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Potential of high pressure homogenisation on probiotic Caciotta cheese quality and functionality

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ABSTRACT

The probiotic *Lactobacillus paracasei* A13, treated at 50 MPa sub-lethal high pressure homogenisation (HPH), was used as adjunct for producing Caciotta cheese. The cell HPH treatment was used because it has been proven to increase the *in vitro* strain functionality. The starters and *Lb. paracasei* A13 viability, the cheese hydrolytic patterns and organoleptic profiles were monitored. After cheesemaking and during ripening, the *Lb. paracasei* A13 gastric acid resistance in cheese and the ability of the cheese, containing HPH-treated or untreated cells, to modulate the gut mucosal immune system in mice were evaluated. Traditional Caciotta was used as controls. The HPH-treated probiotic strain maintained high viability for 14 days whilst the physico-chemical analyses on Caciotta cheese containing HPH-treated cells showed a faster ripening, compared to other cheeses. For functional properties, the 50 MPa treatment increased the *Lb. paracasei* gastric resistance in Caciotta, maintaining high strain viability, but IL-10 producing capacity was lost by HPH-treatment whilst IgA production was not modified.

1. Introduction

Functional foods represent one of the fastest growing areas in the global food industry due to a dietary strategy to reduce the incidence of illness in the humankind (Patrignani, Lanciotti, & Guerzoni, 2011). The European Commission's Concerted Action on Functional Food Science in Europe (FuFoSE), coordinated by International Life Science Institute (ILSI), defined functional foods as "food products having, together with the basic nutritional impact, beneficial effects on one or more functions of the human organism thus either improving the general and physical conditions or/and decreasing the risk of the evolution of diseases". Moreover, "the amount of intake and form

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of the functional food should be as it is normally expected for dietary purposes. Therefore, it could not be in the form of pill or capsule just as normal food form" (Griffiths, Abernethy, Schuber, & Williams, 2009; Siró, Kápolna, Kápolna, & Lugasi, 2008; Tripathi & Giri, 2014).

Considering the different chances available at industrial level, the health-promoting foods can be classified into three categories: (i) foods with specific intrinsic functionality; (ii) foods fortified with natural ingredients able to grant a desired functionality; and (iii) foods with added probiotic microorganisms and prebiotic compounds. In any case, different approaches, based on biotechnology or food processing, can be used to achieve specific functionalities. For this reason, the development of dairy products containing probiotic bacteria has become an important subject in the food industry and several attempts have been made to identify the most suitable carriers and probiotic strains, in terms of viability and functional performances (Burns et al., 2008a; Patrignani et al., 2009; Romano et al., 2014). In addition to the continuous strain selection, another promising area to obtain new foods with new or enhanced functional properties is the use of technologies able to guarantee improved nutritional value and sensory property of food functionality as well as new food physico-chemical and rheological properties (Patrignani et al., 2011). Moreover, the increasing demand for high-value health promoting foods has encouraged the industries, and consequently the research field, to investigate new emerging food processes able to assure functional but also safe and wholesome products (Carroll, Chen, Harnett, & Harnett, 2004). Although most of these technologies were used as alternative to thermal treatment for microbial decontamination (Knorr, 1999; Lado & Yousef, 2002; Senorans, Ibanez, & Cifuentes, 2003; Wan, Conventry, Sanguansri, & Versteeg, 2009), evidence has proven their exploitation to obtain functional foods. Among these, pulsed electric field (PEF), high hydrostatic pressure (HHP) and high pressure homogenisation (HPH) are the most studied for this purpose and they are still being investigated and tested at least in the laboratory scale. In particular, the sector of high-pressure processing is probably one of the most scientifically developed and with already-established applications at industrial level. In particular, HPH has been proposed for several purposes in functional dairy sector, such as the production of probiotic fermented milk, bio-yoghurt and probiotic cheeses with improved sensory or functional properties (Burns et al., 2008a; Patrignani et al., 2007, 2009). Recently, HPH when applied to cells of probiotic bacteria at sub-lethal level (50 MPa), was reported to increase some cell functional properties both *in vitro* and *in vivo* systems (Tabanelli et al., 2012, 2013). These authors demonstrated that this sub-lethal HPH treatment was able to modulate hydrophobicity and auto-aggregation without modification of the viability for *Lactobacillus paracasei* A13 (Tabanelli et al., 2013). Moreover, the HPH treatment increased its *in vitro* resistance to simulated gastric conditions and to simulated stomach duodenum-passage. When sub-lethal HPH treated cells of *Lb. paracasei* A13 were used to feed BALB/c mice, a higher IgA response was observed (Tabanelli et al., 2012). In addition, in previous studies (Burns et al., 2008a; Patrignani et al., 2009) the same strain was used to produce probiotic cheese and yoghurt from HPH treated milk, demonstrating that the cheese was the best carrier for the probiotic strain viability.

Thus, because this strain had basically good *in vitro* functional performances, further enhanced by sub-lethal HPH treatments, we used sub-lethal HPH treated cells of *Lb. paracasei* A13 as adjunct to produce functional Italian Caciotta cheese. Particularly, immediately after cheesemaking and during ripening, we followed the starter (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) and probiotic strain viability and the *Lb. paracasei* A13 gastric resistance in cheese. In order to confirm the encouraging *in vitro* performance of the strain to modulate mouse immune system, BALB/c mice were fed with Caciotta cheese produced using as co-starter *Lb. paracasei* cells, HPH treated or not, to evaluate the IgA, IFN-γ and IL10 production in the mouse small and large intestine. Finally, we monitored also the hydrolytic, textural, sensory and volatile profiles of the functional Caciotta obtained in order to evaluate the effects of a probiotic strain addition on the cheese quality and ripening patterns.

2. Materials and methods

2.1. Strains

Lb. paracasei A13 had been previously tested for its probiotic properties and it is commonly used in commercial functional dairy products (Burns et al., 2008b; Vinderola, Prosello, Ghiberto, & Reinheimer, 2000a). The strain was maintained in de Man, Rogosa and Sharpe (MRS) broth (Biokar, Beauvais, France) with sterile glycerol (20% v/v) at –70 °C in the collection of the Instituto de Lactologiía Industrial (INLAIN, UNL-CONICET, Santa Fe, Argentina). Fresh cultures of the strain were obtained by two consecutive daily transfers in MRS broth (Biokar) using a 1% (v/v) inoculum, incubated at 37 °C in aerobic conditions for 18 h.

The starters *Lb. bulgaricus* and *S. thermophilus* in lyophilised form (Sacco srl, Italy) were provided by the local plant (Mambelli, Bertinoro, Italy) where the cheesemaking was performed.

2.2. High pressure homogenisation treatment

Late exponential phase cells, grown in MRS broth for 18 h at 37 °C, were subjected to a high pressure homogenisation (HPH) treatment at a level of 50 MPa with a high pressure homogeniser PANDA (Niro Soavi, Parma, Italy). The machine was supplied with a homogenising PS type valve; the valve assembly includes a ball type impact head made of ceramics, a stainless steel large inner diameter impact ring and a tungsten carbide passage head. The inlet temperature of samples was 20 °C and the increase rate of temperature was 2.5 °C 10 MPa^{-1}.

2.3. Cheesemaking

Three types of Italian Caciotta cheese were manufactured: type 1 (CC): Caciotta control cheese with the traditional starters; type 2 (NTC): Caciotta with traditional starters and not-HPH treated *Lb. paracasei* A13 cells; and type 3 (TC): Caciotta with traditional starters and HPH-treated *Lb. paracasei* A13 cells. For the production, the cheesemaking protocol of Mambelli dairy farm was followed. For each cheese, 200 l of pasteurised cow milk

kept at 40 °C were used and inoculated with the starters culture at level of 7 log CFU ml^{-1}. The inoculated milk was left at 40 °C for 65 min after that the probiotic strain *Lb. paracasei* A13, HPH treated or not, was inoculated in cheese type 3 and 2, respectively, at level of about 9 log CFU ml^{-1}. After that, rennet was added (1:10,000 25% pepsin:75% chymosin, Bellucci, Modena, Italy) and coagulation was achieved in 20 min. Thus, the curd was cut and left to rest for 45 min, after that it was divided in moulds and, only when the pH reached values of 5.18 ± 0.01, they were stored at 4 °C. Three different productions were performed in three different days and, from each cheesemaking, 100 cheeses resulted.

2.4. *pH, water activity, moisture*

Cheese pH was measured by using a pH-meter Hanna Instruments 8519 (Incofar, Modena, Italy) diluting 10 g of cheese with 10 ml of distilled water. Water activity (A_w) was measured by using an Aqualab Series 4TE (Decagon Device, Inc. Pullman, WA, USA). Grated cheese samples were analysed for moisture (International Dairy Federation, 1982).

2.5. *Microbiological analyses*

Immediately after cheesemaking and during the shelf-life (3 weeks), microbiological analyses were carried out for the enumeration of the starter and probiotic cultures. Twenty grams of cheese were placed in 180 ml 20 g sodium citrate l^{-1} sterile solution and homogenised in stomacher (Lab-blender 80, Pbi International, Milan, Italy) for 3 min. Decimal dilutions of the homogenates were made in 0.9% of ringer solution and 0.1 ml of appropriate dilutions were spread onto the surface of different agar media. *S. thermophilus* was counted on M17 agar (Oxoid, Basingstoke, Hampshire, United Kingdom) (42 °C, 48 h), *Lb. delbrueckii* subsp. *bulgaricus* on MRS agar (Oxoid) acidified with glacial acetic acid (Merck, Darmstadt, Germany) at pH 5.4 (42 °C, 48 h). For the enumeration of *Lb. paracasei* A13, MRS-LP agar (37 °C, 48 h) was used (Vinderola & Reinheimer, 2000b). The Total Lactic Microflora (TLM) was determined in Plate Count Agar (Oxoid) with 10% (w/v) skim milk powder (Oxoid) at 37 °C for 48 h.

2.6. *Proteolysis, lipolysis, volatile profiles and biogenic amines*

Proteolysis was monitored during ripening by using SDS-PAGE and for the protein extraction the method proposed by Kuchroo and Fox (1982) was used. For the running conditions, the method proposed by Lanciotti, Patrignani, Iucci, Saracino, and Guerzoni (2007b) was used.

Free fatty acid (FFA) composition was assessed during ripening. The extraction of cheese lipids and the determination of FFA concentrations were performed as described by Vannini et al. (2008).

The main volatile compounds were monitored during ripening by using a GC/MS/SPME technique according to the method proposed by Burns et al. (2008a). The compounds were identified by use of available mass spectra databases (NIST version 2005).

For the biogenic amine determination, the method proposed by Lanciotti et al. (2007a) was used. Four grams of cheese were homogenised with 15 ml of 0.2 M perchloric acid by means of an Ultraturrax macerator at medium speed. After centrifugation (10,000 rpm for 10 min at 4 °C; Avanti J-25, Beckmann & Coulter, Palo Alto, CA, USA), the sediment was again extracted with 20 ml of 0.2 M perchloric acid and centrifuged. The two supernatant fractions were combined and made to 50 ml with 0.2 M perchloric acid. Amine derivatisation was performed according to Eerola, Hinkkanen, Lindfors, and Hirvi (1993). The derivatising agent used was dansyl-chloride (Sigma–Aldrich, Gallarate, Italy), which was solubilised in acetone for HPLC (Carlo Erba Reagents, Rodano, Italy); the solution was prepared daily and used immediately; to avoid the degradation of the dansyl derivatives all the derivatised samples were protected from light and stored at –18 °C for a maximum of 7 days. The analyses were performed with a PU-2089 Intelligent HPLC quaternary pump, Intelligent UV–VIS multiwavelength detector UV 2070 Plus (Jasco Corporation, Tokyo, Japan) and a manual Rheodyne injector equipped with a 20 µl loop (Rheodyne, Rohnert Park, CA, USA). For the chromatographic separation, an Analytical Cartridge Waters Spherisorb column, 3 l ODS-2 4.6 mm–150 mm, coupled with Guard Cartridge Waters Spherisorb S5 ODS2 column, 4.6–10 mm (Waters Corporation, Milford, MA, USA), was used with the following gradient elution: 0–5 min phosphate buffer (pH 7)/acetonitrile 35:65 5–6 min water/acetonitrile 20/80, 6–15 min water/acetonitrile 10/90, 15–25 min phosphate buffer (pH 7)/acetonitrile 35:65; flow rate 0.8 ml/min. The amount of each amine was expressed as mg kg^{-1} by reference to a calibration curve.

2.7. *Texture profile analysis (TPA)*

Texture analyses were performed after 1, 7, 14 and 21 days of ripening at 6 °C using a Texture Analyser TA DHI (Stable Micro System, Godalming, UK) and according to the method proposed by Patrignani, Lanciotti, Maina Mathara, Guerzoni, and Holzapfel (2006). The results are the means of three repetitions of three different Caciotta cheeses.

2.8. *Sensory analysis*

The three types of Caciotta cheese, after 21 days of ripening, were subjected to a sensory analysis. Twenty-five trained evaluators tasted 20 g of each cheese under controlled conditions of environment and light according to Gallardo-Escamilla, Kelly, and Delahunty (2007). The assessors were asked to evaluate cheese flavour and aroma, appearance and colour, piquant, bitter, creamy, textural features and overall impression attributing a score ranging from 0 (low or poor) to 7 (high or very excellent).

2.9. *Resistance of* Lb. paracasei *A13 to simulated gastrointestinal digestion in Caciotta cheeses*

The resistance *of Lb. paracasei* A13 to simulated gastrointestinal digestion (RSGD) was performed after 1, 14 and 21 days of ripening. Twenty grams of cheese (NTC and TC) were treated according to the method proposed by Vinderola et al. (2011).

2.10. Animal trials

2.10.1. Animals

Forty five 6-old-week male BALB/c mice weighing from 20 to 23 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, Argentina). 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 Institutes of Health (NIH, USA). The animal assays were approved by the Ethical Committee for Animal Experimentation of the Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral.

2.10.2. Feeding procedures

The following groups (five mice/group) were set: a cheese-control group (CC) that received the control cheese; not treated cheese group (NTC) that received cheese with not-HPH treated *Lb. paracasei* A13 cells and treated cheese group (TC) that received cheese containing HPH treated *Lb. paracasei* A13 cells. Mice received daily (by gavage) 0.3 ml of a cheese suspension containing 7.74 log CFU ml^{-1} for NTC and 6.6 log CFU ml^{-1} for TC for 3, 6, or 10 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^{-1}; raw fibre, 60 g kg^{-1}; total minerals, 100 g kg^{-1}; Ca, 13 g kg^{-1}; P, 8 g kg^{-1}; water, 120 g kg^{-1}; and vitamins.

2.10.3. Translocation assay

To determine possible secondary effects of the administration of the cheeses, at the end of each feeding period, mice were anaesthetised and sacrificed by cervical dislocation. Liver was removed and homogenised in sterile PBS. One millilitre was plated in Mac Conkey (Britania, Buenos Aires, Argentina) for detection of enterobacteria. Plates were incubated at 37 °C for 24 h under aerobic conditions. Results were expressed as positive or negative translocation, since liver is a sterile organ under normal conditions.

2.10.4. Evaluation of functionality: immunofluorescence test for IgA-producing cells enumeration and determination of secretory IgA (sIgA), IL-10 and IFN-γ by ELISA

After each feeding period, animals were anaesthetised intraperitoneally (0.2 ml for each mouse) with a rodent cocktail (9 parts ketamine (100 mg ml^{-1}) + 9 parts xylazine (20 mg ml^{-1}) + 3 parts acepromazine (10 mg ml^{-1}) + 79 parts sterile saline). The small intestine was recovered and flushed with 5 ml of cold PBS buffer containing a cocktail of protease inhibitors (Sigma Aldrich, St. Louis, MO, USA). The intestinal fluid was centrifuged (2000 × *g*, 15 min, 4 °C) and stored at –70 °C for secretory IgA quantification by ELISA (Vinderola, Matar, & Perdigón, 2007). Moreover, samples of small and large intestines were recovered and prepared for fixation, histological preparation and paraffin inclusion, according to Vinderola et al. (2005). The number of IgA-producing (IgA+) cells was determined on histological slices. The immunofluorescence test was performed using alpha-chain-specific anti-mouse IgA fluorescein isothiocyanate (FITC) conjugate (Sigma). Histological slices were deparaffinised and rehydrated in a series of decreasing ethanol concentrations (from absolute alcohol to 70% (v/v) alcohol). Deparaffinised 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 (Hg lamp) microscope (Nikon Eclipse E200, Nikon Instruments Inc., Melville, NY, USA). The results were expressed as the number of positive cells (fluorescent cells)/10 fields. Positive (fluorescent) cells were counted with a magnification of 400× (double blind counts). Data were reported as the mean of three counts (each one in a different histological slice) for each animal.

Small and large intestine samples were also obtained and kept frozen (–70 °C) for cytokine determination. Intestine samples were homogenised (Ultra Turrax T8, Ika Labortechnik, Staufen, Germany) in PBS solution containing 1% (v/v) anti-protease cocktail (Sigma), 10 mmol/l EDTA (Sigma) and 0.05% (v/v) Tween 20 (Sigma) in a proportion of 1 ml PBS:100 mg tissue. The samples were then centrifuged (10 min, 10,000 *g*, 4 °C) and the supernatant was collected and kept frozen for cytokine quantification. The concentration of IL-10 and IFN-γ was measured by ELISA using commercially available antibodies (BD Biosciences Pharmingen, San Diego, CA, USA), according to the procedures supplied by the manufacturer.

2.11. Statistical analysis

Data were performed in duplicate on 3 different samples (produced in 3 different assays). The results regarding panel tests are the average values of three different cheesemaking processes.

The data were analysed by one-way ANOVA using the SPSS software (SPSS Inc., Chicago, IL, USA). The differences between means were detected by the Tukey's multiple range and Bonferroni test (version 15.0; SPSS Inc.). Data were considered significantly different when $P < 0.05$.

3. Results

3.1. pH water activity and moisture

In Table 1, the pH and water activity values recorded in the three types of Caciotta cheese are reported, in relation to the ripening time. The addition of *Lb. paracasei* A13, treated at 50 MPa, induced the highest acidification of the product highlighting the role of the HPH treatment in the modification of the strain metabolism, and in particular towards the production of lactic acid. However, in all the three cheese types, after 7 days, the pH decreased significantly with respect to the first day. This is probably due to the activity of the starters used in cheesemaking. Lowest values of pH in TC Caciotta cheese (added of HPH-treated *Lb. paracasei*) were recorded also after 7 and 14 days whilst after 21 days it attained values of 4.87, higher than the traditional product. The product containing the not treated probiotic strain had higher pH values after

Table 1 – pH and water activity (Aw) values in Caciotta cheeses in relation to the adjunction of traditional starters (CC), traditional starters and *Lb. paracasei* A13 (NTC) and traditional starters and high pressure treated *Lb. paracasei* A13 (TC).

Cheese type	Ripening (days)	pH	Aw
CC	1	5.11 ± 0.01[a]	0.991 ± 0.000[a]
	7	4.97 ± 0.03[a]	0.984 ± 0.002[a]
	14	4.93 ± 0.01[a]	0.985 ± 0.001[a]
	21	4.58 ± 0.05[a]	0.975 ± 0.001[a]
NT C	1	5.21 ± 0.03[b]	0.991 ± 0.001[a b]
	7	4.71 ± 0.03[b]	0.984 ± 0.003[a]
	14	4.53 ± 0.07[b]	0.984 ± 0.002[a]
	21	4.60 ± 0.05[a]	0.979 ± 0.002[b]
TC	1	5.07 ± 0.03[a]	0.992 ± 0.000[b]
	7	4.74 ± 0.01[b]	0.984 ± 0.001[a]
	14	4.75 ± 0.04[c]	0.983 ± 0.001[a]
	21	4.87 ± 0.05[b]	0.976 ± 0.002[ab]

Data were performed in duplicate on 3 different samples (produced in 3 different days).
For each row, data at the same storage time must be compared. Different superscript letters mean $P < 0.05$.

cheesemaking but acidification kinetics were higher than the traditional product during the ripening. About Aw, no significant differences were found among the three types of cheese. The initial moisture values of CC, NTC and TC were 54 ± 0.60, 53 ± 0.45 and 53 ± 0.40, respectively.

3.2. Microbiological analyses

As indicated by Table 2, the starter cultures had the same behaviour in all the three types of Caciotta cheese. Particularly, *S. thermophilus* and *L. bulgaricus* maintained cell loads higher than 9 and 6 log CFU g^{-1}, respectively, for all the considered ripening time, independently of the presence of the probiotic strain and the HPH treatment. Differently, the not HPH-treated probiotic strain maintained high viability (>9 log orders) for all the considered ripening time at 4 °C. The sub-lethal treatment applied to the probiotic cells determined a significant viability loss in Caciotta cheese only after 21 days of ripening (4.8 log orders). About the yeast cell loads no differences were underlined among the three Caciotta types. The total coliforms were fairly higher in Caciotta cheeses added with *Lb. paracasei* A13, independently of the HPH treatment applied to the cells.

3.3. Lipolysis and proteolysis

The free fatty acid (FFA) release was monitored during the ripening time in the three types of Caciotta cheese. The results obtained showed a specific profile for each cheese type. FFAs such as C14:0, C16:0, C18:1 (Δ9 cis), C18:2 (Δ7, Δ10 cis cis), C 10:0, and C 12:0 were found, independently of the cheese type considered. Twenty-four hours after the cheesemaking, Caciotta cheeses containing the treated probiotic strain were characterised by 400 ppm of FFAs, whereas the traditional one contained only 83 ppm (Table 3), evidencing a faster lipolysis than the other cheeses. After 7 days of storage at 6 °C, the lipolysis increased in the CC Caciotta cheese and in, minor extent, in NTC Caciotta (containing not HPH treated *Lb paracasei* A13). The level of FFAs remained constant in NTC Caciotta. The addition of the strain *Lb. paracasei* A13 determined a significant increase of C18:1 (Δ 9 cis) (oleic acid), C16:1 (Δ 11 cis), C18:2 (Δ 7, Δ 10 cis cis) concentrations and of palmitoleic, vaccenic and margaric acids.

The SDS-page patterns showed that the TC cheese had a specific profile since the start of ripening. In fact, the results show the presence of a large peptide (100 kDa), the presence of bands ranging between 37 and 75 kDa and bands at 20 kDa. The CC Caciotta cheese evidenced the absence of bands with molecular weight ranging between 75 and 250 kDa associated to the presence of bands between 28 and 75 kDa and two bands corresponding to 20 and 25 kDa. In the NTC cheese only the bands between 37 and 75 appeared. After 7, 14 and 21 days, the proteolytic profiles of TC cheese were characterised by a major presence of bands at low weight (10 kDa) with respect to the other cheeses.

Table 2 – Microbial cell loads detected in Caciotta cheeses in relation to the adjunction of traditional starters (CC), traditional starters and *Lb. paracasei* A13 (NTC) and traditional starters and high pressure treated *Lb. paracasei* A13 (TC).

Cheese type	Ripening (days)	*Streptococcus thermophilus*	*Lactobacillus bulgaricus*	*Lactobacillus paracasei* A13	Yeasts	Total coliforms
CC	1	9.6 (±0.1)[a]	6.9 (±0.1)[a]		–*	1.6 (±0.1)
	7	9.8 (±0.2)[a]	6.6 (±0.1)[a]		–	–
	14	9.1 (±0.2)[a]	6.4 (±0.2)[a]		–	–
	21	9.3 (±0.1)[a]	6.0 (±0.1)[a]		–	–
NT C	1	9.5 (±0.2)[a]	6.9 (±0.1)[a]	8.9 (±0.1)[a]	–	2.3 (±0.1)
	7	9.6 (±0.2)[a]	6.8 (±0.1)[a]	9.6 (±0.1)[a]	–	–
	14	9.1 (±0.1)[a]	6.4 (±0.2)[a]	9.2 (±0.2)[a]	–	–
	21	9.2 (±0.2)[a]	6.1 (±0.2)[a]	9.6 (±0.1)[a]	–	–
TC	1	9.4 (±0.1)[a]	6.8 (±0.1)[a]	9.3 (±0.1)[b]	–	2.2 (±0.1)
	7	9.8 (±0.1)[a]	6.6 (±0.1)[a]	9.3 (±0.1)[b]	–	–
	14	9.2 (±0.1)[a]	6.4 (±0.1)[a]	9.2 (±0.1)[a]	–	–
	21	9.2 (±0.1)[a]	6.1 (±0.1)[a]	4.8 (±0.2)[b]	–	–

Data were performed in duplicate on 3 different samples (produced in 3 different days).
For each row, data at the same storage time must be compared. Different superscript letters mean $P < 0.05$.
* Under the detection limit.

Table 3 – Free fatty acids (ppm) released in the three types of Caciotta cheeses in relation to the adjunction of traditional starters (CC), traditional starters and *Lb. paracasei* A13 (NTC) and traditional starters and high pressure treated *Lb. paracasei* A13 (TC).

Cheese type	Ripening time (days)			
	1	7	14	21
CC	83.30 ± 10.20[a]	1033.40 ± 12.30[a]	118.43 ± 15.80[a]	236.58 ± 12.10[a]
NTC	168.15 ± 35.60[b]	135.60 ± 10.70[b]	207.51 ± 18.50[b]	183.39 ± 15.65[b]
TC	400.95 ± 22.35[c]	707.81 ± 23.45[c]	270.68 ± 12.30[c]	91.27 ± 8.90[c]

Data were performed in duplicate on 3 different samples (produced in 3 different days).
For each column, data with different superscript letters are significantly different ($P < 0.05$).

No significant differences, in relation to the co-starter presence and HPH treatment, were observed for the three Caciotta cheese types in the biogenic amine content whose content was less than 2 ppm for each type of considered cheese during all the storage times.

3.4. *Volatile profiles and sensorial analysis*

The three Caciotta cheese types were subjected to the analysis of volatile molecule profiles by using GC/MS-SPME. Several molecules belonging to different classes of compounds (alcohols, acids, ketones, esters, aldehydes) were detected (data not shown). The HPH sub-lethal treatment performed on *Lb. paracasei* A13 cells significantly affected the cheese volatile molecule profiles. In order to better understand the contribution of the treated cells on cheese volatile profile, all the data were analysed throughout a principal component analysis (PCA). The results obtained are shown in Fig. 1a and b where it is evident that, after 24 h of ripening, the three Caciotta cheese types are separated only by the component 2 able to explain the 18% of the total variance, and thus grouping together. During the ripening, the three cheese types were differentiated also along the component 1. The traditional 7 and 14 day-ripened Caciotta cheeses (Caciotta 1–7 d and Caciotta 1–14 d) were separated along component 2 from all the cheeses containing the probiotic strain, treated or not, and from the traditional 20 day-ripened cheese. The metabolites characterising the different cheese types are reported in Fig. 1b. 2,3 butanedione, 2-heptanone, 2-nonanone characterised the traditional Caciotta cheese, whilst they were less present in the other two types containing the probiotic strain, independently of the treatment. The NTC Caciotta cheese (Caciotta 2) (added of not-HPH treated probiotic strain) was mainly characterised by 2-butanone, 1-pentanone and acetone whilst the functional cheese containing probiotic treated cells differed for the major presence of hydroxy-2-propanone (Caciotta 3). The data regarding the organic acids showed that the sorbic acid was majorly accumulated in the traditional Caciotta cheese whilst the presence of acetic acid from *Lb. paracasei* A13 was affected by the hyperbaric treatment. In fact, major production of acetic acid was found in Caciotta cheese containing not-HPH treated probiotic cells with respect to the functional one with treated cells. On the other hand, in this last cheese type after 21 days of ripening, the acidity descriptor received a lower score compared to the other cheese type by the panellists as shown by Fig. 2. This cheese type was also characterised by major sweetness and improved flavour and overall aroma and, finally, it was the most appreciated by the panellists.

3.5. *Texture*

The textural analyses showed no significant differences among the three types of Caciotta cheese immediately after cheesemaking and after 14 days of ripening. Differently, after 21 days, fair differences were recorded showing highest firmness values for NTC Caciotta cheese (data not shown).

3.6. *Resistance to simulated gastrointestinal digestion*

Table 4 shows the cell load reduction following the simulated gastrointestinal digestion (SDG) of *Lb. paracasei* A13, treated or not, incorporated in both types of cheese (NTC and TC).

Table 4 – Cell load reduction (Log CFU g^{-1}) of *Lb. paracasei* A13, treated (TC) or not (NTC) in Caciotta cheese following the simulated gastrointestinal digestion.

Cheese type	GIT challenge	Cell load reduction of *Lb. paracasei* A13		
		Ripening time (days)		
		1	14	21
NT C	Acid (pH = 3)	0.02	1.29 ± 0.30[a]	2.07 ± 0.40[a]
	Bile (1%)	0.08	1.21 ± 0.10[a]	2.77 ± 0.30[a]
	Bile-pancreatin (0.3–0.1%)	0.40 ± 0.08[a]	1.14 ± 0.20[a]	2.35 ± 0.50[a]
T C	Acid (pH = 3)	0.00	0.91 ± 0.10[a]	1.29 ± 0.20[b]
	Bile (1%)	0.00	1.09 ± 0.08[a]	1.18 ± 0.10[b]
	Bile-pancreatin (0.3–0.1%)	0.26 ± 0.10[b]	1.12 ± 0.10[a]	1.32 ± 0.10[b]

Data were performed in duplicate on 3 different samples (produced in 3 different days).
For each sample, the same treatment applied to cells must be compared. Different superscript letters mean $P < 0.05$.

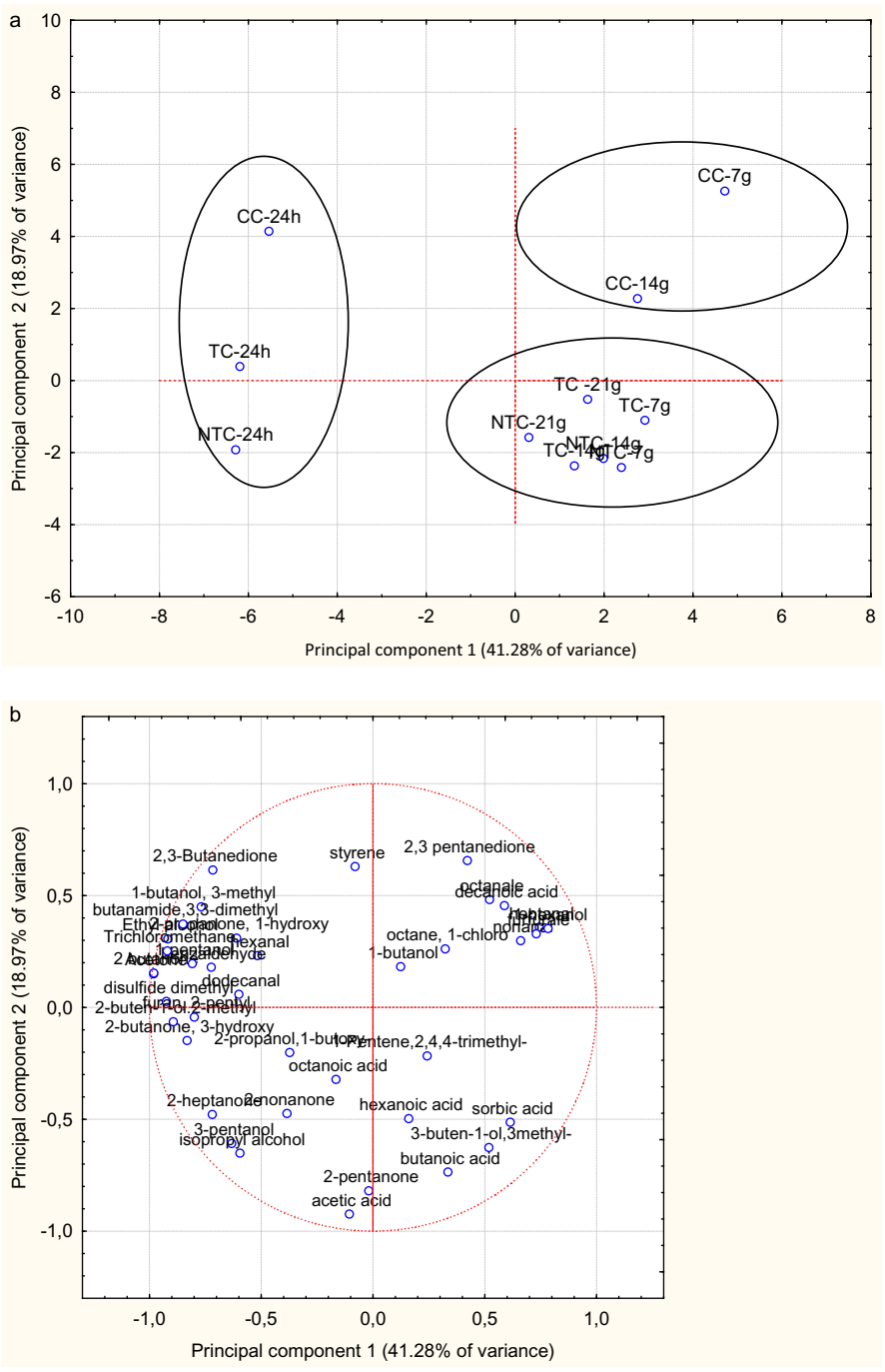

Fig. 1 – Principal component analysis loading plot of the two-first factors relative to volatile profiles of the different Caciotta cheese types (a) and variable factor coordinates for the two-first factors (b). Caciotta1: CC, traditional Caciotta cheese. Caciotta2: NTC, Caciotta cheese added of *Lb. paracasei* A13. Caciotta3: TC, Caciotta cheese added of high pressure treated *Lb. paracasei* A13. Data were performed in duplicate on 3 different samples (produced in 3 different days).

Immediately after cheesemaking (day 1 of ripening) the viability loss of *Lb. paracasei* A13 in TC or NTC, after exposure to bile-pancreatin (0.3–0.1%), were 0.26 and 0.40 log orders, respectively. However, when a fresh culture of *Lb. paracasei* A13 treated or not by HPH (50 MPa) was re-suspended in buffer and submitted to simulated gastrointestinal digestion the viability loss, after exposure to bile-pancreatin (0.3–0.1%), were 4.27 and 4.86 log orders, respectively (data not shown). After 21 days of ripening, *Lb. paracasei* A13 in TC was more resistant than in NTC indicating that the HPH treatment improved the resistance of the strain to the SGD.

3.7. *Translocation assay*

The oral administration of the three types of cheese did not induce undesirable side-effects since there was no

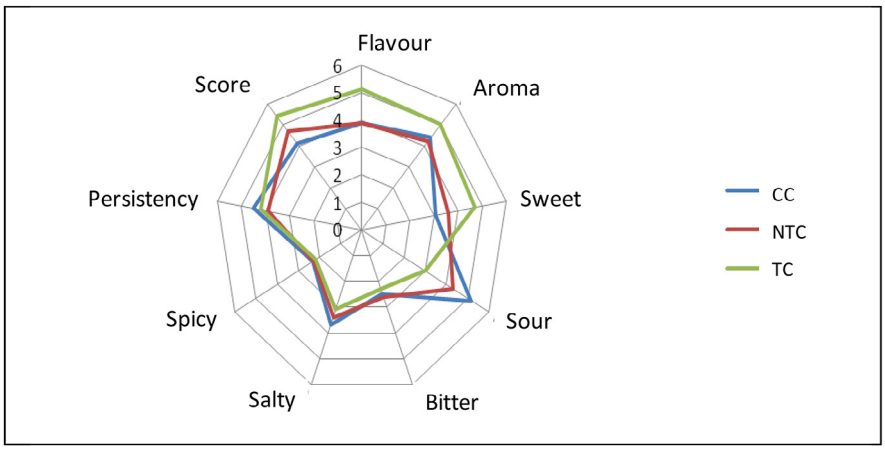

Fig. 2 – Sensory analysis scores obtained for the three types of Caciotta cheese. CC, traditional Caciotta cheese. NTC, Caciotta cheese added of *Lb. paracasei* A13. TC, Caciotta cheese added of high pressure treated *Lb. paracasei* A13. The data reported are the average values resulting from three different cheesemaking.

translocation of enterobacteria to the liver (the translocation assay was negative).

3.8. *Evaluation of functionality*

No differences in the number of IgA+ cells in the small intestinal lamina propria (immunohistochemistry) or in the small intestine content of S-IgA (ELISA) were observed among the groups of mice that received control cheese, or cheese containing HPH-treated and non-treated *Lb. paracasei* A13 cells (3a). Additionally, no differences in the content of IFNγ were observed in small and large intestine homogenates among groups (data not shown) nor in the content of IL-10 in the large intestine. As regards the levels of the regulatory IL-10 in homogenates of small intestine tissue, a significant increase on its concentration was observed in animals that received NTC for 3 consecutive days (Fig. 3b). An increasing, not significant, trend for IL-10 was observed in the CC group from day 3 to 10 of oral administration of the product.

4. Discussion

Probiotic cells are often conveyed throughout fermented products due to their ability to improve the probiotic cell resistance to the human barriers. For example, Wang et al. (2009) reported that the survival rates of freshly prepared cultures of *Lactobacillus casei* Zhang in simulated gastric juice at pH 2.0 and 2.5 were 31 and 69%, respectively, and the delivery of *L. casei* Zhang through fermented soymilk and bovine milk significantly improved the viability of the strain in simulated gastric transit.

Among fermented foods, cheese has been proven to be an optimal carrier to deliver probiotic bacteria, although also in this system there can be loss of viability; because characterised by a solid matrix, higher pH, higher buffering capacity and fat content with respect to yoghurt, joined to its ability to efficiently protect probiotic bacteria during intestinal transit to the site of action (Patrignani et al., 2007; Saxelin, Tynkkynen, Mattila-Sandholm, & de Vos, 2005). In addition, cheeses are perceived positively by consumers because they represent a good source of calcium and vitamins and they are highly digestible. Moreover, the adjunction of probiotic bacteria has contributed to increase their claimed functional features. However, the inclusion of probiotic bacteria in foods, as starter, co-starter or adjunct, can affect their sensorial and quality characteristics to which consumers are used. For this reason, the probiotic strain selection needs to take into consideration their effects on sensory features of the products (Mattila-Sandholm et al., 2002). In fact, when probiotic are used as starters the deriving products are generally lacking of sensorial features, iperacidity e loss of texture (Gobbetti, Di Cagno, & De Angelis, 2010; Patrignani et al., 2006). In this research, we have proposed the use of sub-lethal high pressure homogenisation (HPH) levels for the treatment of *Lb. paracasei* A13, used as adjunct for Caciotta cheese production, in order to increase cheese functionality without negative effect on its quality and texture. The choice to use HPH-treated cells comes out from our previous researches demonstrating that, in *in vitro* system, the application of a 50 MPa treatment to this strain could enhance its probiotic features such as auto-aggregation, hydrophobicity, its gastric and duodenal resistance and its ability to stimulate IgA production in *in vivo* model (Tabanelli et al., 2012, 2013). In this study, the application of a 50 MPa sub-lethal treatment on *Lb. paracasei* A13 has guaranteed high strain viability in cheese up to 21 days of Caciotta ripening, after that its viability decreased. However, the results obtained are in agreement with the literature according to the viability of probiotic strains is negatively affected by refrigerated condition (Patrignani et al., 2007) and so this skill, for cheese products like Caciotta, having probiotic levels of 5 log CFU g^{-1} after 21 days of storage, is considered a positive result. However, recent papers have underlined the positive role of dead probiotic cells for the human well-being (Adams, 2010). In fact many of the effects observed with living cells can be obtained also by dead cells generating in the host beneficial biological responses. Heat-dead cells of *Enterococcus faecalis* were able to stimulate the

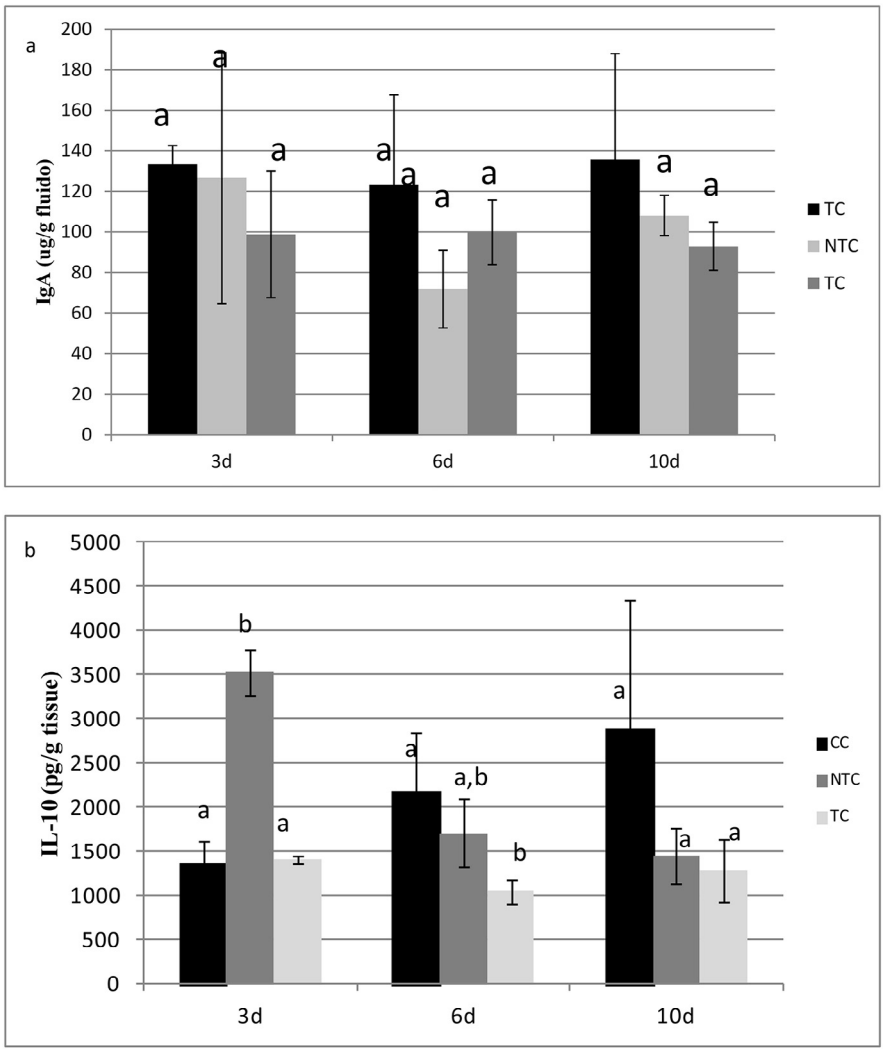

Fig. 3 – Determination of production of IgA (a) and anti-inflammatory IL-10 (b) in mice in relation to the feeding with the three types of Caciotta cheese. CC, traditional Caciotta cheese. NTC, Caciotta cheese added of *Lb. paracasei* A13. TC, Caciotta cheese added of high pressure treated *Lb. paracasei* A13. Data were performed in duplicate on 3 different samples (produced in 3 different days).

immune system in chickens. Thus, in this perspective, the decrease of viability of the HPH-treated strain *Lb. paracasei* A13 in cheese could not be negative. This type of treatment applied to *Lb. paracasei* A13 cells increased the gastric and duodenum resistance of the strain up to the 14th day of ripening and it has permitted, since the first days of refrigerated storage, to modify the ripening patterns of the Caciotta cheese, bringing to precocious proteolysis and lipolysis. In fact, these cheeses, between 7 and 14 days of ripening, had a volatile molecule profile very similar to those of the 21 day ripened traditional control cheeses, demonstrating an acceleration of the ripening processes without detrimental effects on cheese sensory quality and safety. In fact, no significant differences in biogenic amine contents were recorded in relation to the treatment and co-starter presence. Moreover, the application of HPH treatment to *Lb. paracasei* A13 cells permitted to avoid sensory defects related to the production of acetic acid and to increase its overall quality due mainly to the lower scores received for acidity. The major appreciation of cheeses obtained with HPH treated *Lb. paracasei* cells could be attributed also to the modification of proteolytic, lipolytic and volatile molecule profiles. These findings are in agreement with our earlier studies, where we have found that sub-lethal HPH treatments are able to modify the lactic acid bacteria metabolic profiles, leading to obtain attenuated cells, in terms of acidification, and changing their hydrolytic patterns with improved cheese characterising volatile molecule production (Lanciotti et al., 2007b).

IgA is the main immunoglobulin in the gut mucosa where its main function is to exert the immune exclusion of pathogens (Brandtzaeg et al., 1987). As a functional trait, the capacity of enhancing mucosal IgA in the gut after oral administration is a desirable attribute for probiotic bacteria (Galdeano, de Moreno de Leblanc, Carmuega, Weill, & Perdigón, 2009; Vinderola et al., 2007). The results of the present works showed that the traslocation assay was negative and so the use of *Lb. paracasei* A13 as adjunct can be regarded as safe since no colonies appeared in Mac Conkey agar where the liver of mice was plated. Moreover, it did not affect the IgA and InF-y production of the

mice fed with the cheeses taken into consideration. By contast, Medici, Vinderola, and Perdigon (2004) showed in a previous work conducted using fresh cheese containing *Lb. paracasei* A13, *L. acidophilus* and *Bifidobacterium bifidum* that the administration of the probiotic product significantly increased the phagocytic activity of peritoneal macrophages, the number of IgA+ producing cells and the CD4+/CD8+ ratio in the small intestine after 5 days. However, it is well known that the immune modulation ability is species and strain dependent (Medici et al., 2004). The HPH treatment did not affect the IgA and InF-γ production in mice fed with the cheeses taken into consideration. By contrast, the HPH-treatment of *Lb. paracasei* A13 significantly impaired its capacity to induce IL-10 when compared to non-treated cells. The incorporation into the diet of microorganisms able to induce the intestinal production of the anti-inflammatory IL-10 has been signalled also as an interesting tool, among the multiple strategies, to treat inflammatory bowel diseases (Baumgart & Sandborn, 2007). For this strain, the reduction of IL-10 stimulation can be explained with the modification of surface proteins on the cell caused by HPH treatment. On the other hand, the changes of other cell-wall related properties, such as increased hydrophobicity and the interaction capacity with the gut, had been previously reported for this strain as pure culture not included in a food matrix (Tabanelli et al., 2013).

5. Conclusion

The data obtained have underlined the applicative potential of the proposed sub-lethal HPH treatment for the production of probiotic short ripened cheese. Although the HPH treatment affected in negative mode the IL-10 secretion, the IgA production was not influenced by the treatment. Moreover, the resulting probiotic cheese was characterised by improved sensorial features with a reduction of ripening time. Consequently, the application of this technology to probiotic cells used as co-starter for cheese production, can reduce the costs linked to the maturation in refrigerated condition contributing to the entire process sustainability and to the expansion of new functional dairy products, similar to the traditional for safety, but differentiated or improved for quality, sensory and functional features. However, to reach this important skill, an appropriate selection of the probiotic strains and the optimisation of the treatment condition, in relation to the cheese desired features, are fundamental.

REFERENCES

Adams, C. A. (2010). The probiotic paradox: Live and dead cells are biological response modifier. *Nutrition Research Reviews*, *23*, 37–46.

Baumgart, D. C., & Sandborn, W. J. (2007). Inflammatory bowel disease: Clinical aspects and established and evolving therapies. *Lancet*, *12*, 1641–1657.

Brandtzaeg, P., Bjerke, K., Kett, K., Kvale, D., Rognum, T. O., Scott, H., Sollid, L. M., & Valnes, K. (1987). Production and secretion of immunoglobulins in the gastrointestinal tract. *Annals of Allergy, Asthma and Immunology*, 59, 21–39.

Burns, P., Patrignani, F., Serrazanetti, D., Vinderola, G., Reinheimer, J., Lanciotti, R., & Guerzoni, M. E. (2008a). Probiotic Crescenza cheese containing Lactobacillus paracasei and *Lactobacillus acidophilus* manufactured with high pressure-homogenized milk. *Journal of Dairy Science*, *91*, 500–512.

Burns, P., Vinderola, G., Binetti, A., Quiberoni, A., de los Reyes-Gavila, C. G., & Reinheimer, J. (2008b). Bile-resistant derivatives obtained from non-intestinal dairy lactobacilli. *International Dairy Journal*, *18*, 377–385.

Carroll, T., Chen, P., Harnett, M., & Harnett, J. (2004). Pressure treating foods to reduce spoilage. International Patent WO 2004/032655.

Eerola, S., Hinkkanen, R., Lindfors, E., & Hirvi, T. (1993). Liquid chromatographic determination of biogenic amines in dry sausages. *Journal of AOAC International*, *76*, 575–581.

Galdeano, C. M., de Moreno de Leblanc, A., Carmuega, E., Weill, R., & Perdigón, G. (2009). Mechanisms involved in the immunostimulation by probiotic fermented milk. *Journal of Dairy Research*, *76*, 446–454.

Gallardo-Escamilla, F. G., Kelly, A. L., & Delahunty, C. M. (2007). Mouthfeel and flavour of fermented whey with added hydrocolloids. *International Dairy Journal*, *17*, 308–315.

Gobbetti, M., Di Cagno, R., & De Angelis, M. (2010). Functional microorganisms for functional food quality. *Critical Reviews in Food Science and Nutrition*, 50, 716–727.

Griffiths, J. C., Abernethy, D. R., Schuber, S., & Williams, S. L. (2009). Functional food ingredient quality: Opportunities to improve public health by compendial standardization. *Journal of Functional Foods*, *1*, 128–130.

International Dairy Federation (1982). *Determination of the total solids content (cheese and processed cheese). IDF standard 4A*. Brussels, Belgium: International Dairy Federation.

Knorr, D. (1999). Novel approaches in food-processing technology: New technologies for preserving foods and modifying function. *Current Opinion in Biotechnology*, *10*, 485–491.

Kuchroo, C. N., & Fox, P. F. (1982). Soluble nitrogen in Cheddar cheese: Comparison of extraction procedures. *Milchwissenschaf*, *37*, 331–335.

Lado, B. H., & Yousef, A. E. (2002). Alternative food-preservation technologies: Efficacy and mechanisms. *Microbes and Infection*, *4*, 433–440.

Lanciotti, R., Patrignani, F., Iucci, L., Guerzoni, M. E., Suzzi, G., Belletti, N., & Gardini, F. (2007a). Effect of milk high pressure homogenization on biogenic amine accumulation during ripening of ovine and bovine Italian cheeses. *Food Chemistry*, *104*, 693–701.

Lanciotti, R., Patrignani, F., Iucci, L., Saracino, P., & Guerzoni, M. E. (2007b). Potential of high pressure homogenization in the control and enhancement of proteolytic and fermentative activities of some *Lactobacillus* species. *Food Chemistry*, *102*, 542–550.

Mattila-Sandholm, T., Myllarinen, P., Crittenden, R., Mogensen, G., Fonden, R., & Saarela, M. (2002). Technological challenger for future probiotic foods. *International Dairy Journal*, *12*.

Medici, M., Vinderola, C. G., & Perdigon, G. (2004). Gut mucosal immunomodulation by probiotic fresh cheese. *International Dairy Journal*, *14*, 611–618.

Patrignani, F., Burns, P., Serrazanetti, D., Vinderola, G., Reinheimer, J., Lanciotti, R., & Guerzoni, M. E. (2009). Suitability of high pressure-homogenized milk for the production of probiotic fermented milk containing *Lactobacillus paracasei* and *Lactobacillus acidophilus*. *Journal of Dairy Research*, *76*, 74–82.

Patrignani, F., Iucci, L., Lanciotti, R., Vallicelli, M., Maina Mathara, J., Holzapfel, W. H., & Guerzoni, M. E. (2007). Effect of high pressure homogenization, not fat milk solids and milkfat on

the technological performances of a functional strain for the production of probiotic fermented milks. *Journal of Dairy Science*, *90*, 4513–4523.

Patrignani, F., Lanciotti, R., & Guerzoni, M. E. (2011). Emerging Technologies for Probiotic and Prebiotic Foods. In *N. P. SHAH A. GOMES DA CRUZ J. DE ASSIS FONSECCA FARIA. Probiotic and Prebiotic Foods: Technology, Stability and Benefits to Human Health* (pp. 481–518). SAN PAOLO: NOVA SCIENCE PUBLISHER. ISBN: 978-1-61728-825-8.

Patrignani, F., Lanciotti, R., Maina Mathara, J., Guerzoni, M. E., & Holzapfel, W. H. (2006). Potential of functional strains, isolated from traditional Maasai milk, as starters for the production of fermented milks. *International Journal of Food Microbiology*, *107*, 1–11.

Romano, A., Blaiotta, G., Di Cerbo, A., Coppola, R., Masi, P., & Aponte, M. (2014). Spray-dried chestnut extract containing Lactobacillus rhamnosus cells as novel ingredient for a probiotic chestnut mousse. *Journal of Applied Microbiology*, *116*, 1632–1641.

Saxelin, M., Tynkkynen, S., Mattila-Sandholm, T., & de Vos, W. M. (2005). Probiotic and other functional microbes: From markets to mechanisms. *Current Opinion in Biotechnology*, *16*, 204–211.

Senorans, F. J., Ibanez, E., & Cifuentes, A. (2003). New trends in food processing. *Critical Reviews in Food Science and Nutrition*, *43*, 507–526.

Siró, I., Kápolna, E., Kápolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance – A review. *Appetite*, *51*, 456–467.

Tabanelli, G., Burns, P., Patrignani, F., Gardini, F., Lanciotti, R., Reinheimer, J., & Vinderola, G. (2012). Effect of a non-lethal High Pressure Homogenization treatment on the in vivo response of probiotic lactobacilli. *Food Microbiology*, *32*, 302–307.

Tabanelli, G., Patrignani, F., Vinderola, G., Reinheimer, J. A., Gardini, F., & Lanciotti, R. (2013). Effect of sub-lethal high pressure homogenization treatments on the in vitro functional and biological properties of lactic acid bacteria. *LWT - Food Science and Technology*, *53*, 580–586.

Tripathi, M. K., & Giri, S. K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods*, *9*, 225–241.

Vannini, L., Patrignani, F., Iucci, L., Ndagijimana, M., Vallicelli, M., Lanciotti, R., & Guerzoni, M. E. (2008). Effect of a pre-treatment of milk with high pressure homogenization on yield as well as on microbiological, lipolytic and proteolytic patterns of "Pecorino" cheese. *International Journal of Food Microbiology*, *128*, 329–335.

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, C. G., Prosello, W., Ghiberto, D., & Reinheimer, J. A. (2000a). Viability of probiotic (*Bifidobacterium, Lactobacillus acidophilus* and *Lactobacillus casei*) and non probiotic microflora in Argentinian fresh cheese. *Journal of Dairy Science*, *83*, 1905–1911.

Vinderola, C. G., & Reinheimer, J. A. (2000b). Enumeration of *Lactobacillus casei* in the presence of L. acidophilus, *Bifdobacteria* and lactic starter bacteria in fermented dairy products. *International Dairy Journal*, *10*, 271–275.

Vinderola, G., Cespedes, M., Mateolli, D., Cardenas, P., Lescano, M., Aimaretti, N., & Reinheimer, J. (2011). Changes in gastric resistance of *Lactobacillus casei* in flavoured commercial fermented milks during refrigerated storage. *International Journal of Dairy Technology*, *64*, 269–275.

Vinderola, G., Matar, C., & Perdigón, G. (2007). Milk fermented by *Lactobacillus helveticus* R389 and its non-bacterial fraction confer enhanced protection against *Salmonella enteritidis* serovar Typhimurium infection in mice. *Immunobiology*, *212*, 107–118.

Wan, J., Conventry, J., Sanguansri, P., & Versteeg, C. (2009). Advances in innovative technologies for microbial inactivation and enhancement of food safety – Pulsed electric field and low-temperature plasma. *Trends in Food Science and Technology*, *20*, 414–424.

Wang, J., Guo, Z., Zhang, Q., Yan, L., Chen, W., Liu, X. M., & Zhang, H. P. (2009). Fermentation characteristics and transit tolerance of probiotic *Lactobacillus casei* Zhang in soymilk and bovine milk during storage. *Journal of Dairy Science*, *92*, 2468–2476.