Available online at www.sciencedirect.com
ScienceDirect
International Dairy Journal 17 (2007) 525–534
INTERNATIONAL DAIRY JOURNAL
www.elsevier.com/locate/idairyj

Contribution of autochthonous non-starter lactobacilli to proteolysis in Caciocavallo Pugliese cheese

M. Morea[a,*], A. Matarante[a], R. Di Cagno[b], F. Baruzzi[a], F. Minervini[b]

[a]*Istituto di Scienze delle Produzioni Alimentari, CNR, Via Amendola 122/o, 70126 Bari, Italy*
[b]*Dipartimento di Protezione delle Piante e Microbiologia Applicata, Facoltà di Agraria, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy*

Received 23 August 2005; accepted 19 May 2006

Abstract

Caciocavallo Pugliese cheese was manufactured according to a traditional protocol and with added autochthonous *Lactobacillus paracasei* subsp. *paracasei* and *L. parabuchneri* strains, isolated from a well-flavoured Caciocavallo Pugliese cheese, to evaluate their contribution to the cheese biochemical characteristics. Using a "two step REP-PCR" protocol, *L. paracasei* subsp. *paracasei* strains were shown to sustain high viability during ripening, while the *L. parabuchneri* strains were not recovered. The inoculated cheese showed higher levels of free amino acids, as well as differences in the profiles of individual free amino acids in comparison with the control cheese. The addition of autochthonous *Lactobacillus* strains with interesting technological properties in Caciocavallo Pugliese cheese manufacturing could make it feasible to improve cheese processing, while still maintaining the sensory characteristics of this typical pasta-filata cheese.

Keywords: Cheese ripening; *Lactobacillus*; Adjunct strains; Natural microflora; Peptidase activity; REP-PCR

1. Introduction

Caciocavallo, one of the most typical pasta-filata cheeses obtained from natural microflora, is manufactured in three different areas—the Balkans, Russia, and Italy. Various types of Caciocavallo are produced in the south of Italy under names such as Caciocavallo Silano, Molisano, Pugliese, and Corleonese. Caciocavallo Silano, made from cows' milk, is the most important Italian Caciocavallo variety with protected denomination of origin (DOP). Most Caciocavallo cheeses produced in Italy use a natural whey starter, corresponding to a backslopping from a previously successful cheese batch.

Natural whey starters are preferred as they contribute to the typical flavour and aroma of the final cheese, qualities which are attributed to the complex microflora, and to the resistance to phage attack due to the multi-strain culture. For an exhaustive review on this subject, refer to Parente and Cogan (2004).

In recent years, autochthonous cultures have also been used in Tetilla cheese (Menéndez, Godìnez, Hermida, Centeno, & Rodríguez-Otero, 2004), Proosdij-type cheese (Ayad, Verheul, Bruinenberg, Wouters, & Smit, 2003), Reggianito Argentino cheese (Candioti et al., 2002), Turkish White Pickled Cheese (Karakus & Alperden, 1995), Cheddar cheese (Lynch, McSweeney, Fox, Cogan, & Drinan, 1996) and New Zealand Cheddar cheese (Crow, Curry, & Hayes, 2001) in order to study their influence on the ripening process and improve their sensory characteristics.

In a previous paper (Gobbetti et al., 2002), mesophilic lactobacilli such as *Lactobacillus paracasei* subsp. *paracasei* and *L. parabuchneri* were found to make up much of the lactic acid microflora in Caciocavallo Pugliese, after 42 and 60 days of ripening, respectively. Non-starter lactic acid bacteria (NSLAB) have been shown to contribute to flavour development in some varieties of cheeses and could

*Corresponding author. Tel.: +39 080 5929325; fax: +39 080 5929374.
E-mail address: maria.morea@ispa.cnr.it (M. Morea).

0958-6946/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.idairyj.2006.05.010

therefore be considered as a desirable contaminant of either the milk supply or the subsequent cheese (El Soda, Madkor, & Tong, 2000).

To the best of our knowledge, no studies have been conducted on the contribution of autochthonous non-starter lactobacilli to the biochemical properties of different types of Caciocavallo cheese.

In the last few years, various molecular typing methods such as restriction fragment length polymorphism (RFLP), pulsed-field gel electrophoresis, ribotyping, amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD) and other PCR-derived techniques have been used to distinguish the strains present in complex microflora isolated from many sources. Unlike RAPD protocols, in which the patterns are obtained by using a short non-specific primer, repetitive element sequence-based PCR (REP-PCR) protocols are based on the amplification of short repetitive sequence elements, dispersed throughout the chromosome of diverse Gram-negative species (Versalovic, Koeuth, & Lupski, 1991).

In this work, several bacterial strains previously isolated from Caciocavallo Pugliese cheese (Gobbetti et al., 2002) were characterized for their peptidase activities. Four of them, belonging to *L. paracasei* subsp. *paracasei* and *L. parabuchneri* species, were added to natural whey and their growth kinetics in cheese were followed by using a new typing protocol named "two step REP-PCR". The contribution of autochthonous NSLAB added to the microflora present in natural whey to proteolysis of cheese was evaluated in order to standardize Caciocavallo Pugliese cheese processing, safeguarding the peculiar traits of this typical southern Italian dairy product.

2. Materials and methods

2.1. Bacterial strains and peptidase activities

The lactic acid bacterial strains belonging to *L. delbrueckii* subsp. *bulgaricus* (strain B15Z), *L. helveticus* (B26W), *L. gasseri* (B24W), *L. paracasei* subsp. *paracasei* (B44f3t and B25f3), *L. parabuchneri* (B10f5, B23f3, B14f3, B48f3, B51f5, B9f5) and *Pediococcus pentosaceus* (C9f5), used in this study, had been isolated previously from Caciocavallo Pugliese cheese (Gobbetti et al., 2002). All strains were routinely cultured in MRS broth (Difco Laboratories, Detroit, MI, USA) at 37 °C, frozen at −80 °C and stored in the Institute of Sciences of Food Production (ISPA, Bari, Italy) bacterial collection.

Peptidase activity was determined on cells from 12-h-old cultures collected by centrifugation ($8200 \times g$, 5 min), washed twice with 50 mM phosphate buffer pH 7.0 and suspended in the same buffer to give a ten-fold concentrated cellular suspension. Synthetic substrates (Ala-*p*NA, Leu-*p*NA, Lys-*p*NA and Pro-*p*NA; Sigma Chemical Co., St. Louis, MO, USA) were dissolved (20 mM) in absolute ethanol and used for in vitro assaying the aminopeptidase and iminopeptidase activities of non-starter lactic acid bacteria (Gobbetti, Corsetti, Smacchi, De Angelis, & Rossi, 1997). Twenty microlitres of each substrate were incubated with 100 μL of cellular suspension, and 80 μL of phosphate buffer were added to the reaction mixture. Samples were incubated at 37 °C for at least 30 min, and the reaction was stopped by adding 600 μL acetic acid (10% v/v). Cells were removed by centrifugation ($8200 \times g$, 5 min) and absorbance of supernatants was determined at $\lambda = 410$ nm (Ultrospec 3000, Amersham BioSciences, Uppsala, Sweden). A unit of enzymatic activity was defined as the amount of enzyme that produced 1 μmol of para-nitroaniline per min at 37 °C and pH 7.0.

2.2. Caciocavallo Pugliese cheese manufacture

Laboratory-scale Caciocavallo cheese-making trials (control trial C and inoculated trial I) were performed according to standard procedures as follows: goatling rennet (40.0 mg kg^{-1}) was added to 300 L of whole pasteurized bovine milk inoculated with 3% (v/v) natural whey culture (pH 3.80). In control trial C, the cheese was processed traditionally, with milk and curd fermentation being carried out by the microflora present in non-selected whey culture, whereas in trial I, for inoculated cheese, known amounts of four non-starter lactobacilli were added as adjuncts to the natural whey inoculum. After about 30 min at 37–38 °C, the coagulum was cut coarsely and heated under whey at 45 °C for 2 h and then held at room temperature.

When the curd reached a pH value of about 5.30, it was manually stretched in hot water (70–80 °C) producing cheeses weighing about 2 kg. Cheeses were dipped in brine (27–30% NaCl) for 18 h and then ripened at 10–12 °C and 75–80% relative humidity for 2 months.

For trial I, two strains of *L. paracasei* subsp. *paracasei* (B44f3t and B25f3) and two strains of *L. parabuchneri* (B51f5 and B23f3) were subsequently grown for 24 h at 37 °C in 10, 100, 1000 and 10 L of MRS broth. The final 10-L growths were centrifuged ($2460 \times g$ for 20 min), merged in 1 L of 0.05 M Tris–HCl (pH 7.5) containing 0.1 M $CaCl_2$ buffer and then used as starter adjuncts in otherwise traditional cheese-making, thus obtaining trial I. Each strain was added to obtain a final concentration of about log 7.3 cfu mL^{-1}.

Cheeses from two batches of each trial, C and I, were manufactured at different times using completely independent batches of milk on the same day and were analysed in this study.

2.3. Cheese compositional analysis

Samples of cheeses were analysed for protein (macro-Kjeldahl; IDF, 1964), fat (Gerber method; IIRS, 1955), moisture (oven drying at 102 °C; IDF, 1982) and salt (Fox, 1963) content. All determinations were carried out on three different sections of each cheese and values were averaged.