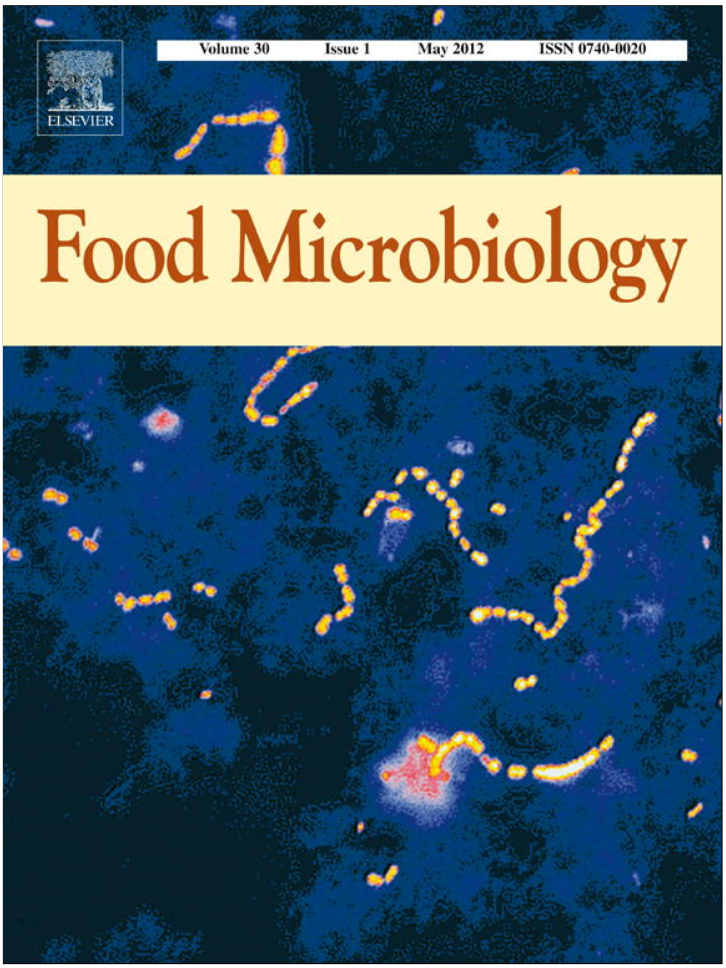

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Technological and probiotic role of adjunct cultures of non-starter lactobacilli in soft cheeses

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ABSTRACT

The influence of two cheese-isolated *Lactobacillus* strains on cheese composition, acceptability and probiotic capacity was assessed. Soft cheeses with and without the addition of *Lactobacillus plantarum* I91 or *Lactobacillus paracasei* I90 were prepared. Gross composition was assessed and secondary proteolysis was described by soluble fractions and free amino acids profiles. Acceptability was determined by a panel of 98 non-trained consumers. Cheeses harboring added *Lactobacillus* strains were also studied *in vivo* to evaluate their probiotic capacity. Gross composition of the cheeses was similar for control and treated (*Lactobacillus*-added) cheeses. Peptidolysis increased in cheeses with added lactobacilli, which was evidenced by a higher free amino acid content. Overall, the acceptability of the cheeses was good: 65%–80% of the consumers said that they "liked very much" or "liked" the cheeses. Cheeses with *L. plantarum* I91 showed the highest changes in composition and proteolysis and were the most accepted ones. On the contrary, composition of cheeses with *L. paracasei* I90 was similar to that of the controls, but these samples were less accepted than cheeses without lactobacilli. The oral administration of cheese containing *L. plantarum* I91 or *L. paracasei* I90 proved to be safe and able to enhance the number of IgA + cells in the small intestine lamina propria of mice. The use of selected strains of NSLAB exerted a technological and probiotic role: it contributed to the standardization of cheese quality and induced benefic health effects at the gut mucosa *in vivo*.

1. Introduction

The development of non-starter lactic acid bacteria (NSLAB) during the ripening of cheeses made in an industrial environment or at a farm-scale is an uncontrolled factor of the cheesemaking process. This should be taken into account when cheese quality is considered since the growth of NSLAB may affect chemical composition and flavor of the cheese. NSLAB are mostly facultative heterofermentative mesophilic lactobacilli including species such as *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and *L. plantarum*, as well as pediococci, *Leuconostoc* and micrococci (De Angelis et al., 2001; Quiberoni et al., 2005). The main source of this component of cheese microflora is the raw milk (Jordan and Cogan, 1999; Poznanski et al., 2004). It has been shown that NSLAB can survive pasteurization at low numbers and slowly grow during cheese ripening up to 10^6–10^7 CFU/g, depending on the cheese ripening period and temperature (Turner et al., 1986; Crow et al., 2002; De Angelis et al., 2004; Christiansen et al., 2006). Post-pasteurization contamination due to a resident flora in the dairy plant has also been showed to contribute to NSLAB proliferation (Somers et al., 2001). NSLAB may overcome lactic acid starters in the cheese and become the dominant microflora, especially if primary lactic acid starters die and lyse simultaneously with NSLAB growth (Crow et al., 2001).

While some authors consider these bacteria as responsible for the "finishing" of cheese ripening, others consider that they are responsible for up to 80% of the defects found in cheeses, mainly related to post-acidification, gas formation (in varieties of cheese that do not require it), lactate racemization with lactate crystal formation and production of off-flavors, among others (Crow et al., 2001). On these grounds, a successful approach has been the addition of selected strains of NSLAB, known as "adjunct cultures" in order to control the adventitious growth of other NSLAB. To obtain these cultures, *Lactobacillus* strains are isolated from cheese of good quality, identified and characterized for their technological

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properties (Crow et al., 2001; Wouters et al., 2002; Ortigosa et al., 2006; Randazzo et al., 2008). The potential effect of these strains on the flavor intensity of the cheese is linked to increased secondary proteolysis, metabolism of remaining carbohydrates and the capacity to produce free amino acids and to metabolize them, with formation of flavor compounds. Not less important is the involvement of these strains in making the environment of the food less favorable for the development of undesirable microorganisms, potentially harmful, thereby contributing to the safety and quality of the cheeses. In this way sensory characteristics and acceptability of the final product, which definitely will determine its acceptance or rejection by consumers, must be taken into account.

Many NSLAB belong to species from which probiotic strains were isolated and characterized (*L. casei, L. paracasei, Lactobacillus plantarum*). Then, recent attention was paid to the possibility that NSLAB may play a role as health promoters, beyond their technological function (Milesi et al., 2009; Settanni and Moschetti, 2010). The addition of selected NSLAB as adjunct cultures could also play a probiotic role in cheese (Bertazzoni Minelli et al., 2004; Bude-Ugarte et al., 2006; Maragkoudakis et al., 2006).

In this paper, we studied the performance of two adjunct cultures of non-starter lactobacilli in a soft cheese. The influence of NSLAB addition on chemical composition, peptidolysis and acceptability and the probiotic capacity of the products manufactured were assessed.

2. Material and methods

2.1. Strains

A commercial direct-to-vat culture of *Streptococcus thermophilus* St-M5 (Chr. Hansen Argentina, Quilmes, Argentina) was used for primary milk fermentation. The adjunct cultures of lactobacilli were *L. plantarum* I91 and *L. paracasei* I90, selected from a collection of ca. 20 strains isolated from ripened, good quality soft and semi-hard cheeses in a previous study (Briggiler-Marcó et al., 2007). The strains were selected on the basis of their satisfactory technological properties for cheese manufacture. Both strains were identified first by a classical approach and then by molecular methods (Bude-Ugarte et al., 2006). *L. plantarum* I91 and *L. paracasei* I90 showed phage resistance and low acidifying activity, they were also moderately proteolytic and able to grow in salt-containing culture medium (NaCl or KCl up to 3% w/v) (Briggiler-Marcó et al., 2007). Additionally, the strains displayed *in vitro* features of interest for their use as probiotics, as result of a previous study in our research group (Bude-Ugarte et al., 2006). Strains were transferred twice in de Man, Rogosa, and Sharpe (MRS) broth (Britania, Buenos Aires, Argentina) at 34 °C for 18 h, from frozen stock cultures (stored at −80 °C in MRS broth with 15% glycerol). An aliquot of the overnight cultures, enough to attain an initial concentration of 10^6 CFU/ml in the milk for cheese manufacture, was centrifuged for 20 min at 8000 × g and 4 °C. The pellet was washed twice with 0.1 M sodium phosphate buffer (pH 7) and then resuspended in the same buffer before adding it to the cheesemilk.

2.2. Cheesemaking

Cremoso cheeses — a high moisture, soft cheese variety - were made at small scale (8 L). Three cheeses were obtained by cheesemaking day: one control cheese without added lactobacilli and two experimental cheeses, one with the addition of *L. plantarum* I91 and the other containing *L. paracasei* I90. Bulk tank milk from a near dairy plant was batch-pasteurized (65 °C, 20 min) and supplemented with $CaCl_2$ 0.014% (w/v). After pasteurization, milk was cooled to 39.5 °C and lactic acid starters were added: *S. thermophilus* St-M5 to control cheeses, and *S. thermophilus* St-M5 and lactobacilli adjunct to the experimental ones. Then coagulant enzyme was added according to the manufacturer's instructions (Chymax, Christian Hansen, Hørsholm, Denmark, 0.35 ml/L milk). Once the milk coagulated and the coagulum strengthened to the appropriate firmness (ca. 18 min), it was cut with a stainless steel wired tool (1 cm wide). Curd was left healing for 7 min, during which, steps of resting and gentle agitation of whey-curds mix were carried out. Whey was separated from the curd and cheese was cast and left at high temperature (ca. 40° C) until a pH of 5.20–5.30 was reached. Cheeses were brined (20% NaCl, pH 5.40) for 1 h at 8 °C. Then, they were left to dry in the ripening chamber at the same temperature for one day, vacuum packed and ripened. After 7 days, half of each cheese was sampled to assess gross composition and for the animal trial. The half cheese remaining was vacuum packed again and continued ripening for the studies of microbiological counts, biochemical changes and consumers acceptability. These samples were maintained for 60 days. Two replicate cheeses were manufactured on different days with different milk.

2.3. Cheese composition and consumer's acceptability

Selective cells counts of *L. plantarum* I91 or *L. paracasei* I90 were performed in cheeses using MRS-LP agar according to Vinderola and Reinheimer (2000), immediately after cheese manufacture and after 10, 30 and 45 days of refrigerated storage. Cheeses were analyzed for gross composition by standard methods to quantify moisture content, fat matter, total protein and pH at day 7 (IDF 4: 1982; IDF 20: B, 1993; IDF, 1997; Bradley et al., 1993, respectively). Peptidolysis was assessed by both, non-specific and specific methods, at 7, 30 and 60 days of ripening. In the first case, the total amount of free amino acids was estimated as the content of soluble nitrogen in the fraction composed of trichloroacetic acid 12% and phosphotungstic acid 2.5% (w/v), which were in turn expressed as a proportion of the soluble nitrogen at pH 4.6 (% SN-TCA/SN pH 4.6 and % SN-PTA/SN pH 4.6, respectively). Nitrogen levels were measured by the micro-Kjeldal method; fractions were extracted and precipitated according to Kuchroo and Fox (1982, modified by Candioti et al., 2002). Specific assay consisted of free amino acids individual contents assessment by HPLC after derivatization (Bergamini et al., 2009).

The overall acceptability of the cheeses was assessed by a panel of over ninety-eight non-trained consumers, age comprised between 25 and 56 years. A hedonic scale of nine levels of liking was used. It ranged from "I like it very much" to "I dislike it very much", centered on "I neither like it nor dislike it".

2.4. Animal trial

2.4.1. Animals

Fifty female BALB/c mice weighing from 18 to 20 g were purchased to the random bred colony of the *Centro de Experimentaciones Biológicas y Bioterio, Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral* (Esperanza, Santa Fe). Animals were allowed to stand at the INLAIN animal facility for 7 days before the animal trial began. Each experimental group consisted of 5 mice housed together in plastic cages and kept in a controlled environment at a temperature of 21 ± 2 °C with humidity at 55 ± 2%, with a 12 h light/dark cycle. Mice were maintained and treated according to the guidelines of the National Institute of Health (NIH, USA).

2.4.2. Feeding procedures

The following groups were set: a control group (five mice) that received no treatment (no cheese). Cheese-control groups, to which control cheese (without added lactobacilli) was offered *ad libitum*

for 2, 5 or 7 consecutive days. Lactobacilli-treated groups which were offered, *ad libitum,* cheese containing *L. plantarum* I91 or *L. paracasei* I90 for 2, 5 or 7 consecutive days. All animals received, simultaneously and *ad libitum*, tap water and a sterile conventional balanced diet (Cooperación, Buenos Aires, Argentina) containing proteins, 230 g/kg; raw fiber, 60 g/Kg; total minerals, 100 g/kg; Ca, 13 g/kg; P, 8 g/kg; water, 120 g/kg; and vitamins.

2.4.3. Evaluation of safety and functionality: translocation assay, ex vivo phagocytosis assay in peritoneal macrophages and immunofluorescence test for IgA-producing cells enumeration

After each feeding period, animals were anesthetized intraperitoneally (0.2 ml/mouse) with a rodent cocktail (9 parts ketamine (100 mg/ml) + 9 parts xylazine (20 mg/ml) + 3 parts acepromazine (10 mg/ml) + 79 parts sterile saline). Animals were sacrificed by cervical dislocation. Peritoneal macrophages were harvested (in sterile conditions) by washing the peritoneal cavity with 5 ml of PBS containing 10 U/ml of Heparin (Sigma–Aldrich, St. Louis, MO, U.S.A.) and 0.1% Bovine Serum Albumin (Jackson ImmunoResearch, West Grove, PA, USA). The macrophage suspension was washed twice with the same buffer, and it was adjusted to a concentration of 10^6 cells/ml. A heat-killed (100 °C, 15 min) *Candida albicans* suspension (10^7 cells/ml) was opsonized with mouse autologous serum (10%) for 15 min at 37 °C. 0.2 ml of the opsonized yeast were added to 0.2 ml of each macrophage suspension. The mixture was incubated for 30 min at 37 °C. The percentage of phagocytosis was measured as the % of activated (with at least one cell of yeast phagocyted) macrophages after a 100-cell count using an optical microscope. Liver was removed and homogenized in 5 ml of sterile PBS. One ml of liver homogenate was pour-plated in MacConkey agar (translocation assay for enterobacteria detection). Plates were incubated at 37 °C for 24 h in aerobiosis. The small intestine was removed for histological preparation and paraffin inclusion, according to previous reports (Vinderola et al., 2005). Paraffin-sections (4 μm) were stained with haematoxilin-eosin followed by light microscopy examination to assess the intestinal architecture. The number of IgA producing (IgA+) cells was determined on histological slices of samples from the ileum, not considering Peyer's patches. The immunofluorescence test was performed using α-chain specific anti-mouse IgA fluorescein isothiocyanate (FITC) conjugate (Sigma–Aldrich). Histological slices were deparaffinized and rehydrated in a series of ethanol concentrations. Deparaffinized histological samples were treated with a dilution (1/100) of the antibody in PBS and incubated in the dark for 30 min at 37 °C. Then, samples were washed two times with PBS and examined using a fluorescent light microscope. The results were expressed as the number of fluorescent, IgA + cells/10 fields. Positive (fluorescent cells) were counted with magnification of 400×. Data were presented as the mean value for three tissue slices for each animal for each feeding period.

2.5. Statistics

Data from chemical composition, microbiological counts, FAA, and IgA producing cell numbers were analyzed by one-way ANOVA with a 95% confidence level. All analyses were made in duplicate. All statistical analyses were performed using the SPSS 10.0 software (SPSS Inc., Chicago, IL).

3. Results

3.1. Cheese characteristics

Gross composition of 7 day-old cheeses containing adjunct cultures of *L. plantarum* I91 and *L. paracasei* I90 was not significantly different from that of control cheeses (Table 1). As for pH, both cheeses containing adjunct cultures showed a significantly lower value than the control ($P \leq 0.05$), although within the normal range for this type of cheese. The cheeses with *L. paracasei* I90 had the lowest pH.

Table 1
Gross composition, pH, overall acceptability and total content of free amino acids of cheeses.

Parameter	Cheese		
	Control	*L. paracasei* I90	*L. plantarum* I91
Moisture[d]	52.12 ± 0.92[a]	53.76 ± 1.93[a]	52.44 ± 1.35[a]
Protein content[d]	18.35 ± 0.65[a]	18.54 ± 0.32[a]	19.18 ± 0.70[a]
Fat matter[d]	23.70 ± 2.10[a]	23.70 ± 1.82[a]	24.18 ± 2.05[a]
pH[d]	5.18 ± 0.03[a]	5.02 ± 0.06[b]	5.10 ± 0.07[b]
Overall acceptability[e]	6.40[a]	5.60[b]	6.59[c]
Free Amino Acids[e]	68.00 ± 8.73[a]	91.74 ± 8.82[b]	100.02 ± 4.10[b]

[a,b,c]Values in rows with different superscript are significantly different ($P < 0.05$).
[d] 7 days-old cheese.
[e] 60 days-old cheese.

Primary proteolysis was similar in all cheeses, which was evidenced by similar values of soluble nitrogen at pH 4.6 (13.54 ± 0.18 in average for all 60 days-old cheeses) and comparable electrophoretic profiles (results not shown). Peptidolysis indirectly described by % SN-TCA/SN pH 4.6 and % SN-PTA/SN pH 4.6 was, in general, similar for control and experimental cheeses (Table 2). At the beginning of the ripening % SN-TCA/SN pH 4.6 was significantly higher in cheeses with adjunct cultures, while at 30 days control and *L. plantarum* I91 containing cheeses were similar, and cheeses with *L. paracasei* I90 had a higher % SN-TCA/SN pH 4.6 content. % SN-PTA/SN pH 4.6 decreased during ripening and was lower in cheese with added lactobacilli than in control cheeses, which suggests a change in nitrogen compounds composition during ripening, with a relative decrease of small compounds (amino acids, dipeptides) against medium size compounds (oligopeptides). However, absolute content of free amino acid was enhanced in samples with *L. plantarum* I91 or *L. paracasei* I90 by the end of the ripening period (Fig. 1, Table 1). The free amino acid profiles found in cheeses with and without added lactobacilli also showed some differences: the levels of Leu, Phe and Arg increased significantly in experimental cheeses ($P \leq 0.05$, Fig. 1).

As for acceptability, it is noteworthy that all samples were scored in the positive side of the liking scale. The cheese containing *L. plantarum* I91 showed even a better performance since it had higher average acceptability (AA), 78.57%, with overall acceptability of 6.59, which significantly exceeded the acceptability of the control (Table 1). Cheese containing *L. paracasei* I90, even if it was considered appropriate, it was placed lower than the control cheese in the liking scale. Cheeses containing *L. paracasei* I90 showed

Table 2
Soluble nitrogen (SN) in 12% trichloroacetic acid (TCA) and 2.5% phosphotungstic acid (PTA), expressed as a proportion of the total soluble nitrogen at pH 4.6 (% SN-TCA/SN-pH 4.6 and % SN-PTA/SN-pH 4.6, respectively).

Type of cheese	% SN-TCA/SN-pH 4.6 (at days)		
	7	30	60
Control	37.53 ± 0.18[a]	41.33 ± 0.33[a]	44.29 ± 0,80[a]
L. plantarum I91	39.22 ± 0.21[b]	41.07 ± 0.38[a]	44.25 ± 0,45[a]
L. paracasei I90	42.34 ± 0.85[c]	43.64 ± 0.26[b]	43.77 ± 0,34[a]
Type of cheese	% SN-PTA/SN-pH 4.6 (at days)		
	7	30	60
Control	12.83 ± 0.67[a]	10.54 ± 0.36[a]	10.01 ± 0.02[a]
L. plantarum I91	12.43 ± 0.97[a]	8.98 ± 0.29[b]	9.51 ± 0.79[b]
L. paracasei I90	12.66 ± 0.48[a]	9.69 ± 0.52[ab]	9.86 ± 0.88[ab]

[a,b,c]Values in columns with different superscript are significantly different ($P < 0.05$).

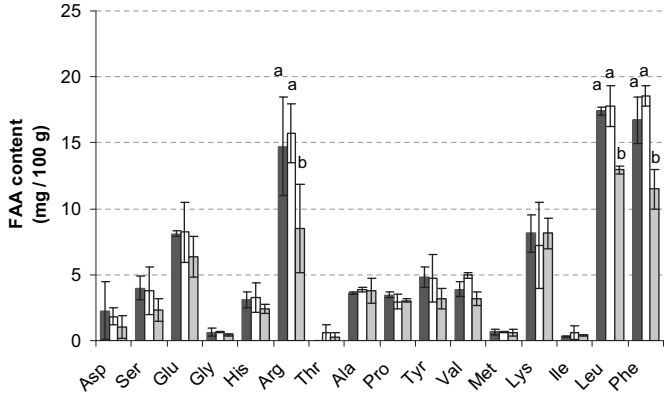

Fig. 1. Individual content of free amino acids at 60 days of ripening in Cremoso cheese containing *L. plantarum* I91 (■) or *L. paracasei* I90 (□) compared to control cheeses (▫). [a,b]Columns with different superscript are significantly different ($P < 0.05$) for the same amino acid.

a good acceptability, being the AA a variation between "I like it moderately" and "I like it slightly" (Fig. 2).

3.2. Animal trial

Control cheeses (with no lactobacilli adjuncts) or cheeses containing individual cultures of *L. plantarum* I91 or *L. paracasei* I90 (ca. 10^7 CFU/g) were offered to mice during 2, 5 or 7 consecutive days. In all cases, treated-animal's behavior during the trial did not differ from the one of the control group, to which no cheese was offered. The oral administration of cheese containing lactobacilli adjuncts induced no undesirable side-effects since there was no translocation of enterobacteria to the liver (the translocation assay was negative). Haematoxilin-eosin staining was used to study the overall architecture of the small intestine in treated and control animals. Microscopy examination by blinded operators showed the absence of lymphocyte infiltrates, edema or mucosal atrophy. The height and size of villi were normal. No significant morphological changes in the general architecture of the small intestine were observed compared with control mice (pictures not shown). In relation to the probiotic properties of cheeses containing lactobacilli, no significant modification of the percentage of phagocytosis of peritoneal macrophages was observed, compared to the control group (5.6 ± 3.2%). In order to study the capacity of the products manufactured to promote gut defenses, the number of IgA + cells were studied in the small intestine lamina propria of mice. The oral administration of control cheese did not modify the number of IgA + cells in the small intestine lamina propria compared to control mice (Fig. 3). However, the oral administration of cheeses harboring *L. plantarum* I91 or *L. paracasei* I90 enhanced the number of IgA + cells in a distinct way. Cheese harboring *L. paracasei* I90 induced a significant enhance in the parameter studied for all feeding periods, without differences between 5 or 7 days of feeding. The oral administration of cheese containing *L. plantarum* I91significantly increased the number of IgA + cells for all feeding periods, reaching a maximum in the immunomodulating capacity after 5 days of feeding, being the magnitude of this maximum different to that achieved by the oral administration of cheese harboring *L. paracasei* I90.

4. Discussion

In this work the technological and probiotic performance of two selected NSLAB were tested in Cremoso cheese, a soft cheese manufactured in Argentina. Both *L. paracasei* I90 and *L. plantarum* I91 showed an adequate performance as adjunct cultures in the cheese matrix since they did not change the overall composition of the products, which had similar dry matter content than the controls. This is due to the maintenance of pH decrease rate during cheesemaking – adjunct cultures did not increase acidification rate. Consequently, protein content and fat matter remained also constant. The pH was slightly lower in the cheeses containing

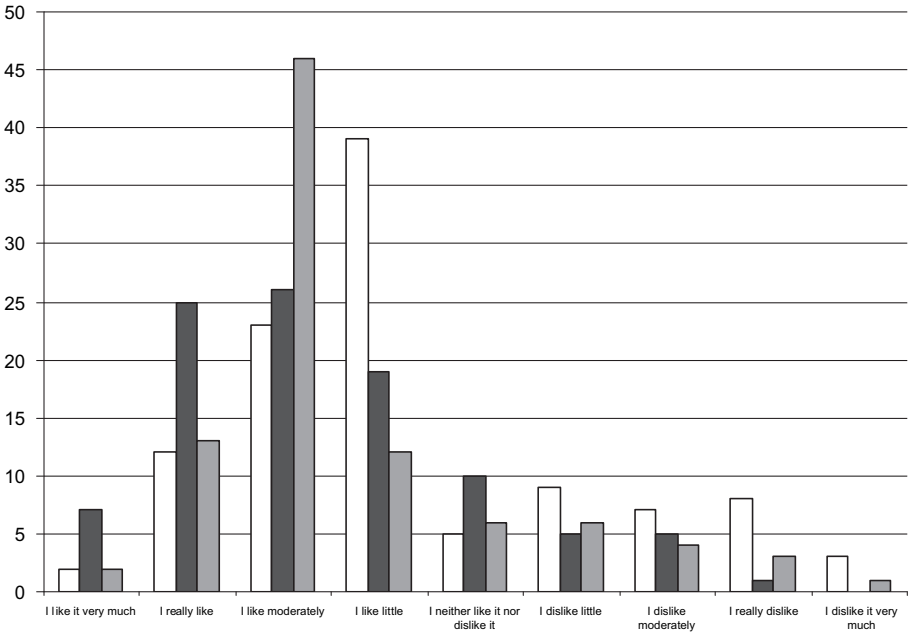

Fig. 2. Overall acceptability, assessed by a consumer's panel, of cheeses containing *L. paracasei* I90 (□) or *L. plantarum* I91 (■) or control cheeses (▫).

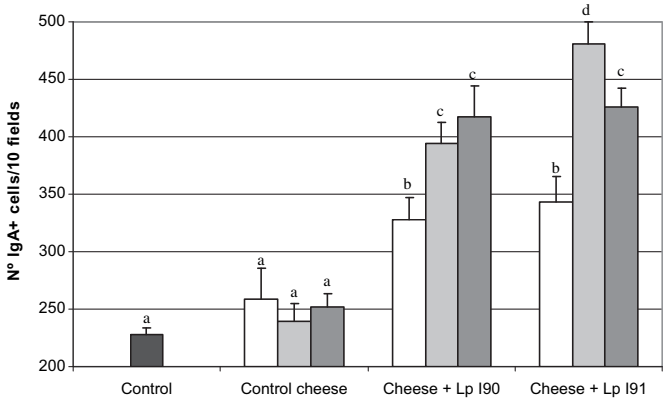

Fig. 3. Effects of the oral administration of control cheese or cheese containing *L. paracasei* I90 (Cheese + Lp I90) or *L. plantarum* I91 (Cheese + Lp I91) for 2 (□), 5 (▫) or 7 (▪) consecutive days, compared to control mice (■), on the number of IgA + cells in the lamina propria of the small intestine. [a,b,c,d]Columns with different superscript are significantly different ($P < 0.05$).

adjunct cultures, however, it was not observed a marked post-acidification during ripening, which has been reported for other probiotic strains, and that usually induces negative changes in texture, flavor and the color of the product (Crow et al., 2002). Cell viability of *L. plantarum* I91 and *L. paracasei* I90 during storage was satisfactory since no changes in colony counts were observed between manufacture and the end of the shelf life, as observed in previous works (Milesi et al., 2008, 2009).

The increase in the production of amino acids is an important starting point for encouraging the development of aroma and flavor of cheeses harboring adjunct cultures, because most of the compounds of cheese aroma come from amino acid metabolism and it is also believed that both free amino acids and small peptides contribute to taste by themselves (McSweeney and Sousa, 2000). In this way, we found that the inclusion of the adjunct cultures changed the peptidolysis pattern during Cremoso cheese ripening. A higher proportion of medium to small-size peptides was found at the beginning of the ripening when adjunct cultures were added, and a relatively lower amount of small nitrogen compounds was found in the soluble fraction both when adjunct cultures were included, with increasing ripening time. In absolute terms, free amino acids total content increased in adjunct-treated cheeses. Both types of experimental cheeses showed an increase in peptidolysis and different amino acid profiles compared to control cheese without lactobacilli adjuncts.

Acceptability was moderate for all samples as resulted from the analysis of the consumers' panel. Control cheeses were classified mainly in the category "I like moderately", which greatly exceeded the proportion of the cheeses with adjunct cultures in the same range. Samples with *L. plantarum* I91, however, showed higher values than control cheeses and cheeses with *L. paracasei* I90 in the categories "I like it" and "I like it very much." In interviews with the panel after the sessions, it was found out that they tended to link the intensity of aroma and flavor with overall acceptability. Cremoso cheese is a mild flavored cheese, which would explain the moderate values reached by all the samples in the categories "like".

The oral administration of probiotic bacteria is a way to stimulate the host non-specific immunity. Probiotics can enhance the mucosal immune response by modulating the functions of immunocompetent cells. Different cheese varieties have been identified as adequate vehicles for probiotic bacteria. Compared to fermented milks, they may confer enhanced protection of viable cells during the transit through the gut due to the more compact matrix compared to fermented milks. In this regard, Argentinian fresh cheese (Vinderola et al., 2000) and Pategras cheeses (Bergamini et al., 2005) were developed in our group and successfully assessed as carriers for viable probiotic bacteria. However, not in all cases the probiotic properties of the strains are demonstrated once they are included in a food matrix such as cheese (Vinderola et al., 2011). The oral administration of cheese containing *L. plantarum* I91 or *L. paracasei* I90 proved to be safe and able to satisfactory enhance the gut mucosal defenses mediated by IgA, but in a distinct way. The role of mucosal IgA is unquestionable, secretory IgA acts to prevent luminal antigens, microorganisms and other foreign proteins from penetrating the intestinal surface, and can neutralize toxins and infectious organisms. IgA can also regulate the composition of the microbial environment of the gut and limit local inflammation induced by pathogen-associated molecular patterns, such as LPS (Wershil and Furuta, 2008). The reach of a maximum in the number of IgA + cells, as observed for cheese containing *L. plantarum* I91, was also observed for Argentinean fresh cheese when its functional capacity was assessed in mice (Medici et al., 2004). This fact may be the result of an autoregulation process that occurs once a maximal response is achieved. This phenomenon would prevent from an inflammatory response, due to an over-stimulation of the intestinal mucosa, to occur. Finally, it was observed only a local response (enhance of IgA + cells in the small intestine) without the product being able to impact on the systemic immune response, since no enhancement of the phagocytic activity of peritoneal macrophages was observed.

5. Conclusions

L. plantarum I91 and *L. paracasei* I90 showed satisfactory properties for their use as adjunct cultures in the cheese industry, achieving the dual role of being secondary starters and probiotic cultures. They neither affected the overall composition of the product nor caused post-acidification during cheese ripening. Cheeses with added lactobacilli were at least as acceptable as the controls, for most consumers. From the two strains studied, *L. plantarum* I91 showed the best performance, both as probiotic and as adjunct culture. The use of selected strains of NSLAB exerted a technological and probiotic role: it contributed to the standardization of cheese quality and induced benefic health effects at the gut mucosa *in vivo*.

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References

Bergamini, C.V., Hynes, E.R., Quiberoni, A., Suárez, V.B., Zalazar, C.A., 2005. Probiotic bacteria as adjunct starters: influence of the addition methodology on their survival in a semi-hard Argentinean cheese. Food Research International 38, 597–604.

Bergamini, C.V., Hynes, E., Candioti, M.C., Zalazar, C.A., 2009. Multivariate analysis of proteolysis patterns differentiated the impact of six strains of probiotic bacteria on a semi-hard cheese. Journal of Dairy Science 92 (6), 2455–2467.

Bertazzoni Minelli, E., Benini, A., Marzotto, M., Sbarbati, A., Ruzzenente, O., Ferrario, R., Hendriks, H., Dellaglio, F., 2004. Assessment of novel Probiotic *Lactobacillus casei* strains for the production of functional dairy foods. International Dairy Journal 14, 723–736.

Bradley, R.L., Arnold, E., Barbano, D.M., Semerad, R.G., Smith, D.E., Vines, B.K., 1993. Chemical and physical methods. In: Marshall, R.T. (Ed.), Standard Methods for the Examination of Dairy Products. American Public Health Association, Washington, DC, USA, pp. 433–531.

Briggiler-Marcó, M., Capra, M.L., Quiberoni, A., Vinderola, G., Reinheimer, J.A., Hynes, E., 2007. Nonstarter *Lactobacillus* strains as adjunct cultures for cheese

making: in vitro characterization and performance in two model cheeses. Journal of Dairy Science 90, 4532–4542.
Bude-Ugarte, M., Guglielmotti, D., Giraffa, G., Reinheimer, J.A., Hynes, E., 2006. Non-starter lactobacilli from Argentinean cheeses. Journal of Food Protection 69, 2983–2991.
Candioti, M.C., Hynes, E.R., Quiberoni, A., Palma, S.B., Sabbag, N., Zalazar, C.A., 2002. Reggianito Argentino cheese: influence of *Lactobacillus helveticus* strains isolated from natural whey cultures on cheese making and ripening processes. International Dairy Journal 12, 923–931.
Christiansen, P., Waagner Nielsen, E., Vogensen, F.K., Brogren, C.H., Ardö, Y., 2006. Heat resistance of *Lactobacillus paracasei* isolated from semi-hard cheese made of pasteurised milk. International Dairy Journal 16, 1196–1204.
Crow, V., Curry, B., Hayes, M., 2001. The ecology of non-starter lactic acid bacteria (NSLAB) and their use as adjuncts in New Zealand Cheddar. International Dairy Journal 11, 275–283.
Crow, V., Curry, B., Christison, M., Hellier, K., Holland, R., Liu, S.Q., 2002. Raw milk flora and NSLAB as adjuncts. Australian Journal of Dairy Technology 57, 99–105.
De Angelis, M., Corsetti, A., Tosti, N., Rossi, J., Corbo, M.R., Gobetti, M., 2001. Characterization of non-starter lactic acid bacteria from Italian ewe cheeses based on phenotypic, genotypic, and cell wall protein analyses. Applied and Environmental Microbiology 67, 2011–2020.
De Angelis, M., Di Cagno, R., Huet, C., Crecchio, C., Fox, P.F., Gobbetti, M., 2004. Heat shock response in *Lactobacillus plantarum*. Applied and Environmental Microbiology 70, 1336–1346.
IDF, 1982. Formaggio e formaggio fuso. Determinazione della materia seca. Metodo di riferimento. N° 4:A. International Dairy Federation, Brussels, Belgium, pp. 184–188.
IDF, 1993. Latte. Determinazione del tenore in azoto. Metodo di riferimento. N° 20:B. International Dairy Federation, Brussels, Belgium. pp.74–107.
IDF, 1997. Lait et produits laitiers. Détermination de la teneur en matière grasse. Guide de directives generales apliquées aux methodes butyrometriques. Norme IDF 152:A. International Dairy Federation, Brussels, Belgium.
Jordan, K.N., Cogan, T.M., 1999. Heat resistance of *Lactobacillus* spp. isolated from Cheddar cheese. Letters in Applied Microbiology 29, 136–140.
Kuchroo, C.N., Fox, P.F., 1982. Soluble nitrogen in Cheddar cheese: comparison of extraction procedures. Milchwissenschaft 37, 331–335.
Maragkoudakis, P.A., Zoumpopoulou, G., Miaris, C., Kalantzopoulos, G., Pot, B., Tsakalidou, E., 2006. Probiotic potential of *Lactobacillus* strains isolated from dairy products. International Dairy Journal 16, 189–199.
McSweeney, P.L.H., Sousa, M.J., 2000. Biochemical pathways for the production of flavour compounds in cheese during ripening. Lait 80, 293–324.
Medici, M., Vinderola, C.G., Perdigón, G., 2004. Gut mucosal immunomodulation by probiotic fresh cheese. International Dairy Journal 14, 611–618.
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.
Ortigosa, M., Arizcun, C., Irigoyen, A., Oneca, M., Torre, P., 2006. Effect of *Lactobacillus* adjunct cultures on the microbiological and physicochemical characteristics of Roncal-type ewes'-milk cheese. Food Microbiology 23, 591–598.
Poznanski, E., Cavazza, A., Cappa, F., Cocconcelli, P.S., 2004. Indigenous raw milk microbiota influences the bacterial development in traditional cheese from an alpine natural park. International Journal of Food Microbiology 92, 141–151.
Quiberoni, A., Guglielmotti, D., Reinheimer, J., 2005. Nuevas y clásicas bacterias causantes de defectos gasógenos en quesos blandos. Revista Argentina de Lactología 23, 19–32.
Randazzo, C.L., Pitino, I., De Luca, S., Scifò, G.O., Caggia, C., 2008. Effect of wild strains used as starter cultures and adjunct cultures on the volatile compounds of the Pecorino Siciliano cheese. International Journal of Food Microbiology 122, 269–278.
Settanni, L., Moschetti, G., 2010. Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. Food Microbiology 27, 691–697.
Somers, E.B., Johnson, M.E., Wong, A.C.L., 2001. Biofilm formation and contamination of cheese by non-starter lactic acid bacteria in the dairy environment. Journal of Dairy Science 84, 1926–1936.
Turner, K.W., Lawrence, R.C., Lelievre, J., 1986. A microbiological specification for milk for aseptic cheesemaking. New Zealand Journal of Dairy Science and Technology 21, 249–254.
Vinderola, C.G., Reinheimer, J.A., 2000. Enumeration of *Lactobacillus casei* in the presence of *L. acidophilus*, bifidobacteria and lactic starter bacteria in fermented dairy products. International Dairy Journal 10, 271–275.
Vinderola, C.G., Prosello, W., Ghiberto, D., Reinheimer, J.A., 2000. Viability of probiotic (*Bifidobacterium*, *Lactobacillus acidophilus* and *Lactobacillus casei*) and Nonprobiotic microflora in Argentinian Fresco cheese. Journal of Dairy Science 83, 1905–1911.
Vinderola, C.G., Duarte, J., Thangavel, D., Perdigón, G., Farnworth, E., Matar, C., 2005. Immunomodulating capacity of kefir. Journal of Dairy Research 72, 195–202.
Vinderola, G., Burns, P., Reinheimer, J., 2011. Technology and stability of probiotics in probiotic and prebiotic cheeses. In: Shah, N.P., Gomes da Cruz, A., Fonsecca Faria, J.A. (Eds.), Probiotic and Prebiotic Foods Technology, Stability and Benefits to Human Health Nova Science. USA, New York, pp. 169–202. ISBN 978-1-61668-842-4.
Wershil, B.K., Furuta, G.T., 2008. Gastrointestinal mucosal immunity. Journal of Allergy and Clinical Immunology 121, 380–383.
Wouters, J.T.M., Ayad, E.H.E., Hugenholtz, J., Smit, G., 2002. Microbes from raw milk for fermented dairy products. International Dairy Journal 12, 91–109.