J. Dairy Sci. 97:5345–5355
http://dx.doi.org/10.3168/jds.2014-8022

Biopreservation of Fior di Latte cheese

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ABSTRACT

In this study a new biopreservation system consisting of an active sodium alginate coating containing *Lactobacillus reuteri* applied to Fior di Latte cheese was studied. The final aim was to extend cheese shelf life by the in situ production of reuterin. Experimental trials were carried out with and without glycerol. How the fermentation time could improve the production of reuterin, enabling Fior di Latte shelf life, was also assessed. To this aim, the experimental analyses were conducted in 2 different trials, using 2 different production batches of samples. In the first one, Fior di Latte samples were dipped into the active sodium alginate solution prepared on the same day of their production, whereas in the second trial, samples were dipped into the active solution prepared 48 h before their production to allow a proper fermentation of the inoculated microorganism. Microbiological and sensory quality indices were monitored to prove the effectiveness of biopreservation on product quality during storage. In the first trial, the combination of the probiotic microorganism with glycerol improved the microbial quality by 1 d compared with the same active solution without glycerol, whereas the 48-h-fermented active alginate solution (second trial) showed a further improved microbial quality. The application of an active coating enriched with *L. reuteri* and glycerol to Fior di Latte cheese is an optimal and innovative way to preserve the product and at the same time, with a combination of an optimal fermentation time, to prolong its microbial quality and thus its shelf life.
Key words: biopreservation, Fior di Latte

INTRODUCTION

Fior di Latte cheese is a typical Mediterranean fresh pasta filata product, made from cow milk and usually packaged in brine. It has high moisture content (from 55 to 64%) and high fat content (>45% fat in DM; Salvadori del Prato, 2001), so it is very susceptible to microbial spoilage, especially under temperature abuse. Although milk is submitted to a further heat treatment during curd stretching to produce Fior di Latte, post-processing contamination may occur, causing cheese spoilage and eventually safety risks to consumers (Spano et al., 2003). Undesirable microorganisms such as pseudomonads, coliforms, yeasts, and molds may cause defects in flavor, texture, and appearance and result in economic losses (Gammariello et al., 2008; Conte et al., 2009; Del Nobile et al., 2009). Fior di Latte shelf life also depends on the quality of raw material and on the process conditions (Brody, 2001).

Current technologies for preservation and shelf-life extension of food include heat processing, chemical preservatives, modified-atmosphere packaging, or refrigeration. Unfortunately, these strategies do not fully control spoilage bacteria. The great availability of nutrients in foods may enable bacteria to repair damaged cells (Gill et al., 2002). Both the intrinsic (fat, protein, water content, antioxidants, pH, salt, and other additives) and the extrinsic properties (temperature; packaging in vacuum, gas, or air; characteristics of microorganisms) of the food can influence bacterial sensitivity to natural and chemical preservatives (Shelef, 1983; Tassau et al., 2000). At present, Fior di Latte cheese shelf life is approximately 5 to 7 d, and many efforts are in progress to prolong this shelf life by means of process innovation and quality improvement of raw materials. Good opportunities came from the use of antimicrobial compounds during milk transformation (Del Nobile et al., 2009). The high consumer attention to food-safety aspects justifies increased research interest in using active agents derived from natural sources, as plant essential oils or plant extracts, considered suitable for food application, able to reduce the microbial count and to control the cell growth during the different steps of the product life (Conte et al., 2007; Gammariello et al., 2008b, 2010). Efforts to prolong the shelf life of Fior di Latte cheese are also made by the optimization of storage and packaging conditions (Conte et al., 2009; Del Nobile et al., 2009, 2010). The potential of modified-atmosphere packaging and active packaging to extend the shelf life of different dairy products has been proposed by various authors (Floros et al., 2000; Pantaleao et al., 2007; Papaioannou et al., 2007).

Received February 6, 2014.
Accepted April 27, 2014.
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The great demand for fresh-like products still promotes the search for new technologies to preserve food. One of the most recent potential approaches to prolong the shelf life of fresh products is the use of biopreservation systems. Biopreservation is the use of natural or controlled microbiota or antimicrobials as a way of preserving food and extending its shelf life (Ananouet al., 2007). Beneficial bacteria or their fermentation products are used in biopreservation to control spoilage and render pathogens inactive in food (Yousef and Carlstrom, 2003). Lactic acid bacteria (**LAB**) have antagonistic properties that make them particularly useful as biopreservatives. When LAB compete for nutrients, their metabolites often include active antimicrobials such as lactic and acetic acid, hydrogen peroxide, and peptide bacteriocins. Biopreservative bacteria must be harmless to humans. Lactic acid bacteria bacteriocins are used as an integral part of hurdle technology. Using them in combination with other preservative techniques can effectively control spoilage bacteria and other pathogens and can inhibit the activities of a wide spectrum of organisms, including inherently resistant gram-negative bacteria. Angiolillo et al. (2013) stated that the addition of *Lactobacillus rhamnosus* in an edible sodium alginate coating applied on the surface of Fior di Latte cheese exerted an antimicrobial activity against *Pseudomonas* spp. and *Enterobacteriaceae.*

Lactobacillus reuteri is a heterofermentative lactobacillus recognized as a normal inhabitant of the human and animal gut (Reuter, 2001). It is also frequently found in fermented and probiotic foods (Vollenweider and Lacroix, 2004). *Lactobacillus reuteri* as a food supplement is accepted and widely used to improve gastrointestinal health and has been granted qualified presumption of safety by the European Food Safety Authority. Probiotic effects of *L. reuteri* have been proposed due to the ability of some strains to produce reuterin (3-hydroxypropionaldehyde) during anaerobic metabolism of glycerol (Rodríguez et al., 2003). Reuterin is an antimicrobial compound soluble in water, resistant to heat, and stable over a wide range of pH values, that inactivates gram-negative and gram-positive bacteria (Vollenweider et al., 2003). Direct addition of reuterin to control food-borne pathogens such *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* has been investigated in milk and dairy products (Arqués et al., 2008a,b), but the gap of these studies consisted of the fact that reuterin was used as a food additive. In this study, we tried to overcome this limit by developing a new biopreservation system consisting of a sodium alginate coating containing *L. reuteri* in combination with glycerol (registered in the European Union as food additive E 422), applied on the surface of Fior di Latte cheese to extend its shelf life by means of in situ production of reuterin. Experimental trials were carried out with and without glycerol. We also studied how the fermentation time could improve the production of reuterin, enabling Fior di Latte shelf life. Microbiological and sensory quality indices were monitored to prove the effectiveness of biopreservation on product quality during storage.

MATERIALS AND METHODS

Sample Preparation

Fior di Latte samples were purchased from a local cheese factory Capurso Azienda Casearia SPA (Gioia del Colle, Bari, Italy) and transported to the laboratory in polystyrene boxes containing ice. The cheese was made from pasteurized cow milk by adding lactic acid bacteria as starters. The curd was obtained after the coagulation of milk by rennet and after a curd-ripening phase (4.0 to 4.5 h at 35 to 37°C); when the optimal pH (4.9 to 5.4) was reached, the drained curd was stretched in hot water (90 to 95°C).

Once transported to the laboratory, the samples were dipped into 3 different sodium alginate solutions. The first one was prepared by dissolving sodium alginate acid (Farmalabor, Canosa di Puglia, Italy; 2% wt/vol) in distilled water; the second one was prepared by dissolving sodium alginate acid (2% wt/vol) in a solution made of 2% (wt/vol) of pure freeze-dried *L. reuteri* (Granarolo, Bologna, Italy) and distilled water; and the third solution was made of 2% (wt/vol) of pure freeze-dried *L. reuteri* (Granarolo), 0.6% of glycerol (Sigma–Aldrich, Milan, Italy), and distilled water. The coated samples were immersed in a 5% (wt/vol) calcium chloride ($CaCl_2$; Sigma–Aldrich) for 1 min, to allowed creating a stable coating on the cheese surface. All samples were dried at room temperature for 2 min and packaged in commercially available polypropylene bags with brine (0.2% wt/vol of NaCl solution). The control samples consisted of Fior di Latte cheese without coating, packaged in trays with brine. All the samples were stored at 9°C. The experimental analyses were conducted in 2 different trials, using 2 different production batches of samples. In the first trial, Fior di Latte samples were dipped into the sodium alginate solutions prepared on the same day of their production, whereas in the second trial, samples were dipped in sodium alginate solutions prepared 48 h before their production. Sodium alginate solutions used in the second trial were prepared in bottles with hermetic seals to avoid evaporation. Immediately after their preparation, sodium alginate solutions were incubated at 25°C for 48 h. Samples will be named as follows: **CNT** (control sample consisting of Fior di Latte cheese without coat-