.06[a]
	Lp S11T3E	ND	ND	ND	1.40 ± 0.38	1.57 ± 0.54	1.63 ± 0.53[a,b]	2.24 ± 0.74	2.72 ± 0.79[a]	2.46 ± 0.21[b]
	Lp S2T10D	ND	ND	ND	0.82 ± 0.04	1.09 ± 0.03	1.40 ± 0.14[a,b]	1.60 ± 0.09	1.68 ± 0.04[a,b]	2.02 ± 0.17[a]
	Control	ND	ND	ND	ND	0.52 ± 0.07	0.66 ± 0.04[b]	0.71 ± 0.01	0.74 ± 0.00[b]	0.71 ± 0.07[c]
Significance					ns	ns	*	ns	*	***
Acetic acid	Lps S3T60C	ND	ND	ND	ND	ND	ND	ND	0.95 ± 0.05[a]	1.62 ± 0.10[a]
	Lp S11T3E	ND	ND	ND	ND	ND	ND	ND	0.51 ± 0.10[b]	0.68 ± 0.09[b]
	Lp S2T10D	ND	ND	ND	ND	ND	ND	ND	ND	0.87 ± 0.10[b]
	Control	ND	ND	ND	ND	ND	ND	ND	ND	ND
Significance									*	**
pH	Lps S3T60C	6.46 ± 0.01	6.44 ± 0.01[c]	6.40 ± 0.01[b]	5.10 ± 0.01[b]	5.14 ± 0.04[a,b]	5.24 ± 0.01	5.27 ± 0.14	5.30 ± 0.28	5.59 ± 0.30
	Lp S11T3E	6.53 ± 0.01	6.50 ± 0.01[b]	6.49 ± 0.01[a]	5.25 ± 0.01[a]	5.16 ± 0.02[a]	5.05 ± 0.14	4.96 ± 0.09	4.88 ± 0.04	5.80 ± 0.01
	Lp S2T10D	6.57 ± 0.06	6.50 ± 0.01[b]	6.48 ± 0.01[a]	5.16 ± 0.02[b]	4.95 ± 0.04[b]	5.14 ± 0.17	5.13 ± 0.21	5.13 ± 0.25	5.66 ± 0.22
	Control	6.56 ± 0.01	6.53 ± 0.01[a]	6.51 ± 0.01[a]	5.18 ± 0.04[a,b]	5.19 ± 0.08[a]	5.21 ± 0.13	5.25 ± 0.10	5.30 ± 0.07	5.71 ± 0.01
Significance		ns	***	**	**	*	ns	ns	ns	ns

[a,b,c,d] Different letters indicate a significant difference, at each sampling point, among the four batches of cheese at $p < 0.05$ (ANOVA with Tukey's post-hoc test).

ND: not detected.

ns: not significant.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

S3T60C showed the highest concentration of acetic acid (1.62 ± 0.10 mg g^{-1}), a value that was significantly higher than the other two cheeses inoculated with *L. plantarum* ($p < 0.05$).

It was possible to observe significant differences in the acidification dynamics between the batches within the first three days ($p < 0.05$), while the pH values recorded for the remaining period of maturation did not show any significant variations between the four cheeses produced.

Finally, as far as the sensory evaluation is concerned, it is worth mentioning that, in relation to the Kruskal–Wallis test, no significant differences were observed among the scores assigned to the four batches of cheeses, whether supplemented or not with the adjunct cultures (data not shown).

4. Discussion

The first and most critical aspect in the development of a probiotic dairy food is the coexistence between SLAB and adjunct cultures, since a negative interaction between them or an interaction between the added cultures and the matrix could determine changes in the process and in the final product stability (da Cruz et al., 2009; Vinderola, Mocchiutti, & Reinheimer, 2002). In this paper, the technological exploitation of Lps S3T60C, Lp S11T3E and Lp S2T10D as adjunct cultures did not significantly affect the SLAB growth compared to the control (Fig. 2A), and this result has also recently been observed in Cheddar cheese supplemented with *L. plantarum* and *L. pentosus* of plant origin (Ciocia, McSweeney, Piraino, & Parente, 2013). As expected, the effect of adjunct cultures had an impact on the lactobacillus dynamics during the first month of ripening (Fig. 2B), while at 60 days of ripening, the dynamics were similar in all the cheeses, due to the progressive growth of NSLAB, as described by Settanni and Moschetti (2010). When the effective presence of the adjunct cultures was analysed in depth, it emerged that all the isolated lactobacilli belonged to the same species of the inoculated strains, i.e. *L. pentosus* in the case of cheeses inoculated with Lps S3T60C and *L. plantarum* for the cheeses made with Lp S11T3E and Lp S2T10D (Table 1). As reported by Fortina et al. (2003), the natural microbiota of Toma Piemontese PDO does not include *L. plantarum* or *L. pentosus* among the NSLAB species responsible for its final organoleptic characteristics. These two species were in fact not identified in any of the control cheeses, thus confirming the previous findings. The presence of Lps S3T60C, Lp S11T3E and Lp S2T10D was also confirmed by means of Rep-PCR coupled to the cluster analysis, which grouped all the profiles of the isolated lactobacilli with those of the three inoculated strains. Hence, it can be asserted that the lactobacilli were able to cope well with the environmental conditions encountered in Toma Piemontese PDO, although they originate from a different fermented product, that is, table olives (Cocolin et al., 2013). Furthermore, the growth of lactobacilli, which is shown in Fig. 2B, reached a final viable count of more than 10^7 CFU per gram, which can be considered satisfactory for a potential probiotic in cheeses (Karimi, Mortazavian, & Da Cruz, 2011). To the best of the authors' knowledge, although *L. plantarum* strains have been used successfully as adjunct cultures in other semi-hard and semi-ripened cheeses (Milesi, McSweeney, & Hynes, 2008; Milesi, Vinderola, Sabbag, Meinardi, & Hynes, 2009; Minervini et al., 2012; Ortigosa, Arizcun, Torre, & Izco, 2005; Songisepp et al., 2012), few studies have been carried out concerning the possible use of *L. pentosus* strains as adjunct cultures in dairy products, especially those isolated from fermented vegetables (Ciocia et al., 2013; Marroki & Bousmaha-Marroki, 2014).

Another fundamental feature that a probiotic added to a food matrix has to possess is the capability of overcoming the conditions found in the digestive tract and of reaching the intestine in adequate amounts. This can primarily be investigated through a simulation of human digestion (Mainville et al., 2005). In the present study the viable counts of lactobacilli, recovered in the partial fraction (after the gastric step) and completely digested fraction (after the duodenal step), together with molecular analysis, have highlighted a massive presence of the adjunct cultures (Table 2). These results are based on the combined use of species-specific-PCR and typing with Rep-PCR, an effective and rapid molecular method to monitor the presence and dynamics of *L. plantarum* and *L. pentosus* strains inoculated in dairy and vegetable fermented food (De Bellis, Valerio, Sisto, Lonigro, & Lavermicocca, 2010; Hurtado, Reguant, Bordons, & Rozès, 2010; Pogačić et al., 2013). As far as the behaviour of the strains during simulated digestion is concerned, an increase in the number of lactobacilli recovered was observed after the duodenal step, compared to the gastric step. As previously observed by other authors, the simulation of gastric transit with its low pH can affect the viability of probiotics more than the subsequent duodenal passage, where the inhibition depends on the content of bile salts, which are often well tolerated by *Lactobacillus* spp. (Faye, Tamburello, Vegarud, & Skeie, 2012; Ricciardi et al., 2014). When the behaviour of the strains is compared, it can be seen that the survival percentage of Lps S3T60C is significantly lower than that of Lp S11T3E, and it is the lowest of the three strains. The higher survival capability of Lp S11T3E than the other two strains has already been observed (Botta et al., 2014), although the bacteria in that case were digested *in vitro,* without being included in a food matrix. The results pertaining to lactobacillus survival have once again highlighted the effective protection of the bacteria by the cheese matrix.

In order to assess the physico-chemical and sensory impacts of the addition of the putative probiotic lactobacilli to Toma Piemontese PDO, the sugars and organic acids were analysed (Table 3), and a sensory characterization was carried out. In agreement with the literature, part of the lactose was lost in the whey during cheesemaking, while the sugar remaining in the curd was hydrolysed into glucose and galactose within the first 24 hours (McSweeney, 2004). The glucose was immediately consumed by the LAB populations (mainly SLAB), and his led to a rapid increase in the lactic acid content at the end of the first day. Overall, lactic acid was the most abundant organic acid in the cheeses and the change in its amount was the primary cause of the observed pH decrease after 90 min and 24 h. The subsequent gradual increase in lactic acid production was closely related to the reduction of galactose, which was poorly consumed in all the cheeses, compared to the values previously reported for Toma Piemontese PDO (Zeppa & Rolle,

2008). However, the differences among the cheeses with respect to the galactose consumption and lactic acid production cannot be attributed directly to the different metabolic activities of the LAB populations, since the milk used for the production of the different batches showed significant differences in the initial concentrations of lactose.

The citric acid concentrations detected in the curds were approximately three times higher than in milk, due to its colloidal concentration, as generally reported in literature (McSweeney, 2004). Its progressive decrease may be related to the presence of citrate positive (Cit^+) strains of lactococci, enterococci, and mesophilic NSLAB, e.g. strains of *Lactobacillus casei*, *Lactobacillus paracasei*, *L. plantarum* or *L. pentosus*, which can convert citrate into acetate, diacetyl and acetoin (Cselovszky, Wolf, & Hammes, 1992; de Figueroa, Alvarez, Holgado, Oliver, & Sesma, 2000; Hugenholtz, 1993). It is worth noting that only in the cheeses made with Lps S3T60C was the citric acid completely consumed, thus determining an increase in acetic acid in the last phases of ripening. It is also important to underline that acetic acid is an important flavour compound that is present in many cheeses, and it has a strong antagonistic effect against Gram-negative bacteria (Piras et al., 2013). On the other hand, the butyric acid contents were markedly higher in all the cheeses made with the adjunct cultures compared to those detected in the control samples and to the literature data (Zeppa & Rolle, 2008), and this could allow us to speculate that its presence is connected to the metabolic activities of the adjunct cultures. As reported by Marilley (2004), high concentrations of butyric acid may determine sweaty odours in cheeses. However, during the liking test, the consumers did not perceive any off-flavours. As observed for the acetic acid, Lps S3T60C was also likely responsible for the high content of butyrate detected at the end of ripening in the cheeses made with this strain. Recently, Lps S3T60C has shown to metabolize higher amounts of butyric and acetic acids in comparison with Lp S11T3E and Lp S2T10D (Pessione, Lo Bianco, Mangiapane, Cirrincione, & Pessione, 2015).

Therefore, considering the pivotal role of these two SCFAs in many probiotic mechanisms of action, further studies on the Lps S3T60C metabolic pathway are necessary (Lan, Lagadic-Gossmann, Lemaire, Brenner, & Jan, 2007; Zhong, Zhang, & Covasa, 2014).

5. Conclusions

To conclude, the putative probiotics Lp S11T3E, Lp S2T10D and Lps S3T60C showed high survival capability during production of Toma Piemontese PDO, as well as after its simulated digestion. Our results highlighted that non-dairy putative probiotics, isolated from table olives, can cope to a completely different fermentation environment, like that of cheeses, actively taking part in the complex biochemical processes of cheesemaking and ripening.

The ability of all three strains to enrich functional cheeses with SCFAs, without damaging their peculiar features, may significantly contribute to the product quality enhancement and deserves further in-depth investigations.

Acknowledgements

This study was founded by the Piedmont Region, Italy, under grant agreement no. 0186000155 (SafeNutriFood Project). The authors would like to thank the research group at Probiotical S.p.A. (Novara, Italy) for the fruitful collaboration as well as the Valle Josina S.a.C. dairy farm (Cuneo, Italy).

REFERENCES

Awaisheh, S. S. (2012). E. Rigobelo (Ed.), *Probiotic food products classes, types, and processing*, Probiotics. ISBN: 978-953-51-0776-7, doi:10.5772/51267 <http://www.intechopen.com/books/probiotics/probiotic-food-products-classes-types-and-processing>; InTech.

Bertolino, M., Dolci, P., Giordano, M., Rolle, L., & Zeppa, G. (2011). Evolution of chemico-physical characteristics during manufacture and ripening of Castelmagno PDO cheese in wintertime. *Food Chemistry*, *129*, 1001–1011.

Bertolino, M., Zeppa, G., Gerbi, V., & McSweeney, P. L. H. (2008). Study of proteolysis in miniature Toma Piemontese cheese made using wild bacteria. *Italian Journal of Food Science*, *20*, 57–74.

Botta, C., Langerholc, T., Cencič, A., & Cocolin, L. (2014). *In vitro* selection and characterization of new probiotic candidates from table olive microbiota. *PLoS ONE*, *9*, e94457.

Ciocia, F., McSweeney, P. L. H., Piraino, P., & Parente, E. (2013). Use of dairy and non-dairy *Lactobacillus plantarum, Lactobacillus paraplantarum* and *Lactobacillus pentosus* strains as adjuncts in cheddar cheese. *Dairy Science and Technology*, *93*, 623–640.

Cocolin, L., Alessandria, V., Botta, C., Gorra, R., De Filippis, F., Ercolini, D., & Rantsiou, K. (2013). NaOH-debittering induces changes in bacterial ecology during table olives fermentation. *PLoS ONE*, *8*, e69074.

Cocolin, L., Rantsiou, K., Iacumin, L., Urso, R., Cantoni, C., & Comi, G. (2004). Study of the ecology of fresh sausages and characterization of populations of lactic acid bacteria by molecular methods. *Applied Microbiology and Biotechnology*, *70*, 1883–1894.

Cselovszky, J., Wolf, G., & Hammes, W. (1992). Production of formate, acetate, and succinate by anaerobic fermentation of *Lactobacillus pentosus* in the presence of citrate. *Applied Microbiology and Biotechnology*, *37*, 94–97.

da Cruz, A. G., Alonso Buriti, F. C., Batista de Souza, C. H., Fonseca Faria, J. A., & Isay Saad, S. M. (2009). Probiotic cheese: Health benefits, technological and stability aspects. *Trends in Food Science & Technology*, *20*, 344–354.

de Figueroa, R., Alvarez, F., Holgado, A., Oliver, G., & Sesma, F. (2000). Citrate utilization by homo- and heterofermentative lactobacilli. *Microbiological Research*, *154*, 313–320.

dos Santos, K. M. O., Bomfim, M. A. D., Vieira, A. D. S., Benevides, S. D., Saad, S. M. I., Buriti, F. C. A., & Egito, A. S. (2012). Probiotic caprine *Coalho* cheese naturally enriched in conjugated linoleic acid as a vehicle for *Lactobacillus acidophilus* and beneficial fatty acids. *International Dairy Journal*, *24*, 107–112.

Dal Bello, B., Rantsiou, K., Bellio, A., Zeppa, G., Ambrosoli, R., Civera, T., & Cocolin, L. (2010). Microbial ecology of artisanal products from North West of Italy and antimicrobial activity of the autochthonous populations. *LWT – Food Science and Technology*, *43*, 1151–1159.

De Bellis, P., Valerio, F., Sisto, A., Lonigro, S. L., & Lavermicocca, P. (2010). Probiotic table olives: Microbial populations adhering on olive surface in fermentation sets inoculated with the probiotic strain *Lactobacillus paracasei* IMPC2.1 in an industrial plant. *International Journal of Food Microbiology*, *140*, 6–13.

Faye, T., Tamburello, A., Vegarud, G. E., & Skeie, S. (2012). Survival of lactic acid bacteria from fermented milks in an *in vitro* digestion model exploiting sequential incubation in human gastric and duodenum juice. *Journal of Dairy Science*, *95*, 558–566.

Fortina, M. G., Ricci, G., Acquati, A., Zeppa, G., Gandini, A., & Manachini, P. L. (2003). Genetic characterization of some lactic acid bacteria occurring in an artisanal protected denomination origin (PDO) Italian cheese, the Toma Piemontese. *Food Microbiology*, *20*, 397–404.

Gomes, A. A., Braga, S. P., Cruz, A. G., Cadena, R. S., Lollo, P. C. B., Carvalho, C., Amaya-Farfán, J., Faria, J. A. F., & Bolini, H. M. A. (2011). Effect of the inoculation level of *Lactobacillus acidophilus* in probiotic cheese on the physicochemical features and sensory performance compared with commercial cheeses. *Journal of Dairy Science*, *94*, 4777–4786.

Gomes da Cruz, A., Alonso Buriti, F. C., Batista de Souza, C. H., Fonseca Faria, J. A., & Isay Saad, S. M. (2009). Probiotic cheese: Health benefits, technological and stability aspects. *Trends in Food Science & Technology*, *20*, 344–354.

Huang, Y., & Adams, M. C. (2004). In vitro assessment of the upper gastrointestinal tolerance of potential probiotic dairy propionibacteria. *International Journal of Food Microbiology*, *91*, 253–260.

Hugenholtz, J. (1993). Citrate metabolism in lactic acid bacteria. *FEMS Microbiology Reviews*, *12*, 165–178.

Hurtado, A., Reguant, C., Bordons, A., & Rozès, N. (2010). Evaluation of a single and combined inoculation of a *Lactobacillus pentosus* starter for processing cv. *Arbequina* natural green olives. *Food Microbiology*, *27*, 731–740.

Kailasapathy, K., & Masondole, L. (2005). Survival of free and microencapsulated *Lactobacillus acidophilus* and *Bifidobacterium lactis* and their effect on texture of feta cheese. *Australian Journal of Dairy Technology*, *60*, 252–258.

Karimi, R., Mortazavian, A. M., & Da Cruz, A. G. (2011). Viability of probiotic microorganisms in cheese during production and storage: A review. *Dairy Science and Technology*, *91*, 283–308.

Karimi, R., Sohrabvandi, S., & Mortazavian, A. M. (2012). Review article: Sensory characteristics of probiotic cheese. *Comprehensive Reviews in Food Science and Food Safety*, *11*, 437–452.

Lan, A., Lagadic-Gossmann, D., Lemaire, C., Brenner, C., & Jan, G. (2007). Acidic extracellular pH shifts colorectal cancer cell death from apoptosis to necrosis upon exposure to propionate and acetate, major end-products of the human probiotic propionibacteria. *Apoptosis: An International Journal on Programmed Cell Death*, *12*, 573–591.

Lavelli, V., Sri Harsha, P. S. C., Torri, L., & Zeppa, G. (2014). Use of winemaking by-products as an ingredient for tomato puree: The effect of particle size on product quality. *Food Chemistry*, *152*, 162–168.

Mainville, I., Arcand, Y., & Farnworth, E. R. (2005). A dynamic model that simulates the human upper gastrointestinal tract for the study of probiotics. *International Journal of Food Microbiology*, *99*, 287–296.

Mancini, A., Lazzi, C., Bernini, V., Neviani, E., & Gatti, M. (2012). Identification of dairy lactic acid bacteria by tRNA *Ala*-23S rDNA-RFLP. *Journal of Microbiological Methods*, *91*, 380–390.

Marilley, L. (2004). Flavours of cheese products: Metabolic pathways, analytical tools and identification of producing strains. *International Journal of Food Microbiology*, *90*, 139–159.

Marroki, A., & Bousmaha-Marroki, L. (2014). Lactobacilli isolated from Algerian goat's milk as adjunct culture in dairy products. *Brazilian Archives of Biology and Technology*, *57*, 410–420.

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

Milesi, M. M., McSweeney, P. L. H., & Hynes, E. R. (2008). Viability and contribution to proteolysis of an adjunct culture of *Lactobacillus plantarum* in two model cheese systems: Cheddar cheese-type and soft-cheese type. *Journal of Applied Microbiology*, *105*, 884–892.

Milesi, M. M., Vinderola, G., Sabbag, N., Meinardi, C. A., & Hynes, E. (2009). Influence on cheese proteolysis and sensory characteristics of non-starter lactobacilli strains with probiotic potential. *Food Research International*, *42*, 1186–1196.

Minervini, F., Siragusa, S., Faccia, M., Dal Bello, F., Gobbetti, M., & De Angelis, M. (2012). Manufacture of Fior di Latte cheese by incorporation of probiotic lactobacilli. *Journal of Dairy Science*, *95*, 508–520.

Ortakci, F., Broadbent, J. R., McManus, W. R., & McMahon, D. J. (2012). Survival of microencapsulated probiotic *Lactobacillus paracasei* LBC-1e during manufacture of mozzarella cheese and simulated gastric digestion. *Journal of Dairy Science*, *95*, 6274–6281.

Ortigosa, M., Arizcun, C., Torre, P., & Izco, J. M. (2005). Use of wild *Lactobacillus* strains in an adjunct culture for a Roncal-type cheese. *Journal of Dairy Research*, *72*, 168–178.

Pessione, A., Lo Bianco, G., Mangiapane, E., Cirrincione, S., & Pessione, E. (2015). Characterization of potentially probiotic lactic acid bacteria isolated from olives: Evaluation of short chain fatty acids production and analysis of the extracellular proteome. *Food Research International*, *67*, 247–254.

Piras, C., Cesare Marincola, F., Savorani, F., Engelsen, S. B., Cosentino, S., Viale, S., & Pisano, M. B. (2013). A NMR metabolomics study of the ripening process of the Fiore Sardo cheese produced with autochthonous adjunct cultures. *Food Chemistry*, *141*, 2137–2147.

Pitino, I., Randazzo, C. L., Cross, K. L., Parker, M. L., Bisignano, C., Wickham, M. S. J., Mandalari, G., & Caggia, C. (2012). Survival of *Lactobacillus rhamnosus* strains inoculated in cheese matrix during simulated human digestion. *Food Microbiology*, *31*, 57–63.

Plessas, S., Bosnea, L., Alexopoulos, A., & Bezirtzoglou, E. (2012). Potential effects of probiotics in cheese and yogurt production: A review. *Engineering in Life Sciences*, *12*, 433–440.

Pogačić, T., Mancini, A., Santarelli, M., Bottari, B., Lazzi, C., Neviani, E., & Gatti, M. (2013). Diversity and dynamic of lactic acid bacteria strains during aging of a long ripened hard cheese produced from raw milk and undefined natural starter. *Food Microbiology*, *36*, 207–215.

Ricciardi, A., Blaiotta, G., Di Cerbo, A., Succi, M., & Aponte, M. (2014). Behavior of lactic acid bacteria populations in Pecorino di Carmasciano cheese samples submitted to environmental conditions prevailing in the gastrointestinal tract: Evaluation by means of a polyphasic approach. *International Journal of Food Microbiology*, *179*, 64–71.

Settanni, L., & Moschetti, G. (2010). Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. *Food Microbiology*, *27*, 691–697.

Sharp, M. D., McMahon, D. J., & Broadbent, J. R. (2008). Comparative evaluation of yogurt and low-fat cheddar cheese as delivery media for probiotic *Lactobacillus casei*. *Journal of Food Science*, *73*, 375–377.

Songisepp, E., Hütt, P., Rätsep, M., Shkut, E., Kõljalg, S., Truusalu, K., Stsepetova, J., Smidt, I., Kolk, H., Zagura, M., & Mikelsaar, M. (2012). Safety of a probiotic cheese containing *Lactobacillus plantarum* Tensia according to a variety of health indices in different age groups. *Journal of Dairy Science*, *95*, 5495–5509.

Sumeri, I., Adamberg, S., Uusna, R., Sarand, I., & Paalme, T. (2012). Survival of cheese bacteria in a gastrointestinal tract simulator. *International Dairy Journal*, *25*, 36–41.

Torriani, S., Felis, G. E., & Dellaglio, F. (2001). Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum* by *rec*A gene sequence analysis and multiplex PCR assay with

*rec*A gene-derived primers. *Applied and Environmental Microbiology*, 67, 3450–3454.
Versalovic, J., Schneider, M., de Bruijn, F., & Lupski, J. R. (1994). Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Methods in Molecular and Cellular Biology*, 5, 25–40.
Vinderola, C. G., Mocchiutti, P., & Reinheimer, J. A. (2002). Interactions among lactic acid starter and probiotic bacteria used for fermented dairy products. *Journal of Dairy Science*, 85, 721–729.
Zeppa, G., & Rolle, L. (2008). A study on organic acid, sugar and ketone contents in typical Piedmont cheeses. *Italian Journal of Food Science*, 20, 127–139.
Zhong, L., Zhang, X., & Covasa, M. (2014). Emerging roles of lactic acid bacteria in protection against colorectal cancer. *World Journal of Gastroenterology*, 20, 7878–7886.