Ministry for Primary Industries
Manatū Ahu Matua

Publications on the effect of processing on the survival of bacterial pathogens in raw milk products

MPI Information Paper No: 2013/12

ISBN No: 978-0-478-42094-4 (online)
ISSN No: 2253-394X (online)

November 2013

New Zealand Government

Growing and Protecting New Zealand

Disclaimer

This publication is a reference list of peer-reviewed articles from international science journals and books concerning pathogens of interest to cheese makers. This reference list is intended to assist the cheese maker in establishing process and understanding risks, but is not exhaustive. The abstracts provided below have been written by the authors of the articles and book chapters. Any view or opinion expressed in the abstracts and in the articles themselves does not necessarily represent the view of the Ministry for Primary Industries.

Requests for further copies should be directed to:

Publications Logistics Officer
Ministry for Primary Industries
PO Box 2526
WELLINGTON 6140

Email: brand@mpi.govt.nz
Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the Ministry for Primary Industries website at
http://www.mpi.govt.nz/news-resources/publications.aspx

Contents

Page

Background 1

1. Reviews and papers with several pathogens discussed 2

2. *Listeria monocytogenes* only 6

3. *Staphylococcus aureus* only 12

4. Pathogenic Escherichia coli only 17

Background

Pathogens can be present in raw milk, and present a hazard that needs to be managed. Processors of raw milk products are required to provide evidence to validate that the process being applied can reduce these hazards to safe levels.
Reference to published reports and cheese challenge studies may be useful in providing evidence of the factors that inhibit growth of pathogens during cheese production and maturation/ripening.

The pathogens of most concern for New Zealand producers are Salmonella, *Listeria monocytogenes* and toxin-producing *Staphylococcus aureus. Campylobacter* may also be a concern but as it has similar survival characteristics to Salmonella, it may not be necessary to consider it separately. In some countries pathogenic *E.coli* are a significant pathogen for dairy products. While this is not the case at the present time in New Zealand, references have been included as its status could change.

1. Reviews and papers with several pathogens discussed

1 Microbial safety of raw milk cheeses traditionally made at a pH below 4.7 and with other hurdles limiting pathogens growth
F. Perez Pacheco and A. Bucio Galindo (2010)
In Current Research, Technology and Education Topics in Applied Microbiology and Microbial biotechnology. A.Mendes-Vilas(Ed.)

Raw milk acid cheeses are manufactured and consumed in some tropical regions in America, North Africa and in the East Mediterranean countries. For tropical cheeses, little information is available on its microbial safety. This review give some insights of the microbiological safety of some raw milk acid cheeses around the world which are traditionally made at a pH below 4.7 and which contain other hurdles which are generally known as limiting factors for pathogens growth. It describes the occurrence of microbial pathogens in acid cheeses; the intrinsic and extrinsic conditions favouring pathogen survival and growth, including pH, moisture, the presence of lactic acid bacteria and temperature. It reviews the published information on outbreaks of human illness linked to consumption of the raw milk cheeses made with acid curds. This information is important at epidemiological level because raw milk cheeses with higher pH values are generally known as vehicles of infection. Cheeses with lower pH values might be considered as a low microbial risk group where pathogen might be at a low level of concern. Microbial safety of these cheeses is due to the bacteriostatic properties given by its manufacture peculiarities. Microbial safety of these cheeses may be enhanced by the usage of good quality raw milk and by following good manufacturing practices in the whole process of cheese making to prevent cross-contamination of the product. Some other strategies used to improve the safety level of this kind of cheeses are mentioned.

2 Quality Control in Processed Cheese Manufacture
A.Tamime, A.miur, M.Wszolek, J.Domagala, L.Metzger, W.Kneifer,K.Durrschmid,K.Domig, A.Hill, A.Smith, T.Guinee and M.Auty (2011)
Chapter 10 in the Processed Cheese and Analogues by Adnan Tamime

3 Application of hazard analysis critical control point (HACCP) system to the cheese-making industry: a review
D.Sandrow &I.Arvanitoyannis (2007)
Food Reviews International, v16, pp 327-368.

Today there is an ever-increasing consumer demand for safe and high-quality foods of prolonged life. Several quality/safety management systems (e.g., ISO 9000, Total Quality Management, and HACCP) were developed for the food industry. The importance of implementing such systems for rather biochemically unstable products like cheese, a product characterized by great variety worldwide, is apparent. The application of HACCP in the cheese-making industry proved to be beneficial and profitable because the industry managed to cut down the raw material (milk) and final product (cheese) losses and to build up consumer confidence by producing safe cheese of enhanced and consistent quality.

4 Microbiological quality of white-brined cheeses: a review
T. Bintsis, P Papademas (2002)
International Journal of Dairy Technology v. 55, pp 113–120.

White-brined cheeses are widely produced in the North-east Mediterranean area and the Balkans. Traditionally, they were manufactured as artisanal cheeses, and nowadays they are manufactured on an industrial scale, and rigorous control of the production and maturation processes is essential. Aspects of the microbiology of white-brined cheeses and their significance with respect to the quality and safety of the final products are discussed in this review.

5 The Fate of Potentially Pathogenic Bacteria in Swiss Hard and Semihard Cheeses Made from Raw Milk
H.P. Bachmann, U. Spahr (1995)
Journal of dairy Science, v 78, pp 476–483

This study examined the ability of potentially pathogenic bacteria to grow and to survive during the manufacture and ripening of Swiss hard and semihard cheese varieties made from raw milk. The results show that hard cheeses are hygienically safe; 1 wk after fabrication, the inoculated pathogens (*Aeromonas hydrophila, Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella spp., Staphylococcus aureus,* and *Yersinia enterocolitica*) could no longer be detected. At the age of commercial ripeness, the semihard cheeses were free from the inoculated pathogens and their toxic metabolites, except for *L. monocytogenes*, which survived the manufacturing and ripening process.

6 Prevalence and sources of cheese contamination with pathogens at farm and processing levels
Maria Kousta, Marios Mataragas, Panagiotis Skandamis, Eleftherios H. Drosinos (2010)
Food Control, v 21, pp 805–815.

Cheeses, even though characterized as safe for consumption, have been implicated in foodborne outbreaks associated with severe symptoms and high fatality rate. The foodborne pathogens in raw milk originate from the farm environment and direct excretion from animals infected udder, whereas in dairy plants the pathogens may enter via contaminated raw milk, colonize the dairy plant environment and consequently contaminate dairy products. Important source of contamination during the handling and processing might be the workers as well. The objective of this study was to review literature on the prevalence of pathogens in various types of cheese, raw milk and dairy environment, identify sources of contamination and present concisely prevention measures for farm and dairy plant.

7 Microbiological quality of white-brined cheeses: a review
T Bintsis and P Papademas (2002)
International Journal of Dairy Technology, v 55, pp 113-120

White-brined cheeses are widely produced in the North-east Mediterranean area and the Balkans. Traditionally, they were manufactured as artisanal cheeses, and nowadays they are manufactured on an industrial scale, and rigorous control of the production and maturation processes is essential. Aspects of the microbiology of white-brined cheeses and their significance with respect to the quality and safety of the final products are discussed in this review.

8 The fate of indigenous microbiota, starter cultures, *Escherichia coli, Listeria innocua* and *Staphylococcus aureus* in Danish raw milk and cheeses determined by pyrosequencing and quantitative real time (qRT)-PCR
Wafa Masoud, Finn K. Vogensen, Søren Lillevang, Waleed Abu Al-Soud ,

Søren J. Sørensen, Mogens Jakobsen (2012)
International Journal of Food Microbiology, v 153, pp 192–202..

The purpose of this work was to study the bacterial communities in raw milk and in Danish raw milk cheeses using pyrosequencing of tagged amplicons of the V3 and V4 regions of the 16S rDNA and cDNA. Furthermore, the effects of acidification and ripening starter cultures, cooking temperatures and rate of acidification on survival of added *Escherichia coli, Listeria innocua* and *Staphylococcus aureus* in cheeses at different stages of ripening were studied by pyrosequencing and quantitative real time (qRT)-PCR.A high diversity of bacterial species was detected in raw milk. *Lactococcus lactis, Streptococcus thermophilus, Lactobacillus casei and Lactobacillus rhamnosus* were the main bacteria detected in raw milk and cheeses. Bacteria belonging to the genera *Brevibacterium, Staphylococcus, Escherichia, Weissella, Leuconostoc, Pediococcus* were also detected in both 16S rDNA and cDNA obtained from raw milk and cheeses. *E. coli*, which was added to milk used for production of some cheeses, was detected in both DNA and RNA extracted from cheeses at different stages of ripening showing the highest percentage of the total sequence reads at 7 days of ripening and decreased again in the later ripening stages. Growth of *E. coli* in cheeses appeared to be affected by the cooking temperature and the rate of acidification but not by the ripening starter cultures applied or the indigenous microbiota of raw milk. Growth of *L. innocua and S. aureus* added to milks was inhibited in all cheeses at different stages of ripening. The use of 16S rRNA gene pyrosequencing and qRT-PCR allows a deeper understanding of the behavior of indigenous microbiota, starter cultures and pathogenic bacteria in raw milk and cheeses.

9 Survivial of Bioluminescent *Listeria monocytogenes* and *Escherichia coli* 0157:H7 in Soft Cheeses

H. Ramsaran, J. Chen, B. Brunke, A. Hill, and M. W. Griffiths (1998)
Journal of Dairy Science, v 81, pp 1810-1817.

Pasteurized and raw milks that had been inoculated at 10^4 cfu/ml with bioluminescent strains of *Listeria monocytogenes* and *Escherichia coli* O157:H7 were used in the manufacture of Camembert and Feta cheeses with or without nisin-producing starter culture. Survival of both organisms was determined during the manufacture and storage of Camembert and Feta cheeses at 2 ± 1°C for 65 and 75 d, respectively.
Bacterial bioluminescence was used as an indicator to enumerate the colonies plated on selective Listeria agar and on MacConkey agar. *Escherichia coli* O157:H7 survived the manufacturing process of both cheeses and was present at the end of the storage period in greater numbers than in the initial inoculum. At the end of 75 d of storage, *E. coli* O157:H7 was found in the brine of Feta cheese. The counts of *L.monocytogenes* increased as the pH of the Camembert cheese increased, and there were significant differences between the counts from samples taken from the inside and the counts from samples obtained near the surface of the cheese. The Feta cheese that contained nisin was the only cheese in which *L. monocytogenes* was at the level of the initial inoculum after 75 d of storage.

10 Survival of selected pathogenic bacteria in white pickled cheese made with lactic acid bacteria or antimicrobials.

Abdalla O M, Davidson P M and Christsen G L (1993)
Journal of Food Protection, v 56, pp 972-976.

Effect of lactic acid bacteria starter culture, nisin, hydrogen peroxide, or potassium sorbate on *Listeria monocytogenes, Staphylococcus aureus*, and *Salmonella typhimurium* in white pickled cheese made from pasteurized milk with 4% salt and preserved in 4% brine solution at 4 degrees C for 60 d was studied. The starter culture inhibited all three pathogens while

antimicrobials did not. Beyond day 50 in curd and day 30 in brine solution, *L. monocytogenes* was not detected by direct plating in cheese with added starter culture. *S. aureus* was not detected after day 30 in curd and day 20 in brine solution in the same cheese. *S. typhimurium* was not detected after day 30 in cheese curd and was not detected in brine solution at any time with lactic acid bacteria starter culture added. The pH of brine solution of starter treatment dropped below 4.7 in all experiments, while antimicrobial treatments all had a pH 5.5

11 Hygienic parameters, toxins and pathogen occurrence in raw milk cheeses.
de Reu K, Debeuckelaere W, Botteldoorn N, de Block J and Herman L (2002)
Journal of Food Safety v 22, pp 183-196.

In total, 71 samples of retail raw milk cheeses produced or imported in Belgium and samples of Belgian farmhouse cheeses were examined for coliforms, β-glucuronidase positive *Escherichia coli, Escherichia coli* O157, *Staphylococcus aureus, Salmonella spp., Listeria spp. and Listeria monocytogenes*. The presence of staphylococcal enterotoxins was investigated on samples with *S. aureus* counts higher than 10^3 cfu/g. The incidence of coliforms, β-glucuronidase positive *E. coli* and *S. aureus* was higher in soft than in blue veined, semi-hard, hard and fresh cheeses. Four mold-ripened soft cheeses were positive for *E. coli* O157. One of the 4 cheeses was positive for verotoxin VT2. Staphylococcal enterotoxins were detected in 1 soft redsmear cheese, which was positive for *L. monocytogenes*. *L. monocytogenes* was also detected in one fresh cheese. Salmonella was not detected in any of the 71 raw milk cheeses.

12 Response of *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella* Typhimurium, and *Staphylococcus aureus* to the thermal stress occurring in model manufactures of Grana Padano cheese.
Ercolini D, Fusco V, Blaiotta G, Sarghini F and Coppola S (2005)
Journal of Dairy Science v 88 pp 3818-3825.

The purpose of this research was to investigate the effect of temperature in the technology of production of Granacheese against *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus*. According to the technology of production, the cheese curds are cooked at 55°C and then cooled at room temperature (25°C). A curd-cooling model was developed to estimate the temperature variation across the curd during cooling, and the thermal stress was applied to the pathogens according to the model in model-scale productions of Grana cheese artificially contaminated with approximately 10^4 cfu/mL of the selected pathogens. According to the numerical results, the initial temperature inside the cheese is kept at almost the initial value (above 50°C) for at least 4 h during cooling, whereas the crust of the curd cools rapidly to 30°C in the first hour. The best case was that of the core of the cheese where the high temperature was able to efficiently eliminate the contaminating pathogens. Moreover, the worst case was where the external ring of the curd in which a more rapid cooling allowed bacterial survival. Therefore, the thermal stress in the technology of production of Grana cheese can be only partially effective in the control of the selected pathogens. However, the whole technology of production includes other hurdles that can affect the survival of the pathogens and that need to be taken into account as a whole to evaluate the safety of Grana Padano cheese.

***12* *Staphylococcus aureus* and *Listeria monocytogenes* in Norwegian raw milk cheese production**.
Jakobsen R A, Heggebø R, Sunde E B and Skjervheim M (2011)
Food Microbiology; 28:492-496.

The aim of this study was to survey the presence of *Staphylococcus aureus* and *Listeria monocytogenes* during the cheese making process in small-scale raw milk cheese production in Norway. The prevalence of *S. aureus* in bovine and caprine raw milk samples was 47.3% and 98.8%, respectively. An increase in contamination during the first 2–3 h resulted in a 73.6% prevalence of contamination in the bovine curd, and 23 out of 38 *S. aureus*-negative bovine milk samples gave rise to *S. aureus*-positive curds. The highest contamination levels of *S. aureus* were reached in both caprine and bovine cheese after 5–6 h (after the first pressing). There was no contamination of *L. monocytogenes* in caprine cheeses and only one (1.4%) contaminated bovine cheese. This work has increased our knowledge about *S. aureus* and *L. monocytogenes contamination during the process of raw milk cheese production and g*ives an account of the hygiene status during the manufacture of Norwegian raw milk cheeses.

13 Laboratory-scale preparation of soft cheese artificially contaminated with low levels of *Escherichia coli* O157, *Listeria monocytogenes*, and *Salmonella enterica* serovars Typhimurium, Enteritidis, and Dublin.
Leuschner, R.G.K., and Boughflower, M.P. (2002).
Journal of Food Protection, v 65, pp 508-514.

The production of cheese with incurred low levels of pathogenic microorganisms stressed by the production process was the aim of the study. A standard protocol for the preparation of artificially contaminated soft cheese on a laboratory scale was developed. Milk for cheese preparation was artificially contaminated with pathogenic target microorganisms at low levels, between 1 and 10 CFU/ml. Two strains of *Escherichia coli* O157:H7, two strains of *Listeria monocytogenes*, and three *Salmonella* spp. (*Salmonella enterica* serovars Typhimurium, Enteritidis, and Dublin) were investigated. The food pathogens in the cheese were exposed to the entire production process. All three microorganism species survived the cheese production process and were detected in the final product at concentrations between 1 and 50 CFU/g. The cheese produced contains target microorganisms that have been exposed to curd formation, drainage, setting, and ripening. This cheese can be used to validate microbiological methods or to examine the target microorganisms in a natural food environment at low concentrations. It represents an alternative to artificial contamination of cheese by adding target microorganisms to a final cheese product.

2. *Listeria monocytogenes* only

1 Risk factors for *L. monocytogenes* contamination of dairy products in Switzerland, 1990–1999
Son-Il Paka, Urs Spahrb, Thomas Jemmic, M.D Salman (2002)
Preventive Veterinary Medicine, v. 53, pp 55–65

Our purpose was to identify the main hazards associated with the spread of *Listeria monocytogene*s in dairy products in Switzerland and to determine the changes in predominant serotypes of the isolates, using databases on dairy-processing and environments from the Swiss Dairy Research Station during the years 1990–1999. Overall, of 76,271 samples collected, 3722 (4.9%) were positive for the presence of *L. monocytogenes*. Cheese-ripening facilities had the highest proportion of positive samples (7.6%), followed by small-scale local dairies (4.4%). By sample type, the highest proportion of positive samples (9.5%) was observed in water samples used for cheese-washing, followed by cheese-surface swabs (5.0%). During the 10-year period, no positive samples were obtained from cream, ice cream, milk powder, yogurt, or fresh cheese. Of 3722 *L. monocytogenes* isolates, 1328 (35.7%) were serologically typeable. Serotypes 1/2a, 1/2b, and 4b accounted for 92.7% of the 1328 isolates.

Until 1995, the most-prevalent serotype was 1/2b (annual proportional prevalence 39.3–72.2%)—whereas since 1996, 1/2a was the most prevalent (34.7–54.7%). During 1996–1999, serotype 1/2a increased by 88%, compared to the average of 1990–1995. In the final random-effect multivariable logistic model, the strongest predictor of a positive culture was samples from cheese-ripening plant (OR=1.54; 95% CI: 1.14, 2.08) and the second-strongest predictor was samples collected by someone who was employed by the plant (OR=1.48; 1.29, 1.71). Hard and semi-hard cheeses were more likely to be associated with serotype 1/2b and soft cheeses with serotype 1/2a.

2 Assuring Growth Inhibition of Listerial Contamination during Processing and Storage of Traditional Greek Graviera Cheese: Compliance with the New European Union Regulatory Criteria for *Listeria monocytogenes*
Samelis, John: Giannou, Eleni; Lianou, Alexandra (2009)
Journal of Food Protection, v. 72, pp. 2264-2271(8)

The current microbiological regulatory criteria in the European Union specify a maximum *Listeria monocytogenes* population of 100 CFU/g allowable in ready-to-eat foods provided the product will not exceed this limit throughout its shelf life.(see note below) The aim of this study was to validate the manufacturing method for traditional Greek Graviera cheese produced from thermized milk. Initial challenge experiments evaluated the fate of inoculated *L. monocytogenes* (ca. 4 log CFU/ml, three-strain cocktail) in thermized Graviera cheese milk (TGCM; 63°C for 30 s) in the presence and absence of a product-specific starter culture (SC) in vitro. Milk samples were incubated for 6 h at 37°C and then for 66 h at 18°C. Experiments were conducted to evaluate the fate of a cocktail of three nonpathogenic *L. monocytogenes* and *L. innocua* indicator strains inoculated (ca. 3 log CFU/g) in Graviera cheeses commercially manufactured from TGCM+SC. Cheeses were brined, ripened at 18°C and 90% relative humidity for 20 days, and stored at 4°C for up to day 60 under vacuum. In TGCM, *L. monocytogenes* increased by ca. 2 log units, whereas in TGCM+SC *L. monocytogenes* growth was retarded ($P < 0.05$) after a ca. 1-log increase within 6 h at 37°C. Populations of *Listeria* indicator strains did not grow in TGCM+SC cheeses at any stage; they declined 10-fold in fresh cheeses within 5 days and then survived with little death thereafter. Thus, growth inhibition but not inactivation of potent natural *Listeria* contaminants at levels below 100 CFU/g occurs in the core of traditional Greek Graviera cheese during fermentation, ripening, and storage.

Note: The review of the microbiological criteria in the Australia New Zealand Food Standards Code will see adoption of this same limit for product.

3 Growth of *Listeria monocytogenes* in Camembert and other soft cheeses at refrigeration temperatures.
Back, JP, Langford SA, Kroll, RG. (1993)
Journal of Dairy Research, v.60, pp 421-429

Listeria monocytogenes survived and, under most conditions, multiplied when inoculated directly into the cheese milk of laboratory made Camembert cheeses. The rate and extent of growth was reduced at lower storage temperatures. Significantly higher rates of growth occurred at the surface compared with the centre of the cheeses, and these were probably associated with increased pH and proteolysis at the cheese surface due to the mould ripening process. Similar results were obtained with Camembert cheeses surface inoculated after manufacture. There was also temperature-dependent growth of *L. monocytogenes* on a range of inoculated commercially manufactured soft cheeses. Significant growth occurred in Cambazola, French and English Brie, blue and white Lymeswold, French Camembert and

Brie with garlic. Little if any growth occurred in blue and white Stilton, Mycella, Chaume and full fat soft cheese with garlic and herbs at the temperatures examined.

4 Survival of *Listeria monocytogenes* during the manufacture and ripening of Swiss cheese.

Buazzi MM, Johnson ME, Marth EH. (1992)
Journal of Dairy Science; v. 75, pp. 380-386*t*

Rindless Swiss cheese was made from a mixture of pasteurized whole and skim milk that was inoculated to contain 10^4 to 10^5 cfu of *Listeria monocytogenes* (strain Ohio, California, or V7)/ml. During clotting of milk, numbers of *L. monocytogenes* remained nearly unchanged. When the curd was heated gradually to attain the cooking temperature (50°C), numbers of *L. monocytogenes* increased by approximately 40 to 45% over those in inoculated milk. Cooking curd at 50°C (122°F) for 30 to 40 min resulted in resilient curd having a pH of 6.40 to 6.45 and decreased *L. monocytogenes* by 48% compared with numbers of the pathogen in inoculated milk. After curd was pressed under whey, numbers of *L. monocytogenes* increased by approximately 52% over those in inoculated milk and reached their maxima at the end of this stage. A sharp decreased in numbers of *L. monocytogenes* occurred during brining of cheese blocks (7°C for 30 h). The population of *L. monocytogenes* continued to decrease during cheese ripening. Average D values for strains California, Ohio, and V7 were 29.2, 24, and 22.5 d, respectively. *Listeria* was not detected (direct plating, and cold enrichment) after 80, 77, and 66 d of ripening of Swiss cheese made from milk inoculated with strains California, Ohio, and V7, respectively. Thus, Swiss cheese made in this study did not permit extended survival of *L. monocytogenes*

5 Evolution of *Listeria monocytogenes* populations during the ripening of naturally contaminated raw ewe's milk cheese

Noémia Gameiroa, Suzana Ferreira-Diasb, Mass Ferreiraa, Luisa Brito (2007)
Food Control, v. 18, pp 1258–1262

The aim of this work was to study, in loco, the evolution of *Listeria monocytogenes* populations, during ripening (7, 42, 60 and 120 days) of naturally contaminated raw ewe's milk cheese. Two batches of cheese consisting of 20 or 16 cheeses were obtained from two farmstead cheesemakers, respectively. A significant increase in numbers of *L. monocytogenes* was observed for both batches, from 7 to 42 days of ripening. These results suggest that this type of cheese has potential to support the survival of *L. monocytogenes*, while stressing the importance of cheese contamination in the dairies by resident strains.

6 Control of *Listeria monocytogenes* in raw-milk cheeses

L. Milleta, M. Saubussea, R. Didiennea, L. Tessierb, M.C. Montel
International Journal of Food Microbiology, v. 108, pp 105–114

The development of *Listeria monocytogenes* in cheeses made with raw-milk originating from six different farms and according to the Saint-Nectaire cheesemaking technology was studied. Milk was inoculated with two strains of L. monocytogenes at 5 to 10 CFU/25 ml. Microbial and chemical analyses were carried out at appropriate intervals during ripening. *L. monocytogenes* did not grow in the cores of cheeses prepared with milk originating from three farms. That inhibition could be partially attributed to the pH values and l-lactate content. There was no growth in cheeses with pH below 5.2 and lactate content around 14 mg/g. In all cheeses, *L. monocytogenes* stopped growing in the cores of cheeses after eight days and some other factors may be involved in the inhibition. No relation was found between *L. monocytogenes* count and other microbial counts. Growth occurred on cheese surfaces

between eight and eighteen days, when the pH significantly increased. The lowest *L. monocytogenes* growth was found on the surface of cheeses with the lowest pH and without any core growth. Further studies will be performed to clarify the involvement of the microbial community in *L. monocytogenes* inhibition, in particular during the ripening period.

7 The fate of *Listeria monocytogenes* during the manufacture of Camembert-type cheese: A comparison between raw milk and milk treated with high hydrostatic pressure

Mark Lintona, Aideen B. Macklea, Vivek K. Upadhyayb, Alan L. Kellyb, Margaret F. Patterson (2008)
Innovative Food Science & Emerging Technologies, v 9, pp 423–428.

Camembert-type cheese was produced from: raw bovine milk; raw milk inoculated with 2 or 4 log CFU/ml *Listeria monocytogenes*; raw milk inoculated with *L. monocytogenes* and subsequently pressure-treated at 500 MPa for 10 min at 20 °C; or uninoculated raw milk pressure-treated under these conditions. Cheeses produced from both pressure-treated milk and untreated milk had the typical composition, appearance and aroma of Camembert. Curd and cheese made from inoculated, untreated milk contained large numbers of *L. monocytogenes* throughout production. An initial inoculum of 1.95 log CFU/ml in milk increased to 4.52 log CFU/g in the curd and remained at a high level during ripening, with 3.85 log CFU/g in the final cheese. Pressure treatment inactivated *L. monocytogenes* in the raw milk at both inoculum levels and the pathogen was not detected in any of the final cheeses produced from pressure-treated milk. Therefore high pressure may be useful to inactivate *L. monocytogenes* in raw milk that is to be used for the production of soft, mould-ripened cheese.

8 Fate of *Listeria monocytogenes* during the manufacture and ripening of blue cheese.

Papageorgiou DK, Marth EH. (1989)
Journal of Food Protection, v 52, pp 459-465.

The ability of *Listeria monocytogenes* to grow during the manufacture of blue cheese and to survive during its ripening was examined. Pasteurized skim milk was standardized to a milk fat content of 3.7% by addition of pasteurized homogenized cream (35% milk fat), was inoculated to contain ca. 1.0-2.0 X 10^3 *L. monocytogenes* [strain Scott A or California (CA)] cfu/ml, and was made into blue cheese according to the modified Iowa method. Blue cheese was ripened at 9-12 degrees C and a relative humidity of 90-98% for 84 d, and then cheese was stored at 4 degrees C. Duplicate samples of milk, curd, whey, and cheese were tested for pH and for numbers of *Listeria* by surface plating of appropriate dilutions [made in Tryptose Broth (TB) with 2% sodium citrate] on McBride Listeria Agar (MLA). Initial TB dilutions were stored at 4 degrees C and surface-plated on MLA after 2, 4, 6, and 8 weeks, if the pathogen was not quantitated in the original sample. Selected Listeria colonies were confirmed biochemically. *L. monocytogenes* was entrapped in curd during cheese-making with the population in curd before hooping being ca. 1.0 $\log^{10}$ cfu/g greater than in the inoculated milk; whey contained an average of 3.6% of the cells in the initial inoculum. *L. monocytogenes* in cheese increased in numbers by 0.58 to 1.22 $\log^{10}$ cfu/g during the first 24 h of the cheese-making process. Only modest growth (0.12 to 0.30 $\log^{10}$ cfu/g) was noted in two lots with rapid acid production. Growth of *L. monocytogenes* ceased when the pH of cheese dropped below 5.0. Populations of both strains of the pathogen decreased significantly (P less than or equal to 0.005) during the first 50 d of ripening, by an average of 2.68 $\log^{10}$ cfu/g compared to populations of 1-d-old cheese. From days 50 to 120 the environment of

blue cheese became more favorable (pH of cheese increased because of growth by *Penicillium roqueforti*), and this resulted in improved survival but no growth of the pathogen.

9 Fate of *Listeria monocytogenes* during the manufacture and ripening of Parmesan cheese.
Yousef A E and Marth E H (1990)
Journal of Dairy Science, v 73, pp 3351-3356.

Parmesan cheese was made from a mixture of pasteurized whole and skim milk that was inoculated to contain ca. 10^4 to 10^5 cells of *Listeria monocytegenes*/ml. Curd was cooked at 51°C (124°F) for ca. 45 min. During cheese making, maximum numbers of *L. monocytogenes* appeared just before cooking; at this point, the increase over initial numbers was a .61 to 1.0 order of magnitude. During cooking of curd, the average decrease in numbers of *L. monocytogenes* was a .22 order of magnitude. During cheese ripening, numbers of *L. monocytogenes* decreased almost linearly and faster than reported for other hard cheeses. *Listeria monocytogenes* strain *California* died faster than did strain V7. *Listeria monocytogenes* were not detected in cheese after 2 to 16 wk of ripening, depending on the strain of the pathogen and the lot of cheese. Parmesan cheese made in this study was not a favorable medium for survival of *L. monocytogenes*.

10 Survival of *Listeria monocytogenes* during manufacture, ripening and storage of soft lactic cheese made from raw goat milk
F. Morgana, V. Bonnina, M-P. Mallereaub, G. Perrinb (1990)
International Journal of Food Microbiology, v 64, pp 217–22.

.
Soft lactic cheeses were manufactured with raw goat milk inoculated with *Listeria monocytogenes*. The physico-chemical and microbiological characteristics of curds and cheeses were determined after each processing step as well as during ripening and refrigerated storage. The fate of *Listeria monocytogenes* was evaluated by enumeration on PALCAM agar and by a qualitative detection after a double selective enrichment procedure. The results showed that the physico-chemical and microbiological characteristics of lactic cheeses caused a decrease of *Listeria monocytogenes* counts. However, this decrease did not lead to the complete disappearance of the pathogen and *Listeria monocytogenes* was able to survive in soft lactic cheeses made with raw goat milk

11 Behavior of *Listeria monocytogenes* during the manufacture and ripening of brick cheese.
Ryser ET, Marth EH. (1989)
Journal of Dairy Science, v 89, pp 948-954.

Brick cheese was made by the washedcurd procedure from pasteurized whole milk inoculated to contain ca. 1×10^2 to 1×10^3 *Listeria monocytogenes* [strain Scott A, Ohio, V7, or California]/ml. Cheeses were ripened (15°C/95% relative humidity) with a surface smear for 2, 3, or 4 wk to simulate production of mild, aged, or "Limburger-like" brick cheese, respectively, and then stored an additional 20 to 22 wk at 10°C. Populations of strains Scott A, Ohio, V7, and California increased 1.89, 1.72, .83, and .86 orders of magnitude, respectively, following completion of brining ca. 32 h after the start of cheese making. All four *L. monocytogenes* strains leached from cheese into brine during 24 h and survived in brine at 10°C at least 5 d after removal of cheese. Strains Scott A and Ohio grew rapidly during the initial 2 wk of smear development and attained maximum populations of ca. 6.6 and 6.2, 7.0 and 6.9, and 5.6 and 5.1 log10/g in 4-wk-old slice (pH 6.0 to 6.5), surface (pH 6.5 to 6.9), and interior (pH 5.6 to 6.2) samples of cheese, respectively. Numbers of strains Scott

A and Ohio generally decreased 1- to 7-fold during 20 to 22 wk at 10°C. Strains V7 and *California* failed to grow appreciably in any cheese during or after smear development, despite pH of 6.8 to 7.4 in fully ripened cheese; the strains were never isolated from 2- and 3-wk-old cheese and with direct plating were detected sporadically at levels generally ≤4.0 log10/g in cheese aged ≥4 wk. Cold enrichment of slice, surface, and interior samples of cheeses aged ≥4 wk generally yielded positive results for *L. monocytogenes*; strains V7 and *California* were detected in all cheeses after 20 to 22 wk at 10°C. At 10 ppm, methyl sulfide, dimethyl disulfide, or methyl disulfide (compounds commonly produced during ripening of brick and Limburger cheese) failed to inhibit appreciably growth of *L. monocytogenes*.

3. *Staphylococcus aureus* only

1 Behaviour and enterotoxin production by *Staphylococcus aureus* during the manufacture and ripening of raw goats' milk lactic cheeses
Christine Vernozy-Rozand, Annie Meyrand, Christine Mazuy, Marie-Laure Delignette-Muller, Guy Jaubert, Gerard Perrin, Christiane Lapeyre and Yves Richard (1998)
Journal of Dairy Research, v.65, pp 273-281

To study the possible presence of staphylococcal enterotoxin A in raw goats' milk lactic cheese, milk was inoculated with an enterotoxigenic *Staphylococcus aureus* strain to a final concentration of 4, 5 and 6 log(cfu/ml). Cheese was prepared following industrial specifications and ripened for 42 d. Detection of the enterotoxins was by the Vidas Staph enterotoxin test (BioMérieux) and by an indirect double-sandwich ELISA technique using anti-enterotoxin monoclonal antibodies. Staphylococcal counts declined markedly after draining, and by the end of ripening they had disappeared from some cheeses. In contrast, aerobic mesophilic organisms grew well. The level of staphylococcal enterotoxin A recovered varied from 1 to 2·5 ng/g cheese made with an initial population of 10^5 or 10^6 cfu/ml. Only traces of enterotoxin A (0·5 ng/g) were detected in cheeses made with the lowest *Staph. aureus* inoculum used in this study. Enterotoxin A was also detected in cheeses from which *Staph. aureus* had disappeared.

2 Unveiling *Staphylococcus aureus* enterotoxin production in dairy products: a review of recent advances to face new challenges
Marina Cretenet & Sergine Even & Yves Le Loir (2011)
Dairy Science & Technology, v. 91, pp127–150.

Staphylococcus aureus is a major food-borne pathogen worldwide and a frequent contaminant of foodstuffs where some strains are able to produce staphylococcal enterotoxins (SE). Consumption of foods containing these SEs is responsible for staphylococcal food poisoning (SFP) outbreaks. Milk and milk products are foodstuffs commonly associated with SFP. Typical SFP symptoms are vomiting with or without diarrhoea and abdominal cramping which reduce after 12 to 72 h. Despite extensive studies, the mechanistic base of SE production is still poorly understood but appears to be quite heterogeneous among the 21 different SEs identified to date. In this review, recent data regarding *S. aureus* and SE detection and quantification in dairy products as well as data about *S. aureus* growth and SE production with regard to parameters relevant for the dairy context and the cheese industry have been summarized. Recent technological developments have allowed the detection of S. aureus and SEs in foodstuffs to be refined. Similarly, molecular approaches have allowed high-throughput investigations of the physiology of *S. aureus* and revealed the complexity of this multi-faceted problem. SFP control must indeed take account of the growth of S. aureus as well as SE production. The wealth of new available data will open up new strategies for a better risk assessment and control of this major pathogen.

3 Conditions of staphylococcal enterotoxin production in milk and milk products
Mantis, A.J.; Papageorgiou, D.K. (2011)
Journal of the Hellenic Veterinary Medical Society, v.54, pp 242-252.

The authors reviewed the existing scientific data, concerning the ability of *Staphylococcus aureus* to grow and produce enterotoxins in milk and in dairy products particularly in cheeses. *S. aureus* can grow well in liquid raw or pasteurized milks and produce enterotoxins if the

product is stored in favorable for the pathogen temperature. Cream also supports growth of *S. aureus* and enterotoxin production, but butter as well as fermented products like yogurt and buttermilk are not favorable substrates for the production of enterotoxins. Cheeses represent a complex environment, due to their great variety in processing technology and environment. Fresh cheeses, soft cheeses and semi-hard and hard cheeses can support growth of *S. aureus* during the first stages of production up to 48 hours. Normally, the pathogen, if it is present in the milk, will multiply for 3-4 logs and after that, when acidity develops, the populations of *S. aureus* decrease and usually disappear by the end of the ripening period. However, if enterotoxins are produced during the multiplication phase of the pathogen, it will remain active in the cheese for a long time. Internal mould ripened cheeses (e.g. blue cheese), pasta filata cheeses or the processed cheeses do not represent favorable substrates for the multiplication of *S. aureus* and enterotoxin production. On the contrary, whey cheeses form a very favorable environment for the enterotoxins' production, because of their high pH and the absence of antagonistic bacterial flora.

4 Effect of Temperature and pH on Growth of *Staphylococcus aureus* in Co-Culture with Lactococcus garvieae

J. Alomar , A. Lebert , M. C. Montel (2008)
Current Microbiology v 56, pp. 408–412

Staphylococcus aureus growth and enterotoxin production in co-culture with *Lactococcus garvieae* were studied in laboratory medium as a function of incubation temperature and pH values. Doehlert experimental design was used to study the effect of *L. garvieae* concentration, temperature, and pH on *S. aureus* growth in laboratory medium. The mathematical model obtained was validated in cheeses. The inhibition of *S. aureus* growth by *L. garvieae* was more important during the first 6 hours of incubation, and its effect increased when its concentration increased. After 24 and 48 hours, the effect of *L. garvieae* decreased, and the growth of *S. aureus* was positively influenced by higher temperature and pH values. Staphylococcal enterotoxins were detected in only one experimental set after 48 hours of incubation at 30˚C at pH 6.8. Our results argue in favor of adding antagonist strain early in the cheese-making process.

5 Growth abilities and enterotoxin production of *Staphylococcus aureus* strains in herby cheese.

Akkaya L and Sancak Y C (2007)
Bulletin of the Veterinary Institute of Pulawy v 51, pp 401-406.

Staphylococcus aureus reference strains, producing A, B, C, and D enterotoxins, were individually or as mixture inoculated at the rate of 10^5 cfu/mL into 10 herby cheese samples, experimentally produced from pasteurised or raw milk. The growth rates and enterotoxin production abilities of the strains were examined during the 90 d ripening period. During the ripening, *S. aureus*, aerobic mesophilic and lactic acid bacteria, aw, pH, acidity, salt levels, and A, B, C, and D enterotoxins were examined at 11 different periods. The level of *S. aureus* in cheese samples produced from pasteurised milk decreased regularly after 15 d, and on the 90th d was reduced to log 2 cfu/g. In cheese made from raw milk, the *S. aureus* levels at the beginning of the ripening period increased up to 10^7 cfu/g, and then decreased to the starting level on the 90th d of the ripening. Throughout the ripening period, enterotoxin A was observed in the curd stage in pasteurised cheese samples inoculated individually with toxin A producing strain. Enterotoxins A, B, C, and D were observed on the third day in pasteurised mixed cheese inoculated with the mixture of *S. aureus* strains. Enterotoxin C was observed in cheese inoculated with toxin C producing strain on the 15th d. No enterotoxin was observed

in cheese samples made from the raw milk during the processing until the end of the ripening period, and the pH levels did not drop compared to those of pasteurised cheeses.

6 *Staphylococcus aureus* growth and enterotoxin production during the manufacture of uncooked, semihard cheese made from cows' raw milk

Delbes C, Alomar J, Chougui N, Martin J-F and Montel M-C (2006)
Journal of Food Protection, v 69,pp 2161-2167.

Staphylococcus aureus growth and enterotoxin production during the manufacture of model Saint-Nectaire, Registered Designation of Origin Saint-Nectaire, and Registered Designation of Origin Salers cheeses, three types of uncooked, semihard, raw milk cheese, were investigated. Coagulase-positive staphylococci (SC^+) grew rapidly during the first 6 h. Between 6 and 24 h, counts increased by less than 0.5 log CFU/ml. Raw milk counts ranged from undetectable (<10 CFU/ml) to 3.03 log CFU/ml. Maximal levels reached in cheese on day 1 ranged from 2.82 to 6.84 log CFU/g. The level of SC^+ after 24 h was mainly influenced by the milk baseline SC^+ level (correlation coefficient, $r > 0.80$) but pH at 6 h influenced the SC growth observed between 6 and 24 h ($r > 0.70$). Thus, the initial level of SC^+ in raw milk should be maintained below 100 CFU/ml and best below 40 CFU/ml. To limit growth, acidification should be managed to obtain pH values around or below 5.8 at 6 h in Saint-Nectaire cheeses and around or below 6.3 at 6 h in Salers cheeses. Enterotoxins were only detected in two Salers cheeses whose SC^+ counts on day 1 were 5.55 log CFU/g and 5.06 log CFU/g, respectively, and whose pH values at 6 h were high (approximately 6.6 and 6.5, respectively).

7 Influence of lactic starter inoculation, curd heating and ripening temperature on *Staphylococcus aureus* behaviour in Manchego cheese.

Gaya P, Medina M, Bautista L and Nuñez M (1988)
International Journal of Food Microbiology, v 6, pp 249-257.

Growth and survival of *Staphylococcus aureus* were investigated in 52 lots of raw ewe's milk Manchego cheese manufactured and ripened under different conditions. A 5.8-fold reduction in *S. aureus* counts after 60 days of ripening was obtained by inoculating milk with 1% *Streptococcus lactis* culture, and a further 2.0-fold reduction could be achieved by adding 0.1% *Lactobacillus plantarum* culture. Curd heating temperature had a significant effect on *S. aureus* survival, with counts 4–5 times lower in cheese from 30° C curd than in cheese from curd heated at 36–40° C. Ripening temperature was the parameter with the greatest influence on *S. aureus* counts, which reached in cheese cured at 10° C or 20° C for 60 days levels 10 and 100 times lower, respectively, than in cheese held at 5° C.

8 Growth and enterotoxin production of *Staphylococcus aureus* during the manufacture and ripening of Camembert-type cheeses from raw goats' milk.

Meyrand A, Boutrand-Loei S, Ray-Gueniot S, Mazuy C, Gaspard C E, Jaubert G, Perrin G, Lapeyre C and Vernozy-Rozand C (1998)
Journal of Applied Microbiology, v 85, pp 537-544.

Tests were carried out to determine the effect of manufacturing procedures for a Camembert-type cheese from raw goats' milk on the growth and survival of *Staphylococcus aureus* organisms added to milk at the start of the process, and to study the possible presence of staphylococcal enterotoxin A in these cheeses. The initial staphylococcal counts were, respectively, 2, 3, 4, 5 and 6 log cfu ml−1. Cheese was prepared following the industrial specifications and ripened for 41 d. Detection of enterotoxins was done by the Vidas SET test and by an indirect double-sandwich ELISA technique using antienterotoxin monoclonal

antibodies. Generally, numbers of microbes increased at a similar rate during manufacture in all cheeses until salting. During the ripening period, the aerobic plate count population and *Staph. aureus* levels remained stable and high. There was an approximately 1 log reduction of *Staph. aureus* in cheeses made with an initial inoculum of *Staph. aureus* greater than 103 cfu ml−1 at the end of the ripening period (41 d) compared with the count at 22 h. The level of staphylococcal enterotoxin A recovered varied from 1 to 3·2 ng g−1 of cheese made with an initial population of 10^3–10^6 cfu ml−1. No trace of enterotoxin A was detected in cheeses made with the lowest *Staph. aureus* inoculum used in this study.

9 Influence of soft cheese technology on the growth and enterotoxin production of *Staphylococcus aureus.*

Necidová L, Šťástková Z, Posp šilová M, Janštová B, Strejček J A N, Dušková M and Karp šková R (2009)
Czech Journal of Food Sciences; 27:127-133.

The aim of this study was to monitor *S. aureus* growth and toxin production in soft cheese during the technological processing. In model experiments, raw milk was inoculated separately with five *S. aureus* strains isolated from milk and milk products. All the strains were producers of staphylococcal enterotoxins (SEs) of types A, B, or C. SEs were detected by the enzyme-linked fluorescence assay (ELFA) performed in the MiniVIDAS device. This study has shown that the amount of SEs varied with the tested strains and stages of the technological process. SEs were detected in soft cheese made from pasteurised milk inoculated with 2.9×10^5 CFU/g of *S. aureus*. The prevention of *S. aureus* contamination and multiplication during the cheese making process is a prerequisite for the production of safe soft cheese. The most important enterotoxin dose build-up factor can be overcome by strict compliance with the cooling requirements during the manufacture, distribution and storage of the product.

10 Factors affecting the multiplication and survival of coagulase positive staphylococci in Cheddar cheese.

Reiter B, Fewins B G, Fryer T F and Sharpe M E (1964)
Journal of Dairy Research, v 31, pp 261-271.

The presence of a thermolabile inhibitor of coagulase-positive staphylococci in milk was confirmed. Starter streptococci significantly suppressed the multiplication of staphylococci in milk not only by their acid production but also by some other competitive effect.
Cheesemaking trials showed that staphylococci multiplied considerably more rapidly in 'slow' or 'sweet' cheese, where the starter was inhibited by phage, than in normal cheese. Little decrease in numbers occurred in 'sweet' cheese even after 18 months, in contrast to the rapid decline in the normal cheese.
Staphylococci subjected in the laboratory to sublethal heat treatments had a prolonged lag phase on all media and their % recovery on selective media was significantly lower than on optimal non-selective media. It is suggested that the low survival rate of the staphylococci in cheese made from milks heated at sublethal temperatures is due to the lag in recovery of heat-shocked cells and their inability to multiply in the unfavourable cheese curd.

11 Production of staphylococcal enterotoxin A in Blue, Brick, Mozzarella, and Swiss cheeses

Tatini S R, Wesala W D, Jezeski J J and Morris H A (1973)
Journal of Dairy Science v 56, pp 429-435.

Staphylococcal enterotoxin A production in Blue, Brick, Mozzarella, and Swiss cheeses from milk inoculated with different initial *Staphylococcus aureus* populations was evaluated. Depending on the type of lactic starter and the strain of S. aureus, enterotoxin was detected under certain conditions in Brick and Swiss cheeses. It was not demonstrated even with higher *S. aureus* populations (5×10^7/g) and a complete starter failure in Blue cheese. Minimal *S. aureus* population associated with presence of detectable enterotoxin was influenced by the environmental conditions in cheese.

12 Relation of cheese-making operations to survival and growth of *Staphylococcus aureus* in different varieties of cheese.
Tuckey S L, Stiles M E, Ordal Z J and Witter L D (1964)
Journal of Dairy Science v 47, pp 604-611.

.

Tests were made to determine the effect of manufacturing procedures for specific varieties of cheese on the growth and survival of *Staphylococcus aureus* (MF31) organisms added to milk at the start of the process. The staphylococcal count was approximately 1×10^5/ml milk. Cheddar, Colby, Swiss-type, Limburger, and Cottage cheese by two methods were made. Conditions of manufacture of each variety were favorable for the growth of *S. aureus* and significant increases occurred at specific stages. In general, the organisms were concentrated in the curd and increased in number until the cheese was salted. After pressing the curd, another increase might occur during the first 21 days of aging. A decrease would then take place, which generally continued throughout aging. At no time, however, did the *S. aureus* count become zero during aging. Staphylococci were not recovered from fresh Cottage cheese after it had been cooked at 130˚F for 40 min at pH 4.5. However, when the curd was stored dry or creamed for ten days at either 40 or 50˚F, viable *S. aureus* organisms were recovered. Nevertheless, the manufacturing procedure for Cottage cheese was the only effective one in destroying *S. aureus*

4. Pathogenic Escherichia coli only

1 An Overview of Molecular Stress Response Mechanisms in *Escherichia coli* Contributing to Survival of Shiga Toxin-Producing *Escherichia coli* during Raw Milk Cheese Production

Peng, Silvio; Tasara, Taurai; Hummerjohann, Jörg; Stephan, Roger (2011)
Journal of Food Protection, v. 74, pp. 849-864(16)

The ability of foodborne pathogens to survive in certain foods mainly depends on stress response mechanisms. Insight into molecular properties enabling pathogenic bacteria to survive in food is valuable for improvement of the control of pathogens during food processing. Raw milk cheeses are a potential source for human infections with Shiga toxin-producing *Escherichia coli* (STEC). In this review, we focused on the stress response mechanisms important for allowing STEC to survive raw milk cheese production processes. The major components and regulation pathways for general, acid, osmotic, and heat shock stress responses in *E. coli* and the implications of these responses for the survival of STEC in raw milk cheeses are discussed.

2 Behavior of *Escherichia coli* O157:H7 during the Manufacture and Aging of Gouda and Stirred-Curd Cheddar Cheeses Manufactured from Raw Milk

Dennis J. D'amico, Marc J. Druart, and Catherine W. Donnelly (2010)
Journal of Food Protection, v. 73, pp 2217–2224

This study was conducted to examine the fate of *Escherichia coli* O157:H7 during the manufacture and aging of Gouda and stirred-curd Cheddar cheeses made from raw milk. Cheeses were manufactured from unpasteurized milk experimentally contaminated with one of three strains of *E. coli* O157:H7 at an approximate population level of 20 CFU/ml. Samples of milk, whey, curd, and cheese were collected for enumeration of bacteria throughout the manufacturing and aging process. Overall, bacterial counts in both cheese types increased almost 10-fold from initial inoculation levels in milk to approximately 145 CFU/g found in cheeses on day 1. From this point, counts dropped significantly over 60 days to mean levels of 25 and 5 CFU/g in Cheddar and Gouda, respectively. Levels of *E. coli* O157:H7 fell and stayed below 5 CFU/g after an average of 94 and 108 days in Gouda and Cheddar, respectively, yet remained detectable after selective enrichment for more than 270 days in both cheese types. Changes in pathogen levels observed throughout manufacture and aging did not significantly differ by cheese type. In agreement with results of previous studies, our results suggest that the 60-day aging requirement alone is insufficient to completely eliminate levels of viable *E. coli* O157:H7 in Gouda or stirred-curd Cheddar cheese manufactured from raw milk contaminated with low levels of this pathogen.

3 Growth and survival of *E. coli* O157:H7 during the manufacture and ripening of a smear-ripened cheese produced from raw milk

M.M. Maher, K.N. Jordan, M.E. Upton and A. Coffey (2001)
Journal of Applied Microbiology, v 90, pp 201-207.

Aims: The behaviour of *Escherichia coli* O157:H7 was studied during the manufacture and ripening of a smear-ripened cheese produced from raw milk.
Methods and Results: Cheese was manufactured on a laboratory scale using milk (20 l) inoculated with *E. coli* O157:H7 and enumeration was carried out using CT-SMAC. From an initial level of 1.52 ± 0.03 log cfu/ml in the milk (34 ± 2 cfu ml/1), the numbers increased to

3.4 ± 0.05 log cfu/g in the cheese at day 1. During ripening, the numbers decreased to <1 cfu/g and <10 cfu/g in the rind and core, respectively, after 21 days, although viable cells were detected by enrichment after 90 days. The presence of *E. coli* O157:H7 in the cheese was confirmed by latex agglutination and by multiplex PCR.
Conclusions: The results indicate that the manufacturing procedure encouraged substantial growth of *E. coli* O157:H7 to levels that permitted survival during ripening and extended storage.
Significance and Impact of the Study: The presence of low numbers of *E. coli* O157:H7 in milk, destined for raw milk cheese manufacture, could constitute a threat to the consumer.

4 Behavior of *Escherichia coli* O157:H7 during the Manufacture and Ripening of Feta and Telemes Cheese.
Govaris, A., Papageorgiou, D.K., and Papatheodorou, K. (2002).
Journal of Food Protection, 65, 600-615.

Pasteurized whole ewe's and cow's milk was used in the manufacture of Feta end Telemes cheeses, respectively, according to standard procedures. In both cases, the milk had been inoculated with *Escherichia coli* O157:H7 at a concentration of ca. 5.1 log CFU/ml and with thermophilic or mesophilic starter cultures at a concentration of ca. 5.3 to 5.6 log CFU/ml. In the first 10 h of cheesemaking, the pathogen increased by 1.18 and 0.82 log CFU/g in Feta cheese and by 1.56 and 1.35 log CFU/g in Telemes cheese for the trials with thermophilic and mesophilic starters, respectively. After 24 h of fermentation, a decrease in *E. coli* O157:H7 was observed for all trials. At that time, the pH was reduced to 4.81 to 5.10 for all trials. Fresh cheeses were salted and held at 16°C for ripening until the pH was reduced to 4.60. Cheeses were then moved into storage at 4°C to complete ripening. During ripening, the *E. coli* O157:H7 population decreased significantly ($P \leq 0.001$) and finally was not detectable in Feta cheese after 44 and 36 days and in Telemes cheese after 40 and 30 days for the trials with thermophilic and mesophilic starters, respectively. The estimated times required for one decimal reduction of the population of *E. coli* O157:H7 after the first day of processing were 9.71 and 9.26 days for Feta cheese and 9.09 and 7.69 days for Telemes cheese for the trials with thermophilic and mesophilic starters, respectively.

5 Survival of Enterohemorrhagic *Escherichia coli* O157:H7 During the Manufacture and Curing of Cheddar Cheese.
Reitsma, C.J. and Henning, D.R. (1996).
Journal of Food Protection, v 59, pp 460-464.

The ability of enterohemorrhagic *Escherichia coli* O157:H7 to survive a standard Cheddar cheese manufacturing process and subsequent curing was determined. Two treatments with added *E. coli* O157:H7 were designed with target levels 1×10^3 CFU/ml and 1 CFU/ml of cheese milk. Cheese samples were analyzed for *E. coli* O157:H7 during manufacture at 14, 28, 42, 60, and 74 days and at 28-day intervals thereafter until the organism could no longer be detected using direct plating or enrichment in two successive samples. Typical colonies on 3M Petrifilm® *E. coli* Count Plates were counted as presumptive *E. coli* O157:H7 and were confirmed with the 3M Petrifilm® Test Kit—HEC for hemorrhagic *E. coli* O157:H7. When no *E. coli* O157:H7 were detected in the cheese with the Petrifilm® plates, a 25-g sample of cheese was enriched in modified EC broth with novobiocin to detect viable *E. coli* O157:H7. Cheese made with 10^3 CFU/ml of milk showed a 2-log-unit reduction after 60 days of ripening, with viable *E. coli* O157:H7 still being detected in 25 g of cheese after 158 days. Cheese made with 1 CFU/ml of milk showed a reduction in *E. coli* Ol57:H7 to 1 or <1 CFU/g in 60 days, with no E. coli being detected in 25 g of cheese at 158 days. However, both

treatments resulted in the survival of *E. coli* O157:H7 during manufacture and for more than 60 days of curing at 2.75 to 3.76% salt in the moisture phase.

6 Fate of *Escherichia coli* O157:H7 during the manufacture of Mozzarella cheese.
Spano G, Goffredo E, Beneduce L, Tarantino D, Dupuy A, and Massa A. (2003).
Letters in Applied Microbiology, 36, 73-76.

Aims: The fate of *Escherichia coli* O157:H7 was investigated during the manufacture of Mozzarella cheese.
Methods and Results: The Mozzarella cheese was made from unpasteurized milk which was inoculated to contain ca 105 cfu ml−1*E.coli* O157:H7. Two different heating temperatures (70 and 80 °C), commonly used during curd stretching, were investigated to determine their effects on the viability of *E. coli* O157:H7 in Mozzarella cheese. Stretching at 80 °C for 5 min resulted in the loss of culturability of *E. coli* O157:H7 strains, whereas stretching at 70 °C reduced the number of culturable *E. coli* O157:H7 by a factor of 10.
Conclusions: The results show that stretching curd at 80 °C for 5 min is effective in controlling *E. coli* O157:H7 during the production of Mozzarella cheese. Brining and storage at 4 °C for 12 h was less effective than the stretching.
Significance and Impact of the Study: Mozzarella cheese should be free of *E. coli* O157:H7 only if temperatures higher than or equal to 80 °C are used during milk processing.

7 Survival of a Five-Strain Cocktail of *Escherichia coli* O157:H7 during the 60-Day Aging Period of Cheddar Cheese Made from Unpasteurized Milk
Schlesser, J.E; Gerdes, R; Ravishankar, S; Madsen, K; Mowbray, J; Teo, A.Y. (2006)
Journal of Food Protection, v 69, pp. 990-998(9)

The U.S. Food and Drug Administration Standard of Identity for Cheddar cheeses requires pasteurization of the milk, or as an alternative treatment, a minimum 60-day aging at ≥2°C for cheeses made from unpasteurized milk, to reduce the number of viable pathogens that may be present to an acceptable risk. The objective of this study was to investigate the adequacy of the 60-day minimum aging to reduce the numbers of viable pathogens and evaluate milk subpasteurization heat treatment as a process to improve the safety of Cheddar cheeses made from unpasteurized milk. Cheddar cheese was made from unpasteurized milk inoculated with 10^1 to 10^5 CFU/ml of a five-strain cocktail of acid-tolerant *Escherichia coli* O157:H7. Samples were collected during the cheese manufacturing process. After pressing, the cheese blocks were packaged into plastic bags, vacuum sealed, and aged at 7°C. After 1 week, the cheese blocks were cut into smaller-size uniform pieces and then vacuum sealed in clear plastic pouches. Samples were plated and enumerated for *E. coli* O157:H7. Populations of *E. coli* O157:H7 increased during the cheese-making operations. Population of *E. coli* O157:H7 in cheese aged for 60 and 120 days at 7°C decreased less than 1 and 2 log, respectively. These studies confirm previous reports that show 60-day aging is inadequate to eliminate *E. coli* O157:H7 during cheese ripening. Subpasteurization heat-treatment runs were conducted at 148°F (64.4°C) for 17.5 s on milk inoculated with *E. coli* O157:H7 at 10^5 CFU/ml. These heat-treatment runs resulted in a 5-log *E. coli* O157: H7 reduction.

8 Potential growth and control of *Escherichia coli* O157:H7 in soft hispanic type cheese
Manuchehr Kasrazadeh, Constantin Genigeorgis (1995)
International Journal of Food Microbiology, v 25, pp 289-300.

This study examined growth and control of two enterohemorrhagic *Escherichia coli* serotype O157:H7 strains in a soft Hispanic type cheese (Queso Fresco). Cheese was made in the laboratory using a commercial procedure and after inoculation it was stored under vacuum at temperatures ranging from 8 to 30 °C. The minimum temperature that allowed growth of *E. coli* in the unmodified normal cheese (pH 6.6) was 10 °C. No growth of either strains was observed during a 2 month storage period at 8 °C. Accumulated data from the growth studies were used for development of models relating square root of 1/LT (LT = lag time) and specific growth rate, to temperature. The effect of selected antimicrobials (potassium sorbate, sodium benzoate, and sodium lactate) added to milk or cheese on the growth arid survival of these pathogens at various storage temperatures was also evaluated. Addition of sodium benzoate (0.3%) to cheese (pH 6.6) or addition of potassium sorbate (0.3%) to cheese (pH 6) made from milk acidified to pH 5.9 with propionic acid, had a significant impact on delaying or preventing growth of the pathogens.

9 Growth and survival of *Escherichia coli* O157:H7 during the manufacture and ripening of raw goat milk lactic cheeses

C. Vernozy-Rozanda, , , C. Mazuy-Cruchaudeta, C. Bavaia, M.P. Monteta, V. Boninb, A. Dernburgc, Y. Richarda (2005)
International Journal of Food Microbiology, v.105, pp 83-88

The behaviour of *Escherichia coli* O157:H7 was studied during the manufacture and ripening of raw goat milk lactic cheeses. Cheese was manufactured from raw milk in the laboratory and inoculated with *E. coli* O157:H7 to a final concentration of 10, 100 and 1000 cfu ml− 1. *E. coli* O157:H7 was counted by CT-SMAC (Mac Conkey Sorbitol Agar with cefixim and tellurite) and O157:H7 ID throughout the manufacturing and ripening processes. When the milk was inoculated with 10, 100 or 1000 cfu ml− 1, counts decreased to less than 1 log10 g− 1 in curds just prior to moulding. However, viable *E. coli* O157:H7 were found in cheeses throughout processing, and even after 42 days of ripening. Results indicate that *E. coli* O157:H7 survives the lactic cheese manufacturing process. Thus, the presence of low numbers of *E. coli* O157:H7 in milk destined for the production of raw milk lactic cheeses can constitute a threat to the consumer.