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ScienceDirect
International Dairy Journal 17 (2007) 67–78
INTERNATIONAL DAIRY JOURNAL
www.elsevier.com/locate/idairyj

Proteolytic pattern and organic acid profiles of probiotic Cheddar cheese as influenced by probiotic strains of *Lactobacillus acidophilus*, *Lb. paracasei*, *Lb. casei* or *Bifidobacterium* sp.

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Received 13 April 2005; accepted 29 December 2005

Abstract

Cheddar cheeses were produced with starter lactococci and *Bifidobacterium longum* 1941, *B. lactis* LAFTI® B94, *Lactobacillus casei* 279, *Lb. paracasei* LAFTI® L26, *Lb. acidophilus* 4962 or *Lb. acidophilus* LAFTI® L10 to study the survival of the probiotic bacteria and the influence of these organisms on proteolytic patterns and production of organic acid during ripening period of 6 months at 4 °C. All probiotic adjuncts survived the manufacturing process of Cheddar cheese at high levels without alteration to the cheese-making process. After 6 months of ripening, cheeses maintained the level of probiotic organisms at $>8.0\log_{10}\text{cfu g}^{-1}$ with minimal effect on moisture, fat, protein and salt content. Acetic acid concentration was higher in cheeses with *B. longum* 1941, *B. lactis* LAFTI® B94, *Lb. casei* 279 and *Lb. paracasei* LAFTI® L26. Each probiotic organism influenced the proteolytic pattern of Cheddar cheese in different ways. *Lb. casei* 279 and *Lb. paracasei* LAFTI® L26 showed higher hydrolysis of casein. Higher concentrations of free amino acids (FAAs) were found in all probiotic cheeses. Although *Bifidobacterium* sp. was found to be weakly proteolytic, cheeses with the addition of those strains had highest concentration of FAAs. These data thus suggested that *Lb. acidophilus* 4962, *Lb. casei* 279, *B. longum* 1941, *Lb. acidophilus* LAFTI® L10, *Lb. paracasei* LAFTI® L26 and *B. lactis* LAFTI® B94 can be applied successfully in Cheddar cheese.
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Keywords: Cheddar cheese; Probiotic; Proteolysis; Ripening

1. Introduction

The application of probiotic bacteria in food products is increasing due to potential health benefits associated with the consumption of these bacteria. A number of health benefits for products containing live probiotic bacteria have been claimed including alleviation of symptoms of lactose intolerance, treatment of diarrhea, anticarcinogenic properties, reduction in blood cholesterol and improvement in immunity (Ballongue, 1993; Shah, 2000a, b; Shah & Wu, 1999). For dietary organisms to be beneficial in food systems, they should maintain viability in the food until the time of consumption and be present in significant numbers, at levels of at least 10^7 viable cells per gram or milliliter of a product (Ishibashi & Shimamura, 1993). For this reason, changes in the numbers of viable bacteria during storage period should be known.

Yoghurt and fermented milk have received most attention as carriers of probiotic bacteria, but foods such as Cheddar cheese (Dinakar & Mistry, 1994; Gardiner, Ross, Collins, Fitzgerald, & Stanton, 1998), Gouda cheese (Gomes, Malcata, & Klaver, 1995), cottage cheese (Blanchette, Roy, Belanger, & Gauthier, 1996), Crescenza cheese (Gobbetti, Corsetti, Smacchi, Zocchetti, & DeAngelis,

Abbreviations: CN, casein; FAA, free amino acids; PTA, phosphotungstic acid; SN, soluble nitrogen; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; TN, total nitrogen; TCA, trichloroacetic acid; WSN, water-soluble nitrogen

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doi:10.1016/j.idairyj.2005.12.009

1997), frozen yoghurts (Laroia & Martin, 1991) and ice-cream (Hekmat & McMahon, 1992) have also been studied as carriers of probiotic microorganisms.

Cheeses have a number of advantages over fresh fermented products such as yoghurt as a delivery system for viable probiotic to gastrointestinal tract as they tend to have higher pH, more solid consistency and relatively higher fat content. These offer protection to probiotic bacteria during storage and passage through the gastro-intestinal tract. Cheeses also have higher buffering capacity than yoghurt (Stanton et al., 1998). Cheddar cheeses, however, have long ripening time hence development of probiotic Cheddar cheese requires a careful examination of the suitability of particular strain(s) to maintain viability throughout the ripening and shelf life (Ross, Fitzgerald, Collins, & Stanton, 2002).

A series of chemical and biochemical reactions occur during Cheddar cheese ripening including glycolysis, lipolysis and most importantly proteolysis (Fox, 1993). Proteolysis plays a critical role in determining the typical sensory characteristics and represents a significant indicator of quality, as shown for Cheddar cheese (Fox & McSweeney, 1996). Proteolysis is caused by enzymes contained in milk (plasmin) and rennet (pepsin and chymosin) or released by microorganisms. The activities of these enzymes reduce the concentration of casein (CN) (α_{s1}, α_{s2}, β and κ-CN) and lead to the formation of large and intermediate size peptides. These peptides may be further hydrolysed by proteolytic enzymes, originating from the microflora (starter bacteria, non-starter lactic acid bacteria and probiotic adjunct) of the cheese, into small peptides and free amino acids (FAA), which are important for the development of Cheddar flavour (Cliffe, Marks, & Mulholland, 1993; Lynch, Muir, Banks, McSweeney, & Fox, 1999).

Six probiotic strains (*Lactobacillus acidophilus* 4962, *Lb. casei* 279, *B. longum* 1941, *Lb. acidophilus* LAFTI® L10, *Lb. paracasei* LAFTI® L26 and *B. lactis* LAFTI® B94) were examined in this study as a potential candidate for incorporation in Cheddar cheeses. These strains have been selected based on their acid and bile tolerance, adhesion to intestinal cell line, anticarcinogenic properties, oxygen sensitivity and ability to modify gut microflora of human subjects (Crittenden et al., 2001; Lankaputhra & Shah, 1998; McIntosh, Royle, & Playne, 1999). Previously these strains were used together as a mix culture into Cheddar cheese and all six probiotics were able to maintain their viability at high level $>7.5\log_{10}\text{cfu g}^{-1}$ at the end of 6 months ripening at 4 °C (Ong, Henriksson, & Shah, 2006). Although there was no direct influence of addition of probiotic organisms on the composition (protein, fat, moisture and salt content) of Cheddar cheese, acetic acid concentration was found to be higher in probiotic cheeses and proteolytic pattern of probiotic cheeses was also significantly different ($P<0.05$) when compared with the control cheese. The probiotic strain that was responsible for the differences in proteolytic pattern and organic acid profile, however, was not identified. The influence of these individual probiotic organisms to the changes in quality of Cheddar cheese has not yet been fully elucidated. The objective of the study was to investigate the performance of individual probiotic strains of *Lb. acidophilus*, *Lb. casei, Lb. paracasei* and *Bifidobacterium* sp. in Cheddar cheeses in terms of their ability to survive during ripening, their influence on the proteolytic pattern and the production of organic acid.

2. Materials and methods

2.1. Starter and probiotic organisms

Cheese starter culture, *Lactococcus lactis* subsp. *lactis* strain 227 and *Lc. lactis* subsp. *cremoris* strains 223 were used in freeze-dried form. The strains were activated by growing at least two times at 30 °C overnight in 12% (w/v) sterile reconstituted skim milk (RSM) containing 2% (w/v) glucose and 1.2% (w/v) yeast extract, prior to inoculation (2%, v/v) of the bulk culture in the same media.

The probiotic strains, *Lb. acidophilus* 4962, *Lb. casei* 279 and *B. longum* 1941 were obtained from the Victoria University Culture Collection (Werribee, Australia), while *Lb. acidophilus* LAFTI® L10, *Lb. paracasei* LAFTI® L26 and *B. lactis* LAFTI® B94 were obtained from DSM Food Specialties Pty. Ltd. (NSW, Australia). *B. longum* 1941 (*B. longum* CSCC 1941) was originally obtained from the Commonwealth Scientific and Industrial Research Organization (CSIRO; Highett, Victoria, Australia) while *Lb. acidophilus* 4962 and *Lb. casei* 279 were both originally obtained from the Australian Starter Culture Collection Center (ASCC; Werribee, Australia). All *Lactobacillus* strains were subcultured (1%, v/v) at least two times at 37 °C overnight in 12% (w/v) sterile RSM prior to use as a bulk culture (2%, v/v). Both *Bifidobacterium* sp. were subcultured similarly using 1% inoculum in sterile RSM supplemented with 0.05% L-cysteine · hydrochloride (Sigma-Aldrich, St. Louis, MO, USA).

2.2. Cheddar cheese manufacture

Cheddar cheeses were made with 10 L pasteurized milk and 1.5% (v/v) inoculum of the mixed strain starter culture using a pair of custom made cheese vats. Seven batches of Cheddar cheeses were made including a control and six different probiotic cheeses as shown in Table 1. The complete set (7 batches) was produced randomly in 4 days with the same batch of pasteurised milk and at least 2 replications were produced the following weeks.

Cheeses were manufactured according to the standard procedures of Kosikowski (1977) as described previously by Ong et al. (2006). All cheeses were packed in oxygen barrier Cryovac® bags (Cryovac® Pty. Ltd., Fawkner, Vic, Australia) and heat-sealed with a Multivac® vacuum packaging equipment (Multivac Sepp Haggenmüller, Wolfertschwenden, Germany) and ripened at 4 °C for 6 months.

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