J. Dairy Sci. 96:1–4
http://dx.doi.org/10.3168/jds.2012-6225

Short communication: Jenny milk as an inhibitor of late blowing in cheese: A preliminary report

C. Cosentino,[1] R. Paolino, P. Freschi, and A. M. Calluso
School of Agricultural, Forest, Food, and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano 10, Potenza 85100, Italy

ABSTRACT

Late blowing on semihard and hard cheese may have an important economic effect on dairy production. Many studies have attempted to prevent this defect by physical treatment, the use of additives, and the use of bacteriocins. In this paper, we look at the effect of jenny milk as an inhibitor of blowing caused by clostridia and coliforms in ewe cheese making. Bulk ewe and jenny milk samples were collected in the morning by mechanical milking and were refrigerated at 4°C. On the collected samples, the count of somatic cells, coliforms, *Clostridium butyricum*, and *Escherichia coli* were determined. The bulk raw milk was divided in two 45-L vats: vat 1 was used as a control, whereas 0.5 L of jenny milk was added to vat 2. Four semihard cheeses, weighing about 2 kg each, were made from each vat. Cheese making was replicated twice. After a ripening period of 60 d, the count of coliforms and of *C. butyricum* was determined. In the treated group, a significant inhibition of coliform bacteria was observed. The addition of jenny milk in cheese making may prove to be a useful and innovative approach for the inhibition of spore-forming clostridia strains.
Key words: jenny milk, lysozyme, cheese making, late blowing

Short Communication

Jenny milk displays good tolerability, as well as high nutritional and functional properties. These characteristics are due to its protein profile, high lactose content, and peculiar FA composition. Moreover, its whey protein fraction is higher than that of cow's milk: α-lactalbumin and β-lactoglobulin range from 35 to 50% of the total nitrogen fraction in jenny milk (Herrouin et al., 2000; Guo et al., 2007). Jenny milk is characterized by a high lysozyme content; lysozyme is a well-known natural antimicrobial agent that may contribute to the inhibition of bacterial growth (Chiavari et al., 2005; Polidori and Vincenzetti, 2007; Cosentino et al., 2012a,b). Additionally, jenny milk provides an array of defense protein factors, such as lactoperoxidase, lactoferrin, lysozyme, and immunoglobulin, all with the capability to kill or to inhibit a large spectrum of pathogens (Zhang et al., 2008; La Torre et al., 2010; Nazzaro et al., 2010). Lysozyme antibacterial activity is due to its capacity to catalyze the hydrolysis of the β 1–4 glycosidic links between *N*-acetylmuramic acid and *N*-acetylglucosamine in the bacterial cell wall of polysaccharides, working in synergy with lactoferrin and immunoglobulins.

The main external source of microbial contamination in milk has been identified in poorly stored silage with bacterial spores that can withstand the pasteurization process (Vissers et al., 2006, 2007a). The anaerobic lactate-fermenting clostridia in cheese (*Clostridium tyrobutyricum* and *Clostridium butyricum*) can reach more than 110 cfu/g within 4 to 6 wk, producing late blowing characterized by excessive gas and off-flavor production (Hughey and Johnson, 1987; Senyk et al., 1989; Kalač, 2011). Growth of *C. butyricum* in cheese is critically affected by different factors, such as salt concentration, pH, ripening time, and temperature, as well as by the presence of other microorganisms (Garde et al., 2011). Previous studies have attempted to prevent late blowing by means of physical treatments, such as bactofugation or microfiltration before processing, or by the use of additives such as nitrate or lysozyme (Wasserfall and Teuber, 1979; Vissers et al., 2007b; Schneider et al., 2010b). In recent studies, the addition of lactic acid bacteria-producing bacteriocins to strains during cheese manufacture was evaluated as an alternative strategy to prevent this defect. These biologically active peptides display a bactericidal mode of action toward specific gram-positive bacteria (Martìnez-Cuesta et al., 2010). Among several methods of prevention, lysozyme, as a commercial additive, has been preferred since 1983; it is extracted from hen egg white (**HEW**; 3.5% of the egg white proteins). Presently, the application of this preservative (E1105) is legal in the entire European Community, according to European Parliament Directive No. 95/2/EC (*quantum satis* in ripened cheese) (Pellegrino and Tirelli, 2000; Scharfen et al., 2007; Schneider et al., 2011). However, lysozyme HEW is becoming less attractive as a preven-

Received October 3, 2012.
Accepted March 1, 2013.
[1]Corresponding author: carlo.cosentino@unibas.it

tion agent, as some studies have shown its allergenic effect in consumers allergic to egg, due to its content in ovomucoid, ovoalbumin, and conalbumin (Frémont et al., 1997; Pérez-Calderón et al., 2007).

In the last decade, several case studies revealed severe allergic reactions due to the presence of lysozyme E1105 in semihard cheeses. Frémont et al. (1997) noted that 35% of patients allergic to eggs had antilysozyme IgE, and a few of them previously experienced a severe reaction after eating Gruyere cheese. In Sweden, it was reported that 5 out of 21 case studies of allergic reactions to eggs were attributed to the presence of this additive in cheese (Kerkaert et al., 2010). In addition, Pérez-Calderón et al. (2007) demonstrated that this additive is likely responsible for episodes of severe edema. In fact, as indicated in recently changed European Commission legislation, the use of lysozyme as an additive has to be declared on the label (EC legislation in Europe 2003/89/ EC, Directive 2000/13/EC). It is necessary, therefore, to reliably detect and quantify this preservative in cheese (Iaconelli et al., 2008; Kerkaert et al., 2010; Dragoni et al., 2011).

In Italy, the use of lysozyme is widespread (Panari and Filippi, 2009); lysozyme has been detected in Grana Padano, in grated hard cheese mixtures (Cocolin et al., 2004; Iaconelli et al., 2008), and in semihard goat and ewe cheeses (Dragoni et al., 2011; Schneider et al., 2010a, 2011). Even though the current commercial source for lysozyme is from HEW, it is present also in milk, blood serum, tears, and saliva (Callewaert and Michiels, 2010). Some authors observed that lysozyme from mare, jenny, and cow milk does not derive from potentially allergenic sources and could be used as a substitute of HEW lysozyme (Cosentino and Paolino, 2012; Galassi et al., 2012). Donkey lysozyme is of the c-type, which is 129 AA long and exhibits 50% homology to the human protein. The lysozyme content in jenny milk ranges between 1.0 and 3.7 mg/mL, according to the lactation stage and the production season (Chiavari et al., 2005; Zhang et al., 2008; Galassi et al., 2012; Vincenzetti et al., 2012), and it is much higher than in cow (0.13 μg/mL), ewe (0.20 μg/mL), or goat milk (0.25 μg/mL; Fratini et al., 2006; Scharfen et al., 2007; Cosentino and Paolino, 2012). The aim of this study was to investigate the effect of lysozyme from jenny milk on blowing defects in artisanal ewe cheese caused by clostridia and coliforms, usually present in ewe cheese produced in traditional cheese factories.

Milk

Bulk ewe and jenny milk were taken on the same day from 2 semiextensive rearing farms that used mechanical milking, both situated in Basilicata at about 750 m above sea level. Jennies were at the second month postfoaling. After collection, milk aliquots were immediately refrigerated at 4°C and transported to the laboratory for analytical determinations.

Table 1. Parameters of ewe and jenny milk

	Milk	
Parameter	Ewe	Jenny
Protein, %	6.56	1.62
Fat, %	8.40	1.10
Clostridium butyricum, cfu/mL	10	<10
Coliform, cfu/mL	166	170
Escherichia coli, cfu/g	<1	<1
SCS, $\log_{10}$ no./mL	5.73	5.18

On raw ewe and jenny milk we measured protein and fat content by Milkoscan FT 6000 (Foss Electric, Hillerød, Denmark) according to the International Dairy Federation standard (ISO, 2000) and SCS ($\log_{10}$ $n \times$ 1,000/mL) according to ISO (2006a). Moreover, we enumerated *C. butyricum* spores according to ISO (2004), coliforms according to ISO (2006b) and *Escherichia coli* as described in ISO (2001). Lysozyme content in ewe and in jenny milk was determined by HPLC (1100 system, Agilent Technologies) according to Pellegrino and Tirelli (2000).

Cheese

Semihard cheeses, produced from 90 L of ewe milk, were manufactured and seasoned on the dairy farm. Milk was heated at 37°C, and then 0.3 g/L of kid rennet (activity 1:10,000; Caglio Camoscio CSC 95/75, DMS Segrate, Italy) was added. Bulk raw milk was divided into two 45-L vats: vat 1 was used as a control and 0.5 L of jenny milk with a lysozyme content of 0.5 mg/L was added to vat 2. This volume of jenny milk ensures the lysozyme quantity ordinarily used in the making of some Italian cheeses (e.g., in Grana Padano cheese making it is permissible to use up to a maximum of 2.5 g/100 kg of milk; Iaconelli et al., 2008; Galassi et al., 2012).

After 35 min, the formed curds were cut, heated at 37°C, and pressed into cylindrical molds. From the 2 vats, 4 semihard cheeses weighing about 2 kg each were obtained. After 24 h of draining, cheeses were salted in containers with sterile brine (200 g/L of NaCl, pH 5.40) for 2 h and then stored at 20°C for 4 d. Molds were then seasoned for 60 d in a ripening room at the temperature of 15°C with an air humidity of 80 to 85%. Cheese making was replicated twice. During ripening, blowing defects were monitored. After the ripening period, the obtained cheeses were sampled and protein and fat