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Technological challenges in the production of a probiotic pasta filata soft cheese

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ABSTRACT

The aim of this study was to adjust technological parameters: acidification of the curd (pH 5.25) and time (2, 5, 10 and 20 min) and stretching temperature (58, 62.5 and 68 °C) in order to make a pasta filata cheese carrying a probiotic bacterium at levels higher than 10^7 CFU/g. A control and a probiotic cheese were produced. *Lactobacillus rhamnosus* GG was used and its resistance to simulated gastrointestinal digestion (SGD) was evaluated. Gross composition and pH, microbiological analysis, proteolysis, physicochemical and sensory characteristics, volatile compounds, organic acids and sugar profiles were also determined. The probiotic remained above 3×10^7 CFU/g during its shelf life and exhibited high resistance to SGD (matrix protection of about 60%). The addition of the probiotic increased secondary proteolysis (about 30% for SN fraction in trichloracetic and phosphotungstic acids) and the production of diacetyl, acetoin, lactic and acetic acids. Sensory characteristics (smell, astringency, acid taste and residual flavor) were also modified. The development of a probiotic Fior di Latte cheese that might contribute to disease prevention and generate improvements in sensory characteristics compared to traditional products would allow expanding the market of functional foods.

1. Introduction

Functional foods are those which contain some health promoting components which go beyond the traditional nutrients (Granato, Branco, Nazzaro, Cruz, & Faria, 2010). One way to achieve this is by adding probiotics. A probiotic food is a processed product which contains viable probiotic microorganisms in a suitable matrix and in sufficient concentration (Castro, Tornadijo, Fresno, & Sandoval, 2014). The definition of probiotic bacteria adopted by the joint FAO/WHO working group (FAO/WHO, 2002) establishes that they are 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'. *Lactobacillus rhamnosus* GG (ATCC 53103, LGG®) is one of the most investigated probiotic strains in the world. It has been widely studied in humans and laboratory animals for a variety of uses. Moreover, it is a well attested clinical bacterial strain widely used as probiotic culture in dairy foods. The ability of *L. rhamnosus* GG to survive and colonize in the gastrointestinal tract (GIT) has been shown for both adults and children (Jia, Chen, Chen, & Ding, 2015).

Nowadays there is a great interest of food industries for the development of dairy products containing probiotic bacteria, which may additionally provide essential nutrients such as calcium and proteins (Angiolillo, Conte, Faccia, Zambrini, & Del Nobile, 2014).

Cheeses have a number of advantages over yogurt and fermented milks as a delivery system for viable probiotic microorganisms, because they generally have higher pH and buffering capacity, a solid and consistent matrix and relatively higher fat content. These attributes protect probiotic bacteria during storage and passage through the GIT (Burns et al., 2008). Even though some cheese varieties have been studied as vehicles for probiotic microorganisms (Albenzio et al., 2013; Burns et al., 2015; Dantas et al., 2016; Felicio et al., 2016; Ong, Henriksson, & Shah, 2007), there are very few published studies (and to our knowledge, no product on the market) in which the incorporation of probiotic microorganisms to fresh pasta filata cheese (Fior di Latte type) was evaluated. Besides, previously reported data suggest the addition of microencapsulated (Angiolillo et al., 2014; Ortakci, Broadbent, McManus,

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& McMahon, 2012) or heat-adapted probiotics (Minervini et al., 2012), strategies that could present some difficulties for their industrial application. The novelty of this work lies in the adaptation of the production process, together with the selection of a suitable bacterium, allowing the manufacture of a probiotic cheese by means of a simple technology which could be easily transferred to the industrial sector.

Fior di Latte (a high-moisture cow milk Mozzarella cheese) can be manufactured through chemical acidification of the curd or by using commercial (*S. thermophilus*) or natural whey starter cultures. It is a white rindless-cheese, with a characteristic shine on the surface and sweet taste (with a slight taste of lactic acid). It presents high humidity (45–55%) and the fat content is about 18–20% (Ghitti, Bianchi, & Rottigni, 1996). It is a cheese that is usually consumed within a few days of being prepared. Nevertheless, several challenges are related to the addition of probiotics to Fior di Latte cheese, the most important being the survival of these bacteria during cheese making (high temperature of water and time of stretching) and storage. Another challenge found when adding a probiotic into a food is to maintain the sensory attributes of the product (Minervini et al., 2012).

The aim of this work was to modify the traditional cheese making technology in order to make a pasta filata soft cheese carrying the probiotic *L. rhamnosus* GG at levels higher than 10^7 CFU/g during its shelf life.

2. Materials and methods

2.1. Strains and growth conditions

Four commercial probiotic bacteria (*Lactobacillus paracasei* A13, *Lactobacillus rhamnosus* GG, *Lactobacillus paracasei* C, Bifidobacterium animalis subsp. lactis D) and the autochthonous Bifidobacterium animalis subsp. lactis INL1 (isolated from human breast milk, Zacarías, Binetti, Laco, Reinheimer, & Vinderola, 2011) belonging to the INLAIN collection were used. Lactobacillus and Bifidobacterium were propagated for 24 h at 37 °C, in the Man, Rogosa and Sharpe broth (MRS; Biokar, Beauvais, France) or MRS with 0.1% (w/v) L-cysteine hydrochloride (Biopack, Argentina) (MRSc), aerobiosis or anaerobiosis (Mitsubishi™ AnaeroPack-Anaero), respectively. Real names of *Lactobacillus paracasei* C and Bifidobacterium animalis subsp. lactis D are not given because of confidentiality reasons.

2.2. Heat resistance

During cheese making the curd is dipped in hot water so, in order to select the most resistant bacterium, their heat tolerance was evaluated in 20% (w/v) skim milk (Difco, Becton, Dickinson and Company, Sparks, MD, USA) acidified with lactic acid (pH 5.25 ± 0.05) at 60 °C for 10 min (to simulate conditions during stretching). An overnight culture of each bacterium (20 h) was centrifuged (6000 g, 10 min, 8 °C), washed twice with PBS buffer (pH 7.20) and resuspended in skim milk at a final concentration of 1×10^7 CFU/mL. Immediately after heat treatment, cells were chilled on ice. Viable cell counts were performed before and after 10 min exposure to 60 °C in MRS or MRSc agar (48 h, 37 °C, aerobiosis or anaerobiosis). Experiments were carried out in duplicate.

2.3. Cheese making and setting of technological parameters

Each cheese making day, 120 L of raw milk at 4 °C (provided by MILKAUT S.A., Franck, Santa Fe, Argentina) were pasteurized at 65 °C for 20 min and cooled to 39 °C (coagulation temperature). Two types of pasta filata cheese were manufactured: (1) Control cheese (CC: without a probiotic bacterium) and (2) Probiotic cheese (PC: with the selected (more heat-tolerant) probiotic bacterium, *L. rhamnosus* GG). The freeze-dried starter culture Streptococcus thermophilus ST-M6 (Chr. Hansen Inc., Denmark) was added at a concentration of 2×10^6 CFU/mL of milk. The probiotic was cultivated in 1 L MRS broth (20 h, 37 °C, aerobiosis). After growth, cells were harvested by centrifugation (6000 g, 10 min, 8 °C), washed twice with PBS buffer (pH 7.20), resuspended in 100 mL of pasteurized milk and added at a final concentration of 5×10^7 CFU/mL. After 10 min, chymosin [(Chy-Max, Inc. Chr Hansen, Denmark. 183 International Milk-Clotting Units (IMCU/mL))] was added. The amount of rennet (0.3 mL/L of milk) was enough to obtain the proper firmness for cutting the curd in 20–25 min. At this time, the curd was cut in the adequate grain size (~20 mm). After 15 min, the mixture was stirred gently for 15 min to achieve proper moisture and left standing for about 70 min until a pH of 5.6. After that, the whey was removed, the curd was placed on table for approximately 30 min until a pH of 5.20 (±0.02) and cut in slices of c.a. 1 cm thick. Stretching of the curd was performed over 10 min in water at 76.0 °C ± 0.5 °C, 81.0 ± 0.5 °C and 86.0 ± 0.5 °C (core temperature of the curd of 58.0 ± 0.5 °C, 62.5 ± 0.5 °C and 68.0 ± 0.5 °C, respectively). The stretched curd cheeses were formed automatically by a mechanical extruder and immediately placed in brine 3.5% (w/v) at 4 °C for 40 min. Finally, cheeses were vacuum-packed and stored in a chamber at 4 °C for 15 days. Cheese making was carried out in triplicate.

2.4. Microbiological analysis

Viable cell count of total lactic acid bacteria (LAB) in milk and curd was performed in plate count agar (PCA, Britania, Buenos Aires, Argentina) + 10% (w/v) skim milk powder (Difco) at 37 °C for 48 h, whereas *L. rhamnosus* GG was enumerated in MRS agar (37 °C, 48 h, aerobiosis). It has been checked that the starter culture would not grow in MRS agar. Microbiological analysis of cheeses was performed after 1, 7 and 15 days of ripening. At the end of the shelf life the level of coliforms and moulds and yeasts was also determined. Coliforms were counted in lactose violet red bile agar (VRBA, Biokar, Beauvais, France) at 37 °C, 24 h, aerobiosis. Moulds and yeasts were enumerated in chloramphenicol glucose agar (Biokar, Beauvais, France) at 25 °C, 7 days, aerobiosis. Microbiological analysis was performed in triplicate.

2.5. Gross composition and pH measurement

Cheese samples were grated and analyzed for fat matter by Gerber van Gulik method (ISO, 2008), moisture by oven-drying at 102 °C (ISO, 2004) and total protein by Kjeldahl method (ISO, 2011) using a Digestion System 6 (1007 Digester, Tekator, Switzerland) and BÜCHI Distillation Unit B-324 (Sweden). The pH of cheese slurry, prepared by blending a mix 1:1 of grated cheese in H_2O according to the American Public Health Association (APHA) (Bradley et al., 1993) was measured with a pH meter (Orion Research Incorporated, United States) after calibrating with fresh pH 4.0 and 7.0 standard buffers. Cheese composition was analyzed at day 1 and 15. Analyses were carried out in triplicate.

2.6. Proteolysis assessment and volatile profiles

2.6.1. Analysis of soluble nitrogen

Cheese samples were treated to obtain crude citrate extract and soluble fractions at pH 4.6, in trichloracetic acid TCA 12% (v/v) and phosphotungstic acid PTA 2.5% (v/v), according to Hynes et al. (2003). The crude cheese extract was obtained by adding 20 mL of sodium citrate 0.5 M–10 g of cheese and grounding to homogeneity using a pestle. Deionized water was added to ~90 mL, and