Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Dairy Journal 16 (2006) 324–334
INTERNATIONAL DAIRY JOURNAL
www.elsevier.com/locate/idairyj

Lysis of starters in UF cheeses: Behaviour of mesophilic lactococci and thermophilic lactobacilli

J.A. Hannon[a,*], S.-M. Deutsch[a], M.-N. Madec[a], J.-Y. Gassi[a], M.-P. Chapot-Chartier[b], S. Lortal[a]

[a]*UMR 1253 Science et Technologie du Lait et de l'Oeuf, INRA-Agrocampus-65, rue de Saint-Brieuc, 35042 Rennes cedex, France*
[b]*INRA, Unité de Biochimie & Structure des Protéines, 78352 Jouy-en-Josas cedex, France*

Received 6 December 2004; accepted 13 April 2005

Abstract

The objective of this work was to study the autolytic behaviour of strains of mesophilic (*Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*) and thermophilic lactic acid bacteria (*Lactobacillus helveticus*, *Lb. delbrueckii* subsp. *lactis* and *Streptococcus thermophilus*) in UF cheese. Cheeses were made from a UF-retentate (milk concentrated by a factor of 6) of microfiltered milk (0.8 μm pore size membrane) using the following starter systems: (1) single inocula of autolytic strains of *L. lactis* (US3, AM2 or AM1), non-autolytic strains of *L. lactis* (AM2-C or CNRZ-144), (2) a co-inocula of strains of *Lb. helveticus* (ITG-LH1, CNRZ-32 or CNRZ-303), *Lb. delbrueckii* subsp. *lactis* (ITG-LL14 or ITG-LL51) with the same strain of *S. thermophilus* CNRZ-1358. Cell viability was monitored over a 28 day ripening period by enumeration on selective media. Degree of lysis was determined by the measurement of the intracellular marker lactate dehydrogenase (LDH) activity, and also by immunodetection of intracellular proteins with species specific antibodies. In UF cheeses, lysis of autolytic strains of *L. lactis* was significantly delayed, showing release of intracellular components after 21 days of ripening. No lysis was observed for non-autolytic *L. lactis* strains or for *S. thermophilus*. Lysis of thermophilic lactobacilli (*Lb. helveticus*, *Lb. delbrueckii*), was observed from the start of ripening, but the onset and the level of lysis observed was strain and species dependent.

Keywords: Lysis; Lactic acid bacteria; UF cheese

1. Introduction

The use of ultrafiltrated (UF) milk for cheesemaking has many advantages: increases in yields of up to 20% from the inclusion of whey proteins, an increase in the nutritive value of the product, a reduced rennet requirement, reduced fat losses in the whey, reduction in cheese weight variations, a simplification of the cheesemaking process, increased plant efficiency, capacity and consequently savings in energy and labour costs (Goudédranche, Maubois, Ducruet, & Mahaut, 1981; Lawrence, 1989; Pedersen & Ottosen, 1992; Saboya, Goudédranche, Maubois, Lerayer, & Lortal, 2001). Ultrafiltration is a membrane separation and concentration process in which macromolecules and solutes of specific shape and molecular weight are retained while low molecular weight constituents pass through the membrane and are separated from the UF milk retentate. The quantity of whey proteins retained depends on the UF technology used as well as on the degree of UF concentration.

Many reports have shown that the ripening of UF cheese, made from five-fold or fully concentrated milk, is retarded in comparison to traditional cheese ripening (Creamer, Iyer & Lelièvre, 1987; de Koning, de Boer, Both, & Nooy, 1981; Harper, Iyer, Knighton, & Lelièvre, 1989; Hickey, van Leeuwen, Hillier, & Jago,

*Corresponding author. Tel.: +33 23485339; fax: +33 23485350.
E-mail address: john.hannon@rennes.inra.fr (J.A. Hannon).

0958-6946/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.idairyj.2005.04.003

1983; Lawrence, 1989). Hypotheses that have been proposed include the presence of extra whey proteins inhibiting the proteolytic activity of rennet (Creamer et al., 1987) and plasmin (Visser, 1981), the inclusion of denatured whey proteins inhibiting plasminogen activity, the presence of undenatured whey proteins that are resistant to proteolysis and an increase in buffering capacity thought to result in the inhibition of starter cell lysis (Goudédranche et al., 1981; Saboya et al., 2001). However hypotheses such as these are controversial (Mistry & Maubois, 2004). In fact, the retarded maturation is probably due to several factors.

Many studies have correlated a positive relationship between autolysis of starter strains and enhanced proteolysis in many types of cheese made using traditional methodology (Chapot-Chartier, Deniel, Rousseau, Vassal, & Gripon, 1994; Crow, Martley, Coolbear, & Roundhill, 1995; Deutsch, Ferain, Delcour, & Lortal, 2002; Hannon et al., 2003; Kawabata et al., 1997; Meyer & Spahni, 1998; Morgan, Ross, & Hill, 1997; O'Donovan, Wilkinson, Guinee, & Fox, 1996; Valence, Richoux, Thierry, Palva, & Lortal, 1998; Wilkinson, Guinee, O'Callaghan, & Fox, 1994). The proteolytic systems of lactococci and lactobacilli are thought to possess similar extracellular proteinases and a range of intracellular peptidases (Sousa, Ardö, & McSweeney, 2001). The activities of these endo- and exopeptidases leads to the production of oligopeptides and free amino acids (which are in turn flavour precursors), improvement of texture due to hydrolysis of the casein matrix, a decrease in bitterness and enhancement of cheese flavour development (Lortal, Lemée, & Valence, 1997). Cell lysis is hence a necessary step to release the cytoplasmic peptidases into the cheese curd and allow direct access to their substrates. According to Law (2001), the rate and extent of starter lysis in young cheese is linked positively to the quality and rate of flavour development in cheese.

Lysis of *Lactococcus lactis* has been shown to be highly strain dependant in Cheddar and St Paulin cheeses (Chapot-Chartier et al., 1994; O'Donovan et al., 1996; Wilkinson et al., 1994). Lysis of *Lactobacillus helveticus* has been demonstrated in Swiss and Cheddar cheeses (Hannon et al., 2003; Madkor, El Soda, & Tong, 1999, 2000; Valence et al., 1998) and also shown to be strain dependant. *Lactobacillus delbrueckii* has been shown to lyse in Swiss cheese (Meyer & Spahni, 1998) and *Streptococcus thermophilus* in Emmental cheese (Deutsch et al., 2002).

Assessment of lysis in the cheese matrix generally employs the use of several techniques simultaneously. Classical enumerations of viability on selective media are commonly used, however this method only demonstrates decreases in viability and not lysis. Cells that are not viable may be metabolically active or perhaps may be dead (i.e., permeabilised with a damaged membrane) but still not lysed (Bunthoff, van Schalkwijk, Meijer, Abee, & Hugenholtz, 2001). The release of intracellular components into the cheese matrix is the principal method to demonstrate lysis. The detection of cytoplasmic enzymes, lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PDH), X-prolyl-dipeptidyl-aminopeptidase (Pep-X) and lysyl-aminopeptidase have been used successfully as lysis markers (Crow et al., 1995; Hannon et al., 2003; Kiernan, Beresford, O'Cuinn, & Jordan, 2000; Wilkinson et al., 1994). The detection of these free enzymes in the cheese by immunological or proteomic analysis of cheese aqueous extracts have unequivocally confirmed lysis (Deutsch et al., 2002; Gagnaire et al., 2004; Saboya et al., 2001; Valence, Deutsch, Richoux, Gagnaire, & Lortal, 2000; Valence et al., 1998). Immunological detection using species specific markers has two main advantages: it does not suffer from activity variations due to enzyme instability and by using species specific antibodies, lysis due to one species in a complex ecosystem can be determined.

Despite the overwhelming evidence that starter lysis enhances ripening of cheese made using traditional methodology and the retarded ripening demonstrated in UF cheeses, very few studies have been dedicated to lysis of starter strains in UF cheese. Saboya et al. (2001) first demonstrated the absence of lactococcal lysis in UF cheeses using a commercial mixture of lactococcal strains (MM 101: *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, and *L. lactis* subsp. *lactis* biovar *diacetylactis*). The authors reported that viable populations of lactococcal strains remained elevated (10^9 cfu g^{-1} cheese) throughout the ripening period and confirmed the absence of lysis until day 27, by immunoblot detection using antibodies anti-lactococcal cytosol, of the aqueous extracts A further study by Saboya et al. (2002) demonstrated the absence of lysis over a 3 week ripening period using *L. lactis* subsp. *cremoris* AM2 as starter in UF-cheese, and compared it with lysis of the same strain in traditionally made cheese. Levels of AM2 were $\sim 10^9$ cfu g^{-1} cheese on day 1 in UF-cheese compared to 10^6 cfu g^{-1} cheese in traditional cheese. Again, the absence of lysis was confirmed in UF-cheese throughout a 21 day ripening period and lysis confirmed from day 1 in traditional cheese by immunoblot detection using antibodies anti-LC cytosol. No information is available, to our knowledge on the autolytic behaviour of other species in UF cheeses.

The objective of this work was to investigate the phenomenon of starter lysis in UF-cheese by studying the lytic behaviour of mesophilic strains of *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis*, thermophilic strains of *Lb. helveticus*, *Lb. delbrueckii* subsp. *lactis*, and *S. thermophilus* in UF cheeses.