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Commercially standardized process for probiotic “Italico” cheese production

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ABSTRACT

Modulation of the intestinal microbiota is one of the potential health-beneficial effects of probiotic foods. The market potential and the consumers' awareness about the impact of these foods on health, annually increases. Dairy industry seems to be a promising sector for new products' development. In the present study, the most suitable probiotic for “Italico” cheese production was assessed. Moreover, the ability as carrier for probiotics as well as the sensory quality of the Italico cheese was evaluated on both laboratory and industrial scale. *Lactobacillus rhamnosus* LbGG and SP1, within cheese samples, showed a remarkable tolerance to acid-gastric and duodenal stresses, up to 40 days of ripening at 4 °C, keeping a viability level higher than 10^8 CFU/g. Furthermore, the odor of the cheese enriched with these two probiotic strains appeared more acidic, creamy, buttery, and characterized by a pleasant matrix deconstruction, resulting in a Stracchino-like product. To authors' knowledge, this is the first study in which probiotic cheese production was followed at industrial level, thus fully describing an attractive system for commercial exploitation.

1. Introduction

The demand for probiotic functional foods is growing rapidly due to increased awareness of consumers about their impact on health. The claim has motivated, as expected, a large number of studies focusing on the incorporation of probiotics into dairy and non-dairy products (Chaves & Gigante, 2016).

Due to its physicochemical characteristics, cheese proved to possess several advantages as a carrier for probiotics when compared to other fermented milk products (Castro, Tornadijo, Fresno, & Sandoval, 2015). Factors such as high pH, low acidity, high buffering capacity, and an anaerobic environment created by the protein-fat matrix have all been ascertained to improve the probiotics viability throughout the cheese shelf-life and during the passage through the gastrointestinal tract (GIT) (da Cruz, Buriti, Batista de Souza, Fonseca Faria, & Isay Saad, 2009”).

As matter of fact, the incorporation of both lactobacilli and bifidobacteria in different kind of cheeses has been widely investigated (da Cruz et al., 2009; Karimi, Mortazavian, & da Cruz, 2011). Studies are mainly focused on the maintenance of probiotic viability during storage and, to a lesser extent, on the survival of probiotics during passage through the GIT, when delivered within the cheese matrix (Pitino et al., 2012).

Indeed, even if cheese has been considered as one of the top carriers for probiotics, the overall manufacturing process has often to be tuned in order to better exploit the microbial activities in the production of dairy products (Heller, 2001). Actually, from a food processing perspective, it is desirable that probiotic strains are suitable for large-scale industrial production and able to withstand the processing conditions (Succi, Tremonte, Reale, Sorrentino, & Coppola, 2007); where it is not possible, other probiotic strains should be applied or new products may have to be developed (Heller, Bockelmann, Schrezenmeir, & deVrese, 2003). So, even if the use of cheese as probiotic food carrier presents potential advantages, the development on industrial scale requires knowledge of all technological steps involved in the traditional processes. Five hurdles have been proven to directly influence the maintenance of the functional activities of probiotic bacteria in cheese: addition of

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the probiotic inoculum, salting, packaging, ripening and storage conditions (da Cruz et al., 2009). To overcome such hurdles, adaptations of the existing manufacturing protocols are usually necessary, but these interventions unavoidably affect the overall sensorial profile of the cheese that often appears in a way different, if compared to the traditional variety to which consumers are used to.

In such light, the aim of this study was to determine the most suitable probiotic strain belonging to *Lactobacillus* spp. to be used in the production of "Italico" cheese, a soft-rind (no surface microflora) and short-ripened (20–30 days) cheese.

Italico cheese was enriched with probiotics endowed with acid and bile salts tolerance, according to FAO/WHO (Food and Agricultural Organization-World Health Organization) guidelines (Prasad, Gill, Smart, & Gopal, 1998). The product was evaluated for its probiotic's carrier ability through a simulated GIT system, and for its sensory quality, taking into account technological process variables not only in cheeses realized on a laboratory scale, but even in cheeses produced at industrial level.

2. Materials and methods

2.1. Probiotic strains tested in the study

Commercial probiotics initially screened were: *Lactobacillus* (*Lb.*) *johnsonii* La1 (NCC533), (Nestlé, Switzerland); *Lb. acidophilus* LA-5 (Christian Hansen, Denmark); *Lb. rhamnosus* LbGG (ATCC 53103) (Valio Ltd., Finland); *Lb. rhamnosus* Sp1 (Sacco, Italy); *Lb. casei* ATCC393 (American Type Culture Collection); *Lb. casei* DN-114 001 (Danone, France); *Lb. casei* Shirota YIT9029 (Yakult Honsha Co., Ltd., Japan); *Lb. reuteri* DSM 17938 (BioGaia AB, Sweden).

2.2. Ability of probiotic strains to grow in cow milk

Population's level reached by probiotic lactobacilli in pasteurized semi skimmed cow milk was evaluated by counting on MRS agar (Oxoid, Basingstoke, United Kingdom) according to the drop method proposed by Collins, Lyne, and Grange (1989). Briefly, MRS overnight cultures of each probiotic strain were used to inoculate (1%) pasteurized milk (100 mL). pH and lactobacilli loads on MRS agar were evaluated at time 0 and after 7 and 24 h of incubation at 37 °C. Each measurement was carried on in triplicate.

2.3. Strains resistance to simulated gastric and intestinal fluids in cow milk

Overnight cultures of probiotics in pasteurized cow milk were subjected to simulated GIT digestion according to method proposed by Ricciardi, Blaiotta, Di Cerbo, Succi, and Aponte (2014) and Ricciardi et al (2015a). Briefly, 30 mL of bacterial cultures were centrifuged (8.160 *g* per 5 min) and pellet was diluted (3:5) with an artificial gastric fluid, consisting of an electrolyte solution (16.2 g/L NaCl, 2.2 g/L KCl, 0.22 g/L $CaCl_2$ and 1.2 g/L $NaHCO_3$) added of 0.3% porcine gastric mucosa pepsin (Sigma, Milan, Italy, activity 3300 U/mg of protein calculated using haemoglobin as a substrate) (pH adjusted to 2.50 with 5 M HCl). After 120 min of incubation at 37 °C, samples were centrifuged and resulting pellets were diluted (1:4) in an artificial duodenal secretion (6.4 g/L $NaHCO_3$, 0.239 g/L KCl, 1.28 g/L NaCl, 0.5% bile salts, 0.1% pancreatin, pH 7.20) and incubated for further 120 min at 37 °C. In all steps, stirring was used to simulate the peristaltic movements. After each step, samples were analysed for viable LAB counts on MRS agar (37 °C, anaerobic incubation, 48 h).

2.4. Production of probiotic Italico cheese

Cheese-making was firstly performed at pilot scale on three batches of 5 L pasteurized cow milk each. The production protocol may be reassumed as follows: milk was warmed at 37 °C under constant stirring and a *Streptococcus (St.) thermophilus* strain was added as starter culture (Lyobac CR, Alce International srl, Italy) according to manufacturer indications; after a 30 min pause to activate the starter, calf rennet was added (1:14 500 025, 1370 IMCU/g Caglificio Clerici Spa, Italy) and the coagulum was cut within 30 min at "corn" size. Salt was then added (1.5%) and, after about 20 min, whey was drained and curd transferred into a hoop. Cheeses were stewed at 39 °C for 2 h and turned 3–4 times. At the end of the process, cheeses were kept in brine (NaCl 18–20°Bé) for 20 min and then transferred at 4 °C for 40 days (Suppl. Fig. 1). After three weeks of ripening, cheese may be marketed. Cheeses produced as above described, served as control; while two further trials were performed following the same protocol except that probiotic strains *Lb. rhamnosus* LbGG and SP1 were individually added 15 min after the starter inoculum. MRS cultures of each probiotic strain in stationary phase were centrifuged at 4 °C (14.000 rpm for 10 min). Cell suspensions in sterile physiological solution were added to the milk to achieve a population's level of about 7 Log CFU/mL. The same set of trials was repeated at farm scale at Iaquilat Trade srl (S. Salvatore Telesino, Benevento, Italy) on 200 L milk batches. A further cheese-making was carried on with milk enriched with *Lb. acidiphilus* LA-5. pH was monitored by a pH-meter (pH50 Lab) through key stages of both cheese-making processes.

2.5. Evaluation of microbial dynamics during cheese production and ripening

Microbial dynamics were monitored during productions both experimental and industrial. Lactobacilli were counted on MRS modified according to Ricciardi et al. (2015b). Plates were read after 48 h of anaerobic (Anaerogen kit, Oxoid) incubation at 30 °C. *St. thermophilus*, used as starter culture, was counted on M17 (Oxoid) with lactose (1%), according to manufacturer's indications, after incubation at 30 °C for 48 h. Plate Count Agar (PCA, Oxoid) was used to evaluate total aerobic microflora after incubation at 30 °C for 48 h. Counts were performed on pasteurized milk in the vat, after the starter addition, on curd after extraction and on curd after stewing. Furthermore, cheeses were analysed three days after brining, and at 7, 14, 21, and 40 days of ripening at 4 °C.

2.6. Evaluation of microbial populations in cheese by PCR-DGGE

Samples of cheese after 7, 21 and 40 days of ripening were used for DNA extraction according to the protocol developed by Aponte et al. (2012). In order to increase the amount of amplicons of hypervariable region V3 within 16S rDNA, nested PCRs were performed. Specifically, the entire 16S rRNA was amplified by means of universal primers (FD1 and RD1) designed by Weisburg, Barns, Pelletier, and Lane (1991) and used as template for PCR amplification of hypervariable regions V3 according to Muyzer, De Waal, and Uitterlinden (1993). Conditions, temperature profile and reaction mixture for amplifications were the same reported by Blaiotta, Di Capua, Romano, Coppola, and Aponte (2014). PCR products were analysed by DGGE using a Bio-Rad D-code apparatus and the procedure first described by Muyzer et al. (1993). DGGE bands to be sequenced were purified in water according to Aponte, Fusco, Andolfi, and Coppola (2008). One μL of the eluted DNA of each DGGE band was re-amplified by using the above-described primers and conditions. PCR products which gave a single band co-migrating with the original band were then purified by QIAquick