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Testing commercial biopreservative against spoilage microorganisms in MAP packed Ricotta *fresca* cheese

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ABSTRACT

Ricotta *fresca* cheese is susceptible to secondary contamination and is able to support the growth of pathogens or spoilage psychotrophic bacteria during storage. The aim of the present study was to evaluate which among three commercial biopreservatives was suitable to be used to control the growth of spoilage microorganisms in sheep's milk MAP ricotta *fresca* cheese. 144 Ricotta *fresca* cheese samples were inoculated either with the bioprotective culture Lyofast FPR 2 (including *Enterococcus faecium*, *Lactobacillus plantarum* e *Lactobacillus rhamnosus*) or Lyofast CNBAL (*Carnobacterium* spp) or the fermentate MicroGARD 430. Not inoculated control and experimental ricotta were MAP packed (30% CO_2 and 70% N_2) and stored at 4 °C. Triplicate samples were analyzed after 5 h and 7, 14 and 21 days after inoculation for total bacterial count, mesophilic lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas* spp, *Listeria monocytogenes*, moulds and yeasts. Among the tested biopreservatives only *Carnobacterium* spp was able to control *Pseudomonas* spp and *Enterobacteriaceae*. The maximum reduction in the concentration of *Pseudomonas* spp and *Enterobacteriaceae* was respectively 1.93 and 2.66 $\log_{10}$ cfu/g, observed 14 days after production. Therefore, *Carnobacterium* spp was selected as the culture of choice to conduct a challenge study against *Pseudomonas* spp.

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1. Introduction

Ricotta fresca is a traditional whey cheese produced by heat coagulation of sheep's milk whey. In Sardinia (Italy) it is generally manufactured from the whey remaining after the production of hard semi-cooked cheeses (Pecorino Romano PDO and Pecorino Sardo PDO). The industrial production follows the traditional batch production process (Pala et al., 2016). *Ricotta fresca* intended for large-scale retail are commercialized in modified atmosphere packaging (MAP), under refrigeration temperature, with a shelf life, determined under the responsibility of the Food Business Operator, varying from 14 up to 21 days. The batch production process exposes *Ricotta fresca* to post-process contamination originating from the dairy plant environment (Greenwood et al., 1991). Due to its naturally poor competitive microflora (Pintado et al., 2001), to its composition, inherent physical and chemical properties and the absence of preservatives, *Ricotta fresca* is an excellent substrate for the growth of pathogens or spoilage psychotropic bacteria during refrigerated storage. Psychotropic microorganisms in refrigerated whey cheeses are mainly represented by *Pseudomonas* spp, yeasts, molds and *Enterobacteriaceae* (Pintado et al., 2001; De Santis and Mazzette, 2002; Pala et al., 2016). The improvement of the hygiene management procedures is a measure that could only reduce the level of initial contamination of ricotta surface. Therefore, the use of bio preservatives (i.e. nisin, other bacteriocins, fermentates or bioprotective cultures), alone or combined with other treatments, has been proposed to compete with contaminants and to preserve the quality and safety of dairy products and other foods (Sobrino-López and Martín-Belloso, 2008; Elsser-Gravesen and Elsser-Gravesen, 2013). Shelf life extension of whey cheeses using bio preservatives have been previously tested against *Listeria monocytogenes* (Davies et al., 1997; Samelis et al., 2003; Martins et al., 2010). However, to date no available studies investigated the use of biopreservatives against psychotropic spoilage microorganism in sheep ricotta cheese. The present study was conducted as a preliminary investigation to assess the potential use of

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biopreservatives to control the growth of spoilage microorganism on the surface of MAP *ricotta fresca* during refrigerated storage. The main objective of the present study was to select which commercial biopreservative, among those available on the market, presents the best adaptation to *ricotta fresca* substrate and is able to control the growth of psychotropic microorganisms. The biopreservative of choice will be used for a successive challenge study against *Pseudomonas* spp.

2. Materials and methods

2.1. Biopreservatives

The protective cultures and the fermentate were selected, among available products on the market, based on the proven activity against spoilage and pathogen microorganisms, their ability to grow at refrigeration temperature and the low development of acidity and aroma in the product. Of the two commercial protective cultures tested, one was Lyofast FPR 2 (Clerici-Sacco Group, Como, Italy) consisting of bacteriocins producing *Enterococcus faecium*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* in the ratio 1:1:1 with an optimum growth temperature of 37 °C (range between 4 °C and 48 °C). The second was Lyofast CNBAL (Clerici-Sacco Group, Como, Italy) consisting of a selected strain of *Carnobacterium spp* producing bacteriocins with an optimum growth temperature between 25 and 45 °C. Both are lyophilized protective cultures indicated for surface treatment of cheeses to inhibit unwanted bacteria, including also *Listeria* spp. The fermentate, the MicroGARD 430 (Danisco, New Century, KS, USA), a microbial fermentation complex obtained through the fermentation of milk by *Propionibacterium shermanii*, is a cultured grade A non-fat dry milk powder. The antimicrobial activity derives from the production of diacetyl, lactic, acetic and propionic acids produced during fermentation (von Staszewski and Jagus, 2008). This type of fermentate has been successfully used to control spoilage and pathogenic microorganisms in cottage cheese and other dairy products (Al-Zoreky et al., 1991).

2.2. Samples

144 Ricotta *fresca* cheese samples were obtained from a local industrial sheep cheese making plant. Ricotta *fresca* were truncated cone shaped (ca.7.5 cm wide at the top and ca. 5.5 cm wide at the bottom) weighing approximately 1.1 kg. 48 ricotta *fresca* samples were randomly selected from each of three different batches (each batch was manufactured in a different day of production). The day after production ricotta fresca samples were packed in rigid polypropylene trays sealed with high-barrier peelable laminated films. Films were made of bioriented polyamide and cast polypropylene with O_2 T.R. (20°- 65% R.H.) of ~30 cc/m2, 24 h. Samples were transported refrigerated to the laboratory. Culture one samples (FRP) were ricotta *fresca* treated with Lyofast FPR 2, culture two samples (CNBAL) were ricotta *fresca* treated with Lyofast CNBAL and Fermentate samples (FERM) were ricotta *fresca* treated with MicroGARD 430. Blank samples (BS) were untreated ricotta *fresca*. According to manufacture's instruction protective cultures were individually rehydrated by dilution in distilled water immediately before their use to a final concentration of 10^6 cfu mL^{-1} (confirmed by count on agar plates) while the fermentate was resuspended in distilled water in order of 0.5–1% of the samples weight. The surface area of Ricotta *fresca* samples to treat was estimated in ca. 292 cm^2, corresponding to ca. 30 g. After the removal of the film 2.5 mL of Lyofast FPR 2 and Lyofast CNBAL were sprayed respectively on the surface of FPR and CNBAL samples and 4 mL of MicroGARD 430 final suspension distributed on the surface of FERM samples. Each inoculum was evenly sprayed on the upper exposed surface of *Ricotta fresca cheese* samples, and repacked in MAP (30% CO_2 and 70% N_2) using the FP Basic Sec tray sealer (Ilpra, Vigevano, Italy). The experimental design describing sample units, testing times and related analysis is summarized in Table 1.

2.3. Microbiological profile intrinsic properties and composition analysis

For each batch, triplicate samples of ricotta *fresca* were analyzed for the determination of microbiological profile, intrinsic properties and composition 5 h (T_0), 7, 14 and 21 days (T_7, T_{14}, T_{21}) after the addition of the biopreservatives. The preparation of the initial suspension and decimal dilution for microbiological examination was conducted according to ISO 6887–1:1999. Briefly, 25 g of samples were aseptically collected from ricotta surface and weighted into a sterile plastic stomacher bag. After the addition of 225 mL of Buffered Peptone Water were homogenized using a stomacher. Transfer of 1 mL of the initial suspension into a tube containing 9 mL of sterile diluent was performed to obtain the 10^{-2} dilution. If required, these operations were repeated using the 10^{-2} to obtain further serial decimal dilution. For the enumeration of aerobic mesophilic bacteria, mesophilic lactic acid bacteria and *Enterobacteriaceae*, yeasts and moulds the pour-plating procedure was used. Briefly, 1 mL of each decimal dilution was aseptically transferred into sterile Petri dishes. Then, 12–15 ml of the appropriate medium were poured at 44–47 °C into each Petri dish. After complete solidification of Plate Count Agar and MRS medium at pH 5.7 (Biolife, Milan, Italy), respectively for the enumeration of aerobic mesophilic bacteria (ISO 4833:2003) and mesophilic lactic acid bacteria (ISO 15214: 1998), plates were incubated at 30 °C ± 1 °C for 72 h ± 3 h. For the enumeration of *Enterobacteriaceae* the plate count technique without resuscitation was used, incubating Violet Red Bile Agar plates (Biolife) at 35–37 °C for 24 h (ISO 21528–1:2004). For the enumeration of yeast and molds (ISO 6611/ IDF094:2004) Chloramphenicol Yeast Glucose Agar plates (Biolife) were incubated at 25 ± 1 °C and the colonies counted on each plate after 3, 4 and 5 days of incubation. For the enumeration of *Pseudomonas* spp (ISO/TS 11059:2009), 0.1 mL of each decimal dilution were spread over the surface of Pseudomonas Agar Plates added with PP supplement (Biolife) and incubated at 25 °C ± 1 °C for 48 h ± 2 h. The detection of *Listeria monocytogenes* was conducted with the two steps enrichment (ISO 11290-1: 1996). The initial suspension was prepared in Fraser Broth Half Concentration (Biolife), after incubation at 30 °C for 24 h (pre-enrichment) 0.1 mL were subcultured into 10 mL of Fraser Broth and incubated at 37 °C for 24–48 h (enrichment). From both, pre-enrichment and enrichment broth, 0.1 mL were streaked onto Agar Listeria Ottaviani Agosti (ALOA, Biolife) and Oxford (Oxoid, Basingstoke, UK) agar plates and incubated at 37 °C for up to 48 ± 3 h. Enumeration was a conducted according to ISO 11290–2:1998, streaking 1 mL volume of the initial dilution both onto 3 ALOA and 3 Oxford agar plates and incubated at 37 °C for up to 48 ± 3 h. Samples inoculated with Lyofast CNBAL at T_0 were also analyzed for the enumeration of *Carnobacterium spp* using MRS modified by increasing the pH to 8.5, omitting acetate, and substituting glucose for sucrose (Hammes et al., 1992).

2.4. Intrinsic properties, composition and headspace gas analysis

PH and a_w were measured using pH meter GLP22 (Crison Instruments SA, Barcelona, Spain) and water activity meter Aqualab 4TE (Decagon, Pullman, WA, USA) respectively. Fat, moisture, protein and total solids were analyzed by using the compositional FoodScan™ device (FOSS, Analytic, Hillerød, Denmark), which uses

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