This is to certify that the

thesis entitled

COMPETITION OF THERMALLY INJURED *LISTERIA MONOCYTOGENES* WITH A MESOPHILIC LACTIC ACID STARTER CULTURE DURING MILK FERMENTATION

presented by

Finny P. Mathew

has been accepted toward fulfillment
of the requirements for

Master of Science degree in Food Science
and Human Nutrition

Elliot T. Ryser
Major professor

Date 12/13/00

COMPETITION OF THERMALLY INJURED *LISTERIA MONOCYTOGENES* WITH A MESOPHILIC LACTIC ACID STARTER CULTURE DURING MILK FERMENTATION

By

Finny P. Mathew

A THESIS

Submitted to
Michigan State University
In partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

2000

ABSTRACT

COMPETITION OF THERMALLY INJURED *LISTERIA MONOCYTOGENES* WITH A MESOPHILIC LACTIC ACID STARTER CULTURE DURING MILK FERMENTATION

By

Finny P. Mathew

The relationship between heat treatment of milk and the ability of sublethally injured *Listeria monocytogenes* to survive mesophilic fermentation in milk was investigated. A three-strain cocktail of *L. monocytogenes*, suspended in 200 ml of tryptose phosphate broth, was heated at 56°C/20 min and 64°C/2 min to obtain low heat-injured (LHI) and high heat-injured (HHI) cells, respectively, showing >99% injury. Flasks containing 200 ml of raw, low heat-treated (56°C/20 min), high heat-treated (64°C/2min), pasteurized or UHT milk were tempered to 31.1°C, inoculated to contain 10^4-10^6 LHI, HHI or healthy *L. monocytogenes* cells and *Lactococcus lactis* subsp. *lactis*/*Lactococcus lactis* subsp. *cremoris* starter culture at 0.5, 1.0 or 2.0% levels. Numbers of healthy and injured *L. monocytogenes* cells were determined using tryptose phosphate agar w/o 4.0% NaCl at selected intervals during the 24h fermentation period along with the numbers of starter organisms. In starter-free controls, ~76-81% and 59-69% of LHI and HHI cells, respectively, were repaired after 8 hours of incubation, with lowest repair in raw milk. Increased injury was observed for healthy *L. monocytogenes* cells at 1.0 and 2.0% starter levels, with less injury seen for LHI and HHI cells. The extent of sublethal injury for all *L. monocytogenes* was inversely related to severity of the milk heat treatment.

Dedicated to my family in India and East Lansing

ACKNOWLEDGMENTS

Firstly, I would like to thank God for giving me the opportunity and wisdom to get my MS degree in Food Science at Michigan State University. I want to extend my heartfelt thanks to my adviser, Dr. Elliot T. Ryser, without whose immense help, guidance and cooperation in academics as well as in research I would not have been able to finish my degree. I am very thankful to him for providing me the opportunity to tap into his expertise on *Listeria monocytogenes*. I want to thank my committee members, Dr. Zeynep Ustunol and Dr. James Pestka, for their kind cooperation and good advice at all times. I would like to thank my lab mates for their cooperation during my research work and for making my time in the lab enjoyable. I would also like to thank Dr. John Partridge, Mr. John Engstrom and his dairy plant team for making my experience in the dairy plant a learning (and fun) experience during the first year of my degree as well as for all help extended towards my research. I thank the National Food Safety and Toxicology Center and the Department of Food Science and Human Nutrition, Michigan State University, for funding my research and providing me with graduate assistantships. Last but not the least, I would like to confer my deepest thanks to my family in India as well as in East Lansing (Alociljas) who constantly supported, helped and guided me in every possible way and prayed for my success at Michigan State University.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS OR ABBREVIATIONS

1. CFU, colony-forming units
2. diameter, diam
3. gram, g
4. high heat-injured, HHI
5. high heat-treated, HHT
6. hour(s), h
7. *Lactococcus lactis* subsp. *lactis*/*Lactococcus lactis* subsp. *cremoris*, LLLC
8. low heat-injured, LHI
9. low heat-treated, LHT
10. minute(s), min
11. modified tryptose phosphate agar, MTPA
12. modified tryptose phosphate agar with 4% salt, MTPNA
13. parts per million, ppm
14. pound, lb
15. revolutions per minute, rpm
16. second, s
17. specie, sp.
18. species, spp.
19. tryptose phosphate agar, TPA
20. tryptose phosphate agar with 4% NaCl, TPNA
21. ultra high temperature, UHT

INTRODUCTION

Most United States "Standards of Identity" for cheese and cheese related products (1948-49) provide cheese manufacturers with the option of pasteurizing [71.6°C (161°F)/15 sec] the milk or holding the cheese for a minimum of 60 days at ≥1.7°C (35°F) to eliminate pathogenic microorganisms. Thus, any cheese prepared from raw or heat-treated milk has to be held at least 60 days. Since 1948, at least 10 foodborne outbreaks have been linked to domestically produced cheese. Reports have shown that three important foodborne pathogens, namely, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 can respectively survive up to 434 days, 210 days and 138 days in Cheddar cheese produced from pasteurized milk inoculated with the pathogen. Consequently, the adequacy of the 60 day hold at ≥ 1.7°C still remains very much in question.

Based on available data, the United States Food and Drug Administration (FDA) is re-examining current regulations. However, given the superior flavor characteristics of raw milk Cheddar Cheese that result from non-starter lactic acid bacteria and enzymes naturally present in the milk, cheese manufacturers as well as certain consumer groups are reluctant to any change in the current aging policy.

Listeria monocytogenes is the hardiest of the three aforementioned foodborne pathogens in terms of heat/acid resistance, temperature, a_w and pH ranges at which it can survive and grow. It can cause abortion in pregnant women and meningitis in immunocompromised individuals. The disease, listeriosis, has a very high mortality rate

among susceptible individuals (~20%). Consequently, United States has a "zero tolerance" policy for *L. monocytogenes* in ready-to-eat foods.

Dairy cows that appear healthy can serve as reservoirs for *L. monocytogenes* with this pathogen reportedly present in 1.6-12.0%, 1.3-5.4%, and 2.5-6.0% of the raw milk produced in the United States, Canada and Western Europe, respectively. In the United States, this pathogen has been responsible for at least 46 class I recalls involving domestically produced cheese, 3 of which were prepared from raw milk. Thus, the current "zero tolerance" policy for *L. monocytogenes* has extracted a particularly heavy toll on the dairy industry.

Fermentation is an age-old food preservation method used to inhibit the growth and survival of pathogenic bacteria. Studies of survival and growth of healthy *L. monocytogenes* in Cheddar, Colby and Cottage cheese indicate that *Listeria* numbers slowly decrease during ripening of the cheese. Incomplete pasteurization can lead to the survival and recovery of sublethally injured cells. Such repair requires an optimal pH near 7.0. Given the low pH of Cheddar cheese (~pH 5.0) combined with high levels of salt in the moisture phase, survival of sublethally injured should be far less than that for healthy cells.

The purpose of the study was to assess the ability of healthy and sublethally injured cells of *L. monocytogenes* to compete with different levels of a mesophilic lactic acid starter culture in milks that have undergone various degrees of thermal processing. The underlying hypothesis was that a sub-pasteurization heat treatment can be identified which will sufficiently injure *L. monocytogenes* to prevent its survival in Cheddar cheese beyond 60 days of ripening and thereby preserve the raw milk cheese industry.

LITERATURE REVIEW

RAW MILK CHEESE REGULATIONS

Present-day laws regarding use of pasteurized, heat-treated (sub-pasteurized), and raw milk for cheesemaking date back to World War II. These standards were established more as a safety measure than from any documented scientific evidence. Most U.S. Standards of Identity for cheese and cheese related products (*Anon. 1949*) specify three safety options: (a) milk pasteurization - min. 71.6°C (161°F)/15 sec, (b) holding finished cheese for a minimum of 60 days at a temperature of 1.7°C (35°F) or greater or (c) neither milk pasteurization nor a 60 days holding period for cheeses used as ingredients in further manufacture. Thus, the holding option is required when cheese is prepared from raw or heat-treated milk. Cheeses that can be made from raw milk with a 60-day hold at ≥35°F include Asiago (soft and fresh, medium, old), blue, Nuworld, Parmesan/Regiano, Roquefort, Swiss/Emmentaler, brick, Cheddar, Colby, cold pack/club cold pack cheese food, cold pack cheese food with meat, fruits and vegetables, Edam, Gouda, Granular and stirred curd, grated American cheese food, Limburger, Provolone, soft ripened cheeses, Samsoe, Caciocavallo siciliano, Gorgonzola, Gruyere, hard grating cheese and Romano.

CHEESEBORNE EPIDEMICS

Since institution of the Federal Standards of Identity for cheese in 1948, some foodborne pathogens have survived longer than 60 days in cheese made from raw or heat-treated milk (i.e. less than legal pasteurization) and caused major outbreaks of illness and/or recalls (*Anon. 1999d*). Post processing contamination of cheese prepared from pasteurized milk is also a problem (*Kornacki 1982, Marier et. al 1973*). Epidemiological

surveys in the United States (*Bryan 1983, 1988, Sharp 1987*), Canada (*D'Aoust et. al 1985b, Sharp 1987*), England and Wales (*Barrett 1986, Galbraith et. al 1982, Sharp 1987*) and several Western European countries (*Sharp 1985, 1987*) have verified that dairy products are a relatively safe class of foods. In the United States, dairy products have been vehicles in only 1-3% of all reported foodborne outbreaks (*Barza 1985, Bryan 1983, 1988, CDC 1985, Finch and Blake 1985, Kaplan et al. 1962, Kleeburg 1975, Parry 1966, Potter 1984, Sharp 1987*). Commercially produced cheeses have been sporadically linked to foodborne illness (Table 1). Since 1948, 10 confirmed outbreaks in the United States were traced to domestically produced cheese (Table 1).

Several pathogens including *Brucella melitensis*, *Clostridium botulinum*, *Staphylococcus aureus*, *Streptococcus zooepidemicus* and *Shigella sonnei* have caused cheese-related outbreaks (Table 1). *S. aureus* growth and enterotoxin production during cheesemaking is a potential problem only if there is subnormal acid development by the starter bacteria (*Stadhouders et. al 1978, Tatini et. al 1971, 1973, Tuckey et. al 1964, Zehren and Zehran 1968*). From 1950 to 1965, a series of food poisoning outbreaks caused by *Staphylococcus aureus* enterotoxins occurred in raw milk Cheddar and some other cheeses in the U.S. (Table 1). Attention will be given to outbreaks caused by *L. monocytogenes*, *Salmonella*, *E. coli* O157:H7 since these pathogens can reportedly survive the mandatory 60-day ripening period in various cheeses made from inoculated pasteurized milk (*Goepfert et al. 1968, Hargrove et. al 1969, Park et al. 1970, Reitsma and Henning 1996, Ryser and Marth 1987a*).

Table 1. Foodborne Illness Associated with Cows' Milk Cheese in the United States, Canada, and Europe

Year	Cheese variety	Pathogen	Cases	Remarks
United States:				
1958	Colby	*S. aureus*	Unknown	Slow or failed
1958	Cheddar	*S. aureus*	200	Lactic starters
1965	Cheddar, Kuminost, Monterey	*S. aureus*	Unknown	
1976	Cheddar	*Salmonella heidelburg*	>339	*Salmonella* source unconfirmed
1983	Homemade "Mexican style"	*Streptococcus zooepidemicus*	16	
1985	"Mexican style" soft cheese	*L. monocytogenes*	>100	Contaminated environment
1989	Mozzarella	*Salmonella javiana/oranienburg*	164	
1997	Unpasteurized Mexican-style	*S.* Typhimurium DT 104	31	
1997	Fresh Mexican-style	*S.* Typhimurium DT 104b	79	*Salmonella* in raw milk
1997	Mexican-style soft cheese	*S.* Typhimurium DT 104/b	54	*Salmonella* in raw milk used
Canada:				
1977	Swiss	*S. aureus*	12	Contaminated lactic cultures
1980	Cheese curds	*S. aureus*	62	Not found
1982	Cheddar, other types	*Salmonella*	Unknown	Raw milk used
1984	Cheddar	*Salmonella*	>2700	Faulty pasteurization practices
1994	Soft cheese	*Salmonella berta*	82	Raw milk used

Table 1 (cont'd).

Table 1 (cont'd).

Year	Cheese variety	Pathogen	Cases	Remarks
Europe:				*Country:*
1971	Camembert	Enteropathogenic *E. coli*	387	France, Consumed in U.S.
1974	Soft cheese	*C. botulinum*	77	France, Switzerland
1975	"Mexican style"	*Brucella*	3	Mexico, Consumed in U.S.
1982	Brie	*Bacillus*	>50	
		Shigella sonnei	>50	Scandinavia
1983	Brie	Enteropathogenic *E. coli*	>350	France, consumed in U.S., Sweden, Netherlands
1983	"Farmhouse"	*Salmonella*	35	Finland
1983	Cheddar	*S. aureus*	2	United Kingdom
1983-84	"Homemade, unripened"	*Brucella*	23	Greece
1985	Vacherin	*Salmonella*	215	Switzerland, France, U.S.
1983-87	Vacherin	*L. monocytogenes*	>31	Switzerland
1989	Irish soft cheese	*Salmonella dublin*	42	Ireland, consumed in England, Wales
1994	Brie de Meaux	*L. monocytogenes*	20	France
1997	Morbier cheese	*S.* Typhimurium	113	France

Source: Adapted from (*Johnson et al. 1990a*).

Salmonella was responsible for five cow-milk cheese-related outbreaks in the United States, three in Canada, and at least four in Western Europe. One United States outbreak in 1976 involving seven lots of Cheddar prepared from pasteurized milk caused 339 cases of salmonellosis (*Fontaine et. al 1980*). The contaminated cheese was traced to a Kansas manufacturer. Low numbers of *Salmonella heidelberg* were isolated from all seven cheese lots and from three vats of cheese at the Kansas factory. Examination of the plant revealed no environmental or employee contamination. Raw milk for cheesemaking was stored unrefrigerated for 1 to 3 days in insulated holding tanks before being pasteurized at 71.7°C (161.5°F)/15 s. The pasteurized milk was not examined for bacterial count or alkaline phosphatase activity. The average pH of contaminated cheese was 5.6 vs. 5.4 for the uncontaminated cheese. Slow acid production leading to an abnormally high cheese pH likely facilitated survival and growth of *S. heidelberg* (*Fontaine et. al 1980*). This outbreak can thus be attributed to poor manufacturing practices and inadequate control programs in the cheese plant.

Salmonella enterica serotype Typhimurium definitive type 104 (*S.* Typhimurium DT104) has emerged as the most common multidrug-resistant *Salmonella* strain in the United States and is resistant to 5 different antibiotics (ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline). During spring of 1997, two cheese-related outbreaks involving *S.* Typhimurium DT104 were investigated in a matched case-control study The first outbreak peaked in February 1997; 31 patients were culture-positive for a strain of *S.* Typhimurium var. Copenhagen that exhibited the same pulsed-field gel electrophoresis (PFGE) pattern. This strain was identified as phage type DT104. In a subsequent case-control study, 15 of 16 *S.* Typhimurium var. Copenhagen cases

compared with 14 of 24 matched controls reported eating unpasteurized Mexican-style cheese. Enhanced surveillance uncovered a second outbreak, which peaked in April 1997 and was caused by a non-Copenhagen variant of *S.* Typhimurium. During this second outbreak, *S.* Typhimurium was isolated from 79 people who ate fresh Mexican-style cheese from street vendors, as well as from some cheese samples and raw milk used in cheesemaking. The PFGE pattern of the milk isolate matched 1 of 3 patient strains with all isolates identified as phage type DT104b (*Cody et. al 1999*).

In early 1997, a 5-fold increase in salmonellosis among Hispanics was observed in Yakima County, Washington. Bacterial strains and risk factors for infection with *S.* Typhimurium in Yakima County were investigated in laboratory, case-control and environmental studies. Between January and May 1997, 54 culture-confirmed cases of *S.* Typhimurium were reported. The median age of patients was 4 years and 91% were Hispanic. Overall, 77% of the cases reported eating unpasteurized Mexican-style soft cheese in the 7-day period preceding onset of illness, compared to 28% of the controls. All case isolates were phage type DT104 or DT104b. Cheese consumed by two unrelated patients was made from raw milk, which was traced back to the same local farm. Milk samples from nearby dairies yielded *S.* Typhimurium DT104 (*Villar et. al 1999*).

During 1980 to 1982, several outbreaks of salmonellosis were traced to raw milk Cheddar that was produced in Ontario, Canada (*Wood et. al 1984*). The milk used for cheesemaking came from a farm where one cow was shedding *Salmonella muenster* in her milk. This naturally contaminated raw milk was then used to determine survival of *S. muenster* during commercial preparation of raw milk cheese. Curd from 11 of 181 vats tested positive with two of these lots still positive after pressing. During curing at 41°F,

one lot was negative after 30 days, but one lot was positive after 125 days. No significant compositional differences were observed between the lots of contaminated cheese.

Another large Canadian outbreak of salmonellosis involving Cheddar cheese occurred during March-July 1984 in the four Maritime Provinces (*D'Aoust 1985a, D'Aoust et. al 1985b, Ratnam and March 1986*). Over 2700 people were infected with *S.* Typhimurium. Epidemiological evidence implicated Cheddar cheese that was manufactured by a single plant on Prince Edward Island. *Salmonella* Typhimurium was sporadically detected in Cheddar cheese that was manufactured at this facility from either pasteurized milk [73.8°C (165°F)/16 s] or heat-treated milk [66.7°C (152°F)/16 s]. *Salmonella* was first confirmed in a cheese trim bucket. One of the employees who used their hands to transfer curds to a forming machine also tested positive for *S.* Typhimurium. Testing of the raw milk supply ultimately identified two cows in separate herds, one shedding *S.* Typhimurium from one quarter of her udder, the other shedding *S. heidelberg.* A thorough evaluation of the pasteurization process revealed that the pasteurizer operator manually over-rode the electronic controller, thereby shutting down the pasteurizer while milk continued to flow through the unit and into a vat, leading to *Salmonella*-positive vats. This only occurred when raw milk from the infected cow shedding *S.* Typhimurium was used.

D'Aoust et al. (*D'Aoust et. al 1985b*) investigated survival of *S.* Typhimurium in the contaminated cheese lots. *Salmonella* Typhimurium was present in mild Cheddar made from either heat-treated or pasteurized milk. Analysis of six contaminated cheese lots indicated that the cheeses contained 0.39 to 9.3 *Salmonella* CFU/100 g. *Salmonella* Typhimurium was detected in 1 of 6 cheese lots cured for eight months at 5°C (41°F).

However, some cheeses also showed heavy mold growth, which may have contributed to survival of *S.* Typhimurium (*D'Aoust et. al 1985b*).

Four *Salmonella* outbreaks in Europe have been traced to cheese (Table 1). The latest community-wide outbreak of salmonellosis was reported in France during 1997. A total of 113 cases were identified in a case control study with one batch of Morbier cheese (soft raw milk cheese) from one processing plant identified as the source of *S.* Typhimurium (*Valk et al. 2000*). These studies demonstrate that *Salmonella* can survive past the 60 day holding requirement at ≥35°F. The outbreaks also indicate that soft cheese made from unpasteurized milk is an important vehicle for *S.* Typhimurium transmission. The need for good manufacturing practices and adequate process control programs in the cheese factory is also underscored.

A major outbreak of gastrointestinal illness caused by enteropathogenic *E. coli* occurred in the United States in 1971 (*Kornacki 1982, Marier et. al 1973*). This outbreak which included at least 387 cases was traced to Camembert cheese prepared by a single manufacturer in France. All the contaminated cheese was manufactured during a 2-day period at one plant and contained 10^5 to 10^7 *E. coli* O:124 per gram. The same serotype was found in stool specimens. The attack rate was >94% for people who consumed the cheese. Although the source of contamination was never confirmed, the filtration system for river water used in washing equipment was not working properly when the contaminated cheese was manufactured. Enteric pathogens were not isolated from the water or from those employees that were available for examination. While the epidemic strain was never isolated from the starter culture, salt, or the equipment, this organism was recovered from the curdling tank which suggests post-pasteurization contamination.

In 1983, three outbreaks of enteropathogenic *Escherichia coli* affected 45 persons who attended office parties in Washington DC. Additional cases were later identified in Illinois (75 cases), Wisconsin (35 cases), Georgia (10 cases), and Colorado (4 cases). Brie cheese imported from France was identified as the vehicle by epidemiological and laboratory investigations. Stools of the victims contained enterotoxigenic *E. coli* serotype O27:H20. Cultures of cheese did not yield *E. coli* O27:H20 although coliform counts ranged from 10^2 to 10^8 CFU/g (*MacDonald et. al 1985*). Isolation from stools but not from cheese suggests that other foods or mishandling of cheese during distribution may have contributed to the outbreak.

Listeria monocytogenes was not identified as a serious foodborne pathogen until 1981 when 41 cases of listeriosis in Canada, including 17 deaths, were linked to consumption of contaminated coleslaw (*Gravani 1999*). Despite further evidence 2 years later suggesting possible involvement of pasteurized milk in an outbreak of listeriosis in Massachusetts, the presence of *L. monocytogenes* in food was not yet regarded as a major threat to public health. However, this situation changed dramatically in June of 1985 when a major listeriosis epidemic occurred in California (*Linnan et. al 1988*). As many as 300 cases, including 85 fatalities were reported (*Gravani 1999*). In 1988, Linnan and his team published their findings concerning 142 cases in Los Angeles County that were linked to this outbreak. Ninety-three (65.5%) cases involved pregnant women or their off-spring with the remaining 49 (34.5%) cases involving non-pregnant adults. Forty-eight of the 142 listeriosis victims died giving a mortality rate of 33.8%. *L. monocytogenes* serotype 4b comprised over 80% of the patient isolates (*Linnan et. al 1988*). *Listeria*-contaminated Mexican-style cheese from a single factory was confirmed as the vehicle of

transmission, since the serotypes and phage types of isolates from cheese and the clinical cases were identical. *Listeria* was not detected in raw milk samples from dairy herds that produced milk for the factory. However, these samples were taken after the cheese factory closed. The factory environment and equipment were grossly contaminated with *L. monocytogenes*, including a vat pasteurizer that yielded the organism after clean up. This pasteurizer, used to process a milk-vegetable fat premix used in cheese, had neither controls nor a head space heater. Final reports indicated that *L. monocytogenes* most likely entered the cheese during manufacture through direct addition of raw milk. Since the plant received 10% more raw milk than could be pasteurized by their pasteurizer, unpasteurized milk was possibly mixed deliberately with pasteurized milk for cheesemaking (*Linnan et. al 1988*).

Considerable evidence indicates that *L. monocytogenes* and *Listeria innocua* are primarily introduced into cheese during curing. Such contamination has occurred in cheeses produced from either pasteurized or raw milk (*Bradshaw et. al 1987, Mossel 1987, Prentice and Neaves 1987*). Outbreaks of listeriosis associated with soft and semi-soft ripened cheeses prepared from either pasteurized or raw milk have been reported in France (*Anon. 1988b*), Switzerland (including a large listeriosis outbreak traced to Vacherin Mont d'Or soft-ripened cheese) (*Breer 1987, Bula et al. 1988, Malinverni et. al 1985, Mossel 1987*), and the United Kingdom (*Azadian et. al 1989, Bannister 1987*). Investigations in the U.K. have shown that listeriosis can be transmitted via soft cheese to immunocompetent, healthy individuals (*Azadian et. al 1989, Bannister 1987*). In 1987, a woman was hospitalized with symptoms of fever, back pain, aching legs, and neck stiffness which led to severe meningitis (*Bannister 1987*). *Listeria monocytogenes*

serotype 4b was isolated from cerebrospinal liquid (CSF) and the remaining portions of some French soft cheese from her refrigerator. However, *L. monocytogenes* was not recovered from unopened packages of commercial cheese, which were prepared from pasteurized milk. In another case, a 40-year-old immunocompetent woman was hospitalized with a 4-day history of headache, fever, and one episode of vomiting. She had consumed most of a 4 oz (114 g) package of goat's milk whey cheese about 24 h before developing symptoms. *Listeria monocytogenes* serotype 4b was isolated from the patient's CSF and from four packages of cheese (30 to 50 million organisms/g). *Listeria* populations reportedly increased while the cheese was stored in a display cabinet at 8°C (46.4°F) (*Azadian et. al 1989*). These incidents emphasize that post-pasteurization contamination and growth of *L. monocytogenes* are important risk factors in cheese-borne listeriosis. Standard sanitation operating procedures for the factory, good manufacturing practices, use of active starter cultures, good personal hygiene, and careful cheese handling until consumption must be followed, especially when cheese is prepared from raw and subpasteurized milk.

LISTERIA MONOCYTOGENES

Listeria monocytogenes, one of six species of *Listeria*, is generally hardier than the aforementioned foodborne pathogens including *S.* Typhimurium, *E. coli* O157:H7 and *S. aureus*, in terms of heat/acid resistance, temperature, a_w and pH ranges for survival and growth. *Listeria monocytogenes* is a small (0.5μm x 1-2μm), gram-positive, non-spore forming rod with rounded ends. Cells are usually found singly, or in short chains, or may be arranged in V and Y forms. *Listeria* is motile by peritrichous flagella when cultured at 20-25°C, but not motile when grown at 37°C. *Listeria* grows well on most

commonly used bacteriological media. The growth rate is increased by the presence of fermentable sugars, particularly glucose. Normal temperature limits for growth are +1-2°C to 45°C (*Gray and Killinger 1966*) with an optimum range of 30-37°C (*Petran and Zottola 1989, Seeliger 1961*). Growth is slow at refrigeration temperatures, with generation times of 30-40 h at 4°C. *Listeria* is one of the few foodborne pathogens that can grow at an a_w value of 0.93 (*Rocourt 1999*). *Listeria monocytogenes* is ubiquitous in nature, being commonly found in soil and water and on plant material, particularly that which has undergone decay. The organism can survive longer under adverse environmental conditions than many other non spore-forming foodborne pathogens. This resistance, together with the ability to colonize, multiply, and persist on processing equipment makes *L. monocytogenes* a major threat to the food industry (*Fenlon 1999*).

Listeriosis, the human disease caused by *L. monocytogenes* usually occurs in certain well-defined high-risk groups including pregnant women, neonates and immunocompromised adults (elderly people and those suffering from diseases like AIDS) but may occasionally occur in people who have no predisposing underlying condition. Unlike infection with other common foodborne pathogens, listeriosis has a mortality rate of ~20% (*Gellin and Broome 1989*). Manifestations include septicemia, meningitis (or meningoencephalitis), encephalitis, and bacteremia in immunocompromised individuals; sepsis or meningitis in neonatal infection (depending on onset time) (*Gray and Killinger 1966, Seeliger 1961*) and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion (2nd/3rd trimester) or stillbirth. Overall mortality may be as high as 70, 80 and 50% in cases of meningitis, septicemia and perinatal/neonatal infections, respectively (*FDA/CFSAN*). The onset of the aforementioned disorders is

usually preceded by influenza-like symptoms including persistent fever. Gastrointestinal symptoms such as nausea, vomiting, and diarrhea may precede more serious forms of listeriosis or may be the only symptoms expressed in normal hosts that consume foods contaminated with *L. monocytogenes* (*Dalton et al. 1997, Slutsker and Schuchat 1999*). The onset time for the most serious forms of listeriosis is unknown but may range from a 3 to 70 days. The onset time for the gastrointestinal form of listeriosis is far shorter, ranging from 12 hours to a few days (*FDA/CFSAN*). This uncertainty in onset time leads to obvious difficulties in identifying cases of foodborne listeriosis.

According to the Food, Drug and Cosmetic Act of 1938, a food may be considered adulterated and therefore unfit for human consumption if the product contains harmful substances (e.g., pathogenic organisms). While still unknown, the oral infective dose of *L. monocytogenes* varies widely with the strain and susceptibility of the individual. Evidence from cases contracted through raw or supposedly pasteurized milk as well as the California cheese outbreak suggests that the number of *L. monocytogenes* cells needed to induce listeriosis may be quite low – perhaps as few as several hundred to a few thousand cells in certain high-risk segments of the population. Consequently, because of the moral obligation to the public, the FDA has adopted and continues to uphold the current policy of "zero tolerance" for the presence of *L. monocytogenes* in ready-to-eat foods (*Ryser 1999c*).

L. monocytogenes may invade the gastrointestinal epithelium. Once the bacterium enters the host's monocytes, macrophages, or polymorphonuclear leukocytes, it is bloodborne (septicemic) and can grow. Its presence intracellularly in phagocytic cells also permits access to the brain and probably transplacental migration to the fetus in

pregnant women. The pathogenesis of *L. monocytogenes* centers on its ability to survive and multiply in phagocytic host cells (*FDA/CFSAN*).

Sporadic cases of listeriosis in dairy cows (symptoms include encephalitis, abortion and septicemia) in which *L. monocytogenes* was intermittently shed in milk over several lactation periods have been recorded in the literature for more than 50 years. The apparently normal appearance of milk and consumption of raw milk on farms could be important factors in the transmission and epidemiology of milkborne listerial infection (*Wesley 1999*). Dairy cows that appear healthy can serve as reservoirs for *L. monocytogenes* and secrete the organism in milk (*Ryser 1999b*). Milk and milk products have been linked to cases of foodborne listeriosis for over 17 years. Following the pasteurized milk outbreak in Massachusetts and the California cheese outbreak, FDA officials in cooperation with state governments and the dairy industry intensified their surveillance programs under the Dairy Safety Initiative Program, which began April 1, 1986 (*Kozak 1986*). FDA surveys in 1986 indicated that an average of 2.5 % of all dairy products manufactured from pasteurized milk contained *L. monocytogenes* (*Anon. 1986*). A subsequent report in February 1987 indicated generally similar contamination rates with 2.6% of dairy processing facilities manufacturing finished products containing *L. monocytogenes* (*Anon. 1987c*). *Listeria monocytogenes* is reportedly present in 1.6 to 12.0% of all raw milk produced in the United States (4% average) (*Donnelly et al. 1988, Hayes et. al 1986, Liewen and Plantz 1988, Lovett 1987*). Incidence rates outside the United States are generally similar with 1.3 to 5.4% of Canadian and 2.5 to 6.0% of Western European raw milk yielding *L. monocytogenes* (*Ryser 1999a*). While some early reports indicated that *L. monocytogenes* could survive pasteurization (*Bearns and Girard*

1958), others proved these findings to be false (*Bradshaw et. al 1985, Farber 1989, Mackey and Bratchell 1989*). As mentioned before, this pathogen has thus far been responsible for four major soft cheese-related outbreaks that included over 100 deaths (*Ryser 1999a*). Owing to the current "zero tolerance" policy, *L. monocytogenes* has extracted a particularly heavy toll on the dairy industry in terms of Class I recalls.

SURVEILLANCE FOR *L. MONOCYTOGENES* IN CHEESE

As a result of the 1985 listeriosis outbreak in California, FDA officials added *L. monocytogenes* to their list of bacterial pathogens that should be of concern to cheesemakers and began surveying various soft domestic cheeses for listeriae. Less than one month after the first nationwide Class I *Listeria*-associated recall (Table 2) was issued for 22 varieties of Mexican-style cheese (~500,000 lb) contaminated with *L. monocytogenes*, the FDA developed a series of programs designed to prevent the reoccurrence of such an outbreak (*Skinner 1989*) (Figure 1).

The Domestic Soft Cheese Surveillance Program-the first of the Dairy Initiative Programs-was instituted by the FDA in July of 1985 and involved on-site inspection of firms manufacturing soft cheese (*Anon. 1985*). Priority was given to manufacturers of Mexican-style soft cheese, followed by firms producing other ethnic-type soft cheeses such as Edam, Gouda, Liederkranz, Limburger, Monterey Jack, Muenster, and Port du Salut from raw, heat-treated [<71.7°C (161°F)/15 sec] or pasteurized [>71.7°C (161°F)/15 sec] milk. Between June 1985 and October 1988, FDA inspectors collected cheese samples to be analyzed for *L. monocytogenes* using the original FDA procedure (*Ryser 1999a*).

Less than 2 months into this program, FDA officials isolated a pathogenic strain of *L. monocytogenes* from one sample of domestically produced Liederkranz cheese. In general, FDA inspections of other soft cheese factories uncovered problems similar to those encountered during inspections of Grade A fluid milk factories: (a) potential bypasses of the pasteurizer, (b) post pasteurization blending of product, and (c) a general lack of education and/or training of factory personnel (*McBean 1988*). Items of particular concern to cheesemakers that were not generally found during the above visits included defects in the pasteurization process, discrepancies in pasteurization/production records, and a higher incidence (than in Grade A milk factories) of pathogenic microorganisms (including *L. monocytogenes*) on environmental surfaces in production and storage areas (*Ryser 1999a*).

Inspections of domestic cheese factories continued throughout 1986, 1987, and 1988 under four separate programs (Figure 1) with FDA officials reaching nearly half of the 400 soft cheese factories in the United States by April of 1986 and the remaining factories (including follow-up inspections of problem factories) by late 1987 (*Anon. 1987b*). According to FDA records (*Archer 1988*), *L. monocytogenes* was identified in 12 of 586 (1.82%) domestic cheese samples analyzed during 1986. During these inspection programs, six Class I recalls were issued for various ethnic-type soft and semi-soft cheeses contaminated with *L. monocytogenes* (Table 2). Given the ability of *L. monocytogenes* to grow in these soft cheeses during refrigerated storage and marketing, Hispanic-style cheeses continue to constitute a significant public threat, with these varieties thus far accounting for 13 of 38 recalls issued (Table 2), including one large recall in 1990 involving approximately 500,000 lb of product.

Table 2: Chronological List of Class I Recalls in the United States for Domestic Cheese Contaminated with *L. monocytogenes*

Type of cheese	Date recall initiated	Origin	Distribution	Quantity (lb)	Ref.
Jalisco brand soft Mexican-style: Cotija, Queso Fresco, and 20 other varieties	6/13/85	California	Arizona, Arkansas, California, Colorado, Georgia, Guam, Hawaii, Idaho, Illinois, Kansas, Louisiana, Marshall Islands, Massachusetts, Nevada, New Jersey, New Mexico, New York, Oklahoma, Oregon, Rhode Island, Samoa, Texas, Utah, Washington State	~500,000	(*Ryser 1999a*)
Liederkranz (Brie,[a] Camembert)	8/14/85	Ohio	Nationwide, Puerto Rico	~10,000	-do-
Soft Mexican-style: Queso Fresco and 5 other varieties	3/5/86	California	Arizona, California, Oregon, Texas	127,607	-do-
Semisoft Salvador-style white	9/11/86	Virginia	Virginia, Washington, DC	10,850	-do-
Soft-ripened: Old Heidelberg	4/17/87	Illinois	Illinois, North Carolina, Ohio, Pennsylvania	1150	-do-
Soft-ripened: Bonbel and Gouda	5/6/87	Kentucky	Nationwide	~13,800	-do-
Raw milk sharp Cheddar	8/21/87	Wisconsin	California, Washington State	~1400	-do-
Soft Mexican-style: Cotija, Queso Fresco, and 8 other varieties + Baby Jack and Monterey Jack	1/29/88	California	Arizona, California, Florida, Texas, Washington State	Unknown	-do-
Mexican-style soft cheese	11/6/90	California	Arizona, California, Idaho, Nevada, Oregon, Washington State	500,000	-do-

Table 2 (cont'd).

Type of cheese	Date recall initiated	Origin	Distribution	Quantity (lb)	Ref.
Cheese spread	2/l/91	Florida	Southeastern United States	~1362	(*Ryser 1999a*)
Mozzarella	2/14/91	Wisconsin	Connecticut, Georgia, Illinois, Michigan, New York, Ohio, Pennsylvania, Texas, West Virginia, Wisconsin	>89,000	-do-
Ricotta	7/11/91	New York	Florida, New York	1109	-do-
Jack	10/28/91	Wisconsin	Iowa, Minnesota, Wisconsin	12,500	-do-
Cold-pack cheese food	3/10/92	Wisconsin	Arizona, California, Colorado, Florida, Georgia, Illinois, Indiana, Maryland, Michigan, Minnesota, New York, Ohio, Pennsylvania, Tennessee, Texas, Vermont, Virginia, Wisconsin	Unknown	-do-
Queso fresco	10/14/92	Washington	Oregon, Washington State	Unknown	-do-
Limburger	12/18/92	Wisconsin	Wisconsin	1500	-do-
Cheese spread	3/4/93	Tennessee	Alabama, Illinois, Indiana, Kentucky, Mississippi, Tennessee	11,789	-do-
Cream cheese	10/19/93	Wisconsin	California, Florida, Georgia, Illinois, Indiana, Iowa, Minnesota, Nebraska, North Carolina, Ohio, South Carolina, South Dakota, Tennessee, Wisconsin	3075	-do-
Queso prensado	4/15/94	Wisconsin	Florida, New Jersey, Wisconsin	1429	-do-
Cream cheese and lox	5/11/94	Massachusetts	Connecticut, Georgia, Massachusetts	20	-do-
Mexican-style soft white	5/20/94	Texas	Texas	Unknown	-do-
Mexican-style soft white	5/21/94	Texas	Texas	Unknown	-do-
Mexican-style soft white	5/23/94	Texas	Texas	Unknown	-do-

Table 2 (cont'd).

Type of cheese	Date recall initiated	Origin	Distribution	Quantity (lb)	Ref.
Queso blanco	5/24/94	Wisconsin	New Jersey	1220	(*Ryser 1999a*)
Goat milk cheese	6/15/94	California	California, Colorado, Georgia, Illinois, Massachusetts, Michigan, New York, Oregon, Texas	~5,682	-do-
Torte loaf cheese	8/11/94	Missouri	Illinois, Indiana, Kansas, Louisiana, Missouri, Texas	301	-do-
Swiss cold-pack cheese food	8/11/94	Wisconsin	Missouri, Ohio	510	-do-
Swiss	10/28/94	Ohio	Pennsylvania	2270	-do-
Gorgonzola	2/2/96	Wisconsin	California, Colorado, Florida, Georgia, Illinois, Minnesota, New Jersey, New York, North Carolina, Pennsylvania, Tennessee, Washington State, Wisconsin	4500	-do-
Cream cheese with vegetables	10/30/97	Massachusetts	Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Rhode Island, Pennsylvania, Vermont	7,340	-do-
Cream cheese	11/14/97	Massachusetts	Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Rhode Island, Vermont	Unknown	-do-
Queso fresco	2/4/98	Wisconsin	Nationwide	248,938	-do-
Queso fresco	3/23/98	Domestic	Alabama, Florida, Georgia, North Carolina, South Carolina, Tennessee, Virginia	Unknown	-do-
Mozzarella	3/27/98	California	California, Colorado, Nevada	Unknown	-do-
Blue cheese	4/10/98	Wisconsin	Nationwide	4,122	(*Anon. 1998a, 1998d*)
Blue cheese salad dressing	5/1/98	Louisiana	Nationwide	Unknown	(*Ryser 1999a*)

Table 2 (cont'd).

Type of cheese	Date recall initiated	Origin	Distribution	Quantity (lb)	Ref.
Spanish White Cheese	5/26/98	New York	New York	105	(*Anon. 1998c*)
Mexican Style Fresh White Cheese	7/27/98	New York	New York	Unknown	(*Anon. 1998b*)
Pimento Cheese Spread	1/7/99	North Carolina	North Carolina, South Carolina	312	(*Anon. 1999f*)
Shredded Monterey Jack	3/29/99	Iowa	Texas	6000	(*Anon. 1999e*)
Raw milk Chives Colby	5/22/99	Missouri	Arizona, California, Colorado, Georgia, Illinois, Maryland, Missouri, New York, Pennsylvania, South Carolina, Tennessee, Utah	135	(*Anon. 1999d*)
Raw milk mild Cheddar	6/11/99	Missouri	Alabama, Arkansas, Florida, Georgia, Michigan, Missouri, North Carolina, Pennsylvania, Tennessee, Washington State	228	(*Anon. 1999c*)
Queso Fresco	6/1/99	New Jersey	New Jersey, New York	500	(*Anon. 1999a*)
Pimento Spread	3/17/00	South Carolina	Alabama, North Carolina, South Carolina, Tennessee, Virginia,	Unknown	(*Anon. 2000c*)
Medium Cheddar	7/24/00	Oregon	Alaska, California, Oregon, Washington State	1,260	(*Anon. 2000a*)
Colby Jack and Mexican Blend cheese	11/6/00	Wisconsin	Nationwide distribution	Unknown	(*FDA News Relsease*)

[a] Later found to contain only *L. innocua*.

Source: Adapted from (*Ryser 1999a*)

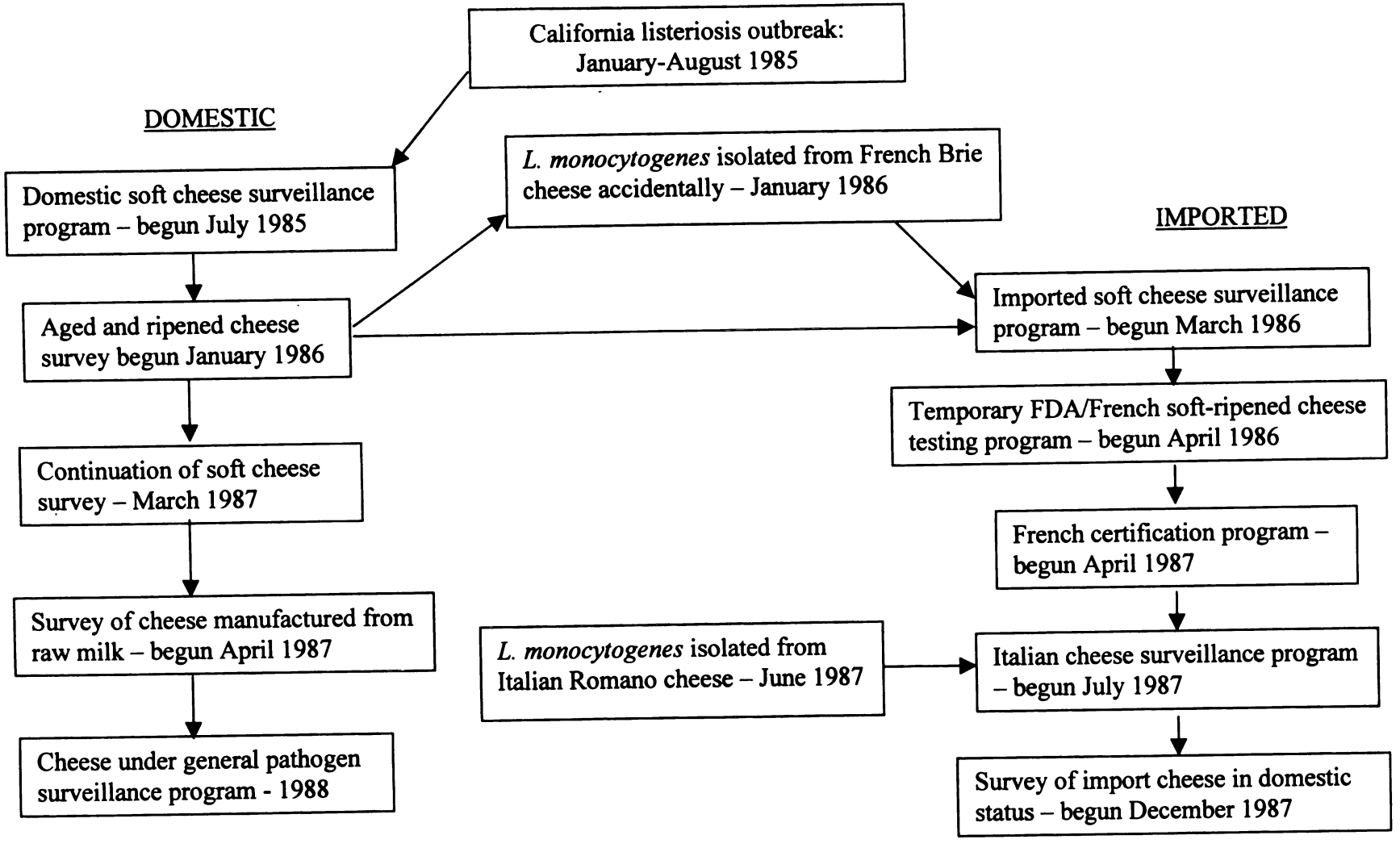

Figure 1. Surveillance programs for *Listeria* spp. in domestic and imported cheese. Source: Reprinted from (*Ryser 1999a*).

While all products contaminated with *L. monocytogenes* must be retrieved from the marketplace, formal Class I recalls do not have to be issued for contaminated products that have not yet left the factory. Since such situations typically lead to nonpublished "internal recalls" issued by the manufacturer, more cheese was likely destroyed during this 12-year period than has actually been reported (*Ryser 1999a*).

Following a report by Ryser and Marth (*Ryser and Marth 1987a*) that *L. monocytogenes* can survive more than one year in Cheddar cheese (i.e., well beyond the mandatory 60-day aging period for Cheddar cheese manufactured from raw milk), the FDA modified its Domestic Cheese Program in August of 1987 to include cheese prepared from unpasteurized milk (*Anon. 1987b*). Between April and October of 1987, 181 samples of domestic aged [held a minimum of 60 days at $\geq$1.7°C (35°F)] natural cheese manufactured from raw milk, as well as similar imported cheeses in domestic status, were collected from retail stores by FDA field personnel and analyzed for *L. monocytogenes*. These efforts uncovered one positive sample-a sharp Cheddar cheese manufactured in Wisconsin, which was subsequently recalled from the market in July of 1987 (Table 2).

Isolation of *L. monocytogenes* from several imported Brie cheeses between 1986 and 1988 led to the eventual recall of approximately 300,000 tons of Brie cheese imported from France (Table 3) which prompted a real concern about the incidence of this pathogen in other European cheeses. Recall of the Brie cheese led to two corrective measures: (a) adoption of a cheese certification program by the United States and France to prevent importation of *Listeria*-contaminated cheese and (b) initiation of numerous large-scale surveys to determine the extent of *Listeria* contamination in virtually all types

of cheese manufactured in the United States, Canada, and Western Europe. After first isolating listeriae from a hard cheese (Italian Pecorino Romano cheese prepared from goat's milk) in June of 1987, (Figure 1) (*Skinner 1989*), the previous import alert was extended to include both soft and hard varieties of Italian cheese (*Anon. 1987a*). Subsequently, the FDA ordered intensified sampling of soft and hard cheese for the next two months as part of the ongoing imported cheese surveillance program (Figure 1) (*Anon. 1988b*). This action prompted the recall of several Danish cheeses in early 1988, four separate Class I recalls for *Listeria*-contaminated soft cheeses manufactured in Cyprus (Table 3) as well as an import alert for contaminated soft and hard cheeses produced by two Italian firms in the latter half of 1989 (*Farber et al. 1988*). The overall situation regarding presence of *L. monocytogenes* in imported cheese has greatly improved since 1986 (*Anon. 1990*) with only four additional recalls of imported cheese since 1990 (Table 3).

Sporadic detection of listeriae in domestic and imported cheeses suggests that surveillance of such products is still necessary to safeguard public health. Moreover, as mentioned earlier, class I recalls lead to heavy economic loss in terms of product retrieval, product disposal and consumer lawsuits as well as possible loss of market share for the company's products, lost productivity and related medical expenses. Thus, faulty cheesemaking practices could have devastating effects on consumers as well as the company.

Table 3: Chronological List of Class I Recalls in the United States for Imported Cheese Contaminated with *L. monocytogenes*

Type of cheese	Date recall initiated	Country of manufacture	Distribution	Quantity	Ref.
Brie	2/12/86	France	Bermuda, Nationwide	57,000 2-6-lb wheels	(*Ryser 1999a*)
Brie	2/14/86	France	Georgia, New Jersey	40 cases	-do-
Brie	2/14/86	France	Colorado, Connecticut, Florida, Louisiana, Maryland, Massachusetts, New Jersey, New York, Ohio, Washington, DC	100 cases	-do-
Brie	2/14/86	France	Florida, New York, Washington, DC	10 cases	-do-
Brie	2/14/86	France	Nationwide	Unknown	-do-
Brie	2/21/86	France	Oregon, Washington State	Unknown	-do-
Brie	2/24/86	France	Illinois, Minnesota, New Jersey	909 cases	-do-
Brie	3/14/86	France	Nationwide	~660 million lb	-do-
Brie	4/l/86	France	Colorado, Connecticut, Georgia, New Jersey, New York, North Carolina, Texas, Washington, DC	~230 lb	-do-
Soft-ripened: Tourre de l'Aubier and Fromage des Burons	6/23/86	France	New York, Ohio, Pennsylvania	Unknown	-do-
Soft-ripened: Tourre de l'Aubier	8/13/86	France	California, Illinois, Maine, Massachusetts, New Jersey, New York, Oregon	1056 lb	-do-
Semişoft: Morbier Rippoz	8118/86	France	Illinois, Massachusetts, Michigan	~1600 lb	-do-
Soft-unripened, full fat	4/16/87	France	New Jersey, New York, Texas	15 wheels	-do-
Semisoft	1/27/88	Italy	Nationwide	410 cartons	-do-
Semisoft: L'Amulette Danish Esrom	2/11/88	Denmark	California	Unknown	-do-

Table 3 (cont'd).

Type of cheese	Date recall initiated	Country of manufacture	Distribution	Quantity	Ref.
Semisoft: L'Amulette Danish Esrom	2/18/88	Denmark	Florida, Massachusetts, New Jersey, New York	~1,150 lb	(*Ryser 1999a*)
Blue	4/6/88	Denmark	California, Florida, Illinois, Massachusetts, Michigan, Minnesota, New Jersey, New York, North Carolina, Oregon, Pennsylvania, Texas	~5,000 lb	-do-
Anari	5/26/89	Cyprus	New York	50 cases	-do-
Anari	7/27/89	Cyprus	Illinois, Texas	79 cases	-do-
Anari	8/9/89	Cyprus	New York	80 cases	-do-
Hallounii	9/15/89	Cyprus	Florida, New Jersey, New York	14,400 lb	-do-
Italian soft ripened and semisoft	7/27/90	Italy	California, Connecticut, New Jersey, New York, Pennsylvania	Unknown	-do-
Fontina	4/6/93	Sweden	California, Connecticut, Maryland, Massachusetts, Minnesota, New Hampshire, New York, North Carolina, Pennsylvania, Rhode Island, Washington State	85,080 lb	-do-
Limburger	2/29/96	Germany	Florida, Indiana, Maine, Massachusetts, New Jersey, New York, Ohio, Utah, Virginia	813 lb	-do-
Jarlsberg	6/7/96	Norway	Alaska, California, Guam, Hawaii, Idaho, Montana, Nevada, Oregon, Utah, Washington State	30,727 lb	-do-
Ricotta Salata	5/25/99	Italy	Connecticut, Delaware, Florida, Georgia, Indiana, Maryland, Massachusetts, Nebraska, New Hampshire, New Jersey, New York, North Carolina, Pennsylvania, South Carolina, Tennessee, Virginia.	164 cases (8 oz wedge and 0.6 lb wheel)	(*Anon. 1999b*)

Source: Adapted from (*Ryser 1999a*).

BEHAVIOR OF *L. MONOCYTOGENES* IN DIFFERENT PRODUCTS

Cheeseborne listeriosis outbreaks prompted scientists on both sides of the Atlantic Ocean to determine the incidence of *Listeria* spp. in various cheeses and examine the behavior of *L. monocytogenes* during manufacture and storage of fermented dairy products (*Ryser 1999a*). While results from two Yugoslavian studies concerned with behavior of *L. monocytogenes* in various fermented dairy products were published in 1964 (*Ikonomov and Todorov 1964*) and 1981 (*Stajner et al. 1979*), neither surveys dealing with the incidence of listeriae in fermented dairy products nor research on behavior of *L. monocytogenes* in cheese was conducted before contaminated Mexican-style cheese was linked to the California listeriosis outbreak in June of 1985.

Milk Fermentation

Schaack and Marth (*1988a*) investigated the fate of *L. monocytogenes* in sterile skim milk that was fermented with *Lactococcus lactis* subsp. *lactis* (LL) and *Lactococcus lactis* subsp. *cremoris* (LC) in sterile skim milk. Milk samples containing different levels of LL or LC (5.0, 1.0, 0.5 or 0.1%) was inoculated to contain one of two *L. monocytogenes* strains at a level of 10^3 CFU/ml. Inoculated milks were fermented for 15 h at 21 or 30°C, followed by refrigeration at 4°C. *Listeria monocytogenes* survived all fermentations and grew to some extent. Incubation at 30°C with 5.0% LL was most inhibitory to *L. monocytogenes*. At 30°C, LC was less inhibitory to *L. monocytogenes* than LL at inoculum levels of 0.1 and 5.0%. Growth of *L. monocytogenes* generally ceased when the pH dropped below 4.75.

In a similar study by El-Gazzar et al. (*1992*), *L. monocytogenes* was inhibited by a four strain mixture of LC in sterile skim milk but survived the 36-h fermentation at 30°C.

When this milk was stored at 4°C, *L. monocytogenes* survived 4 to 6 weeks, with the length of survival dependant on the *Listeria* strain. Both of these studies show that *L. monocytogenes* can survive in milk fermented by mesophilic lactic acid bacteria used in cheesemaking, thus, suggesting potential public health problems if post-processing contamination of cheesemilk occurs.

Behavior in cheese

Ryser and Marth (*1987a*) studied the fate of *L. monocytogenes* during Cheddar cheesemaking and ripening. Pasteurized whole milk inoculated to contain ~2.5 logs of *L. monocytogenes* CFU/ml was made into stirred-curd Cheddar cheese. Cheese was ripened at 6 or 13°C. During cheese manufacture, *Listeria* counts remained relatively constant. After overnight pressing, numbers of *L. monocytogenes* increased to about 3 logs/g of curd. Highest numbers of *Listeria*, ~3.5 logs/g, were detected in cheese after 14 days of ripening. The three different *L. monocytogenes* strains studied survived as long as 224, 154 and at least 434 days in Cheddar cheese of normal composition with greatest survival generally seen in cheese ripened at 6 rather than 13°C. Additional studies conducted with *Salmonella* Typhimurium and *Escherichia coli* O157:H7 showed that these pathogens could survive up to 210 days (*Goepfert et al. 1968, Hargrove et. al 1969, Park et al. 1970*) and 138 days (*Reitsma and Henning 1996*), respectively, in Cheddar cheese produced from artificially contaminated pasteurized milk.

Yousef and Marth (*1988*) prepared Colby cheese from pasteurized milk that was inoculated to contain 10^2-10^3 *L. monocytogenes* CFU/ml. Up to 3.2% of the *Listeria* population was recovered in the whey and the mean count in the curd was 1.27 log higher than in the milk. The cheese was ripened at 4°C for 140 days. *Listeria* populations

remained fairly constant during the first 3 to 5 weeks of ripening. Thereafter, numbers of *Listeria* decreased almost linearly. The D-values were 143 and 105 days in 2 cheeses having >40% moisture and 51-67 days in 4 cheeses with <40% moisture. After 140 days, survival differences were observed between the two strains with higher initial numbers of *Listeria* in milk leading to greater survival. Hence, both of these studies indicate the ability of *L. monocytogenes* to survive beyond the mandatory 60-day ripening for Cheddar and Colby cheese.

Parmesan cheese, a hard cheese with a low moisture content, was prepared by Yousef and Marth (*1989*) from pasteurized milk inoculated to contain ~10^4-10^5 *L. monocytogenes* CFU/ml (2 strains studied). Unlike the previous cheeses, a lipolytic enzyme (lipase) is often added to cheesemilk to produce the characteristic flavor of fully ripened Parmesan cheese. The coagulum was cut into very small particles and cooked at ~52°C (125°F) for 45 min until the pH decreased to 6.1, producing a dry, rice-like curd which was pressed to form a very dense, low-moisture cheese. Following manufacture, the cheese was brine salted (22% NaCl) for 7 days at 13°C, dried 4-6 weeks in a humidity-controlled chamber at 13°C, vacuum-packaged, and ripened at 13°C for a minimum of 9 months. During the first 2 h of cheesemaking, populations of both *Listeria* strains increased approximately 6- to 10-fold. Although *Listeria* counts remained relatively stable during cooking, populations decreased appreciably during pressing of the curd. During brining, drying and ripening at 13°C, numbers of both *Listeria* strains decreased almost linearly, with estimated D-values ranging between 8 and 36 days. Using direct plating, the 2 strains were no longer detected in cheese after ~14-112 days of ripening at 13°C. Despite large differences in survival of *L. monocytogenes* between

different batches of cheese, both *Listeria* strains decreased at a faster rate in Parmesan than in Colby or Cheddar cheese (*Ryser and Marth 1987a, Yousef and Marth 1988*) during ripening.

Decreased viability of *L. monocytogenes* in Parmesan cheese is probably related to a combination of factors, including action of lipase added to the milk, cooking of the curd during cheesemaking, and low water activity of the fully ripened cheese. To decrease the moisture content and develop proper flavor, the present regulation in the United States requires that Parmesan cheese be aged a minimum of 10 months regardless of whether or not the cheese is prepared from raw or pasteurized milk thus ensuring its safety.

Buazzi et al. (*1992a*) examined the fate of *L. monocytogenes* during manufacture and ripening of Swiss cheese, which involves cooking of the curd at 50-53°C and ripening the finished cheese at an elevated temperature for "eye" development. When rindless Swiss cheese was prepared from pasteurized milk inoculated to contain 10^4-10^5 *L. monocytogenes* (1 of 3 strain) CFU/ml, the pathogen was generally unable to grow during cheesemaking, with populations increasing 43% during the early stages of cooking owing to physical concentration and curd shrinkage. Thereafter, about 57% of the population in the curd was inactivated after 30-40 min of cooking at 50°C. After pressing, the curd contained 50% fewer listeriae, with this population decreasing most sharply after 30 h of brining at 7°C. Storing the finished cheese (pH 5.2-5.4) for 10 days at 7°C reduced the *Listeria* population to very low numbers. Complete inactivation of the pathogen occurred after 66-80 days of ripening at 24°C, with production of propionate by eye-forming bacteria likely contributing to the death of listeriae. Two studies conducted

in Switzerland (*Bachmann and Spahr 1995, Kaufmann 1990*) demonstrated that the environments within Emmentaler and Gruyere cheese (other varieties of Swiss cheese) also are not conducive to *Listeria* survival, with the pathogen no longer being present in 24-h-old cheeses (pH 5.2-5.4) prepared from raw milk inoculated to contain 10^4 *L. monocytogenes* CFU/ml. These studies indicate that the manufacturing steps involved in Swiss cheesemaking should ensure the safety of cheese made from raw, heat-treated or pasteurized milk.

In another study, Brick cheese was made from pasteurized whole milk inoculated to contain ~10^2-10^3 *L. monocytogenes* CFU/ml (4 strains) (*Ryser and Marth 1989*). Cheeses were ripened at 15°C and 95% RH with a surface smear for 2, 3 or 4 weeks to simulate production of mild, ripened and Limburger-like Brick cheese, respectively. Cheeses were then stored an additional 20-22 weeks at 10°C. Populations of the four *Listeria* strains increased 1-2 orders of magnitude following completion of brining ~32 h after the start of cheesemaking. All 4 strains leached from cheese into the 22% brine solution during 24 h and survived in the brine at 10°C for at least 5 days after cheese removal. During initial smear development, two strains grew rapidly to different levels depending on the type of sample and the pH - i.e. 6.6 and 6.2 logs/g in 4-week-old slice sample (pH 6.0-6.5); 7.0 and 6.9 logs/g in the surface (pH 6.5-6.9) samples; and 5.6 and 5.1 logs/g in the interior (pH 5.6-6.2) samples. Numbers of these two strains generally decreased 1-to 7-fold during 20-22 weeks at 10°C. The two remaining strains failed to grow appreciably in any cheese during or after smear development, despite a pH of 6.8-7.4 in fully-ripened cheese, and were not isolated from 2- and 3-week-old cheeses. Using direct plating, both strains were detected sporadically at ~4 log CFU/g in 4-week old

cheese. Cold enrichment of 4–week old slice, surface and interior samples generally yielded positive results for *L. monocytogenes*. Inhibition of these two strains could have been due to smear-ripening organisms, which can reportedly produce bacteriocin-like substances active against listeriae (*Ennahar et al. 1996, Ryser et al. 1994*), or heightened sensitivity of these *L. monocytogenes* strains to the inhibitory effects of certain listeriocidal fatty acids (i.e., linoleic) and monoglycerides (*Wang and Johnson 1992*) produced during cheese ripening.

In 1995, Bachmann and Spahr (*1995*) manufactured Tilsiter cheese, a semi-firm, slightly yellow, smear-ripened variety similar to brick cheese from milk inoculated to contain 10^4 *L. monocytogenes* CFU/ml. Overall, their findings were similar to those observed for two of the *L. monocytogenes* strains in brick cheese (*Ryser and Marth 1989*), with *Listeria* populations varying between 10^3 and 10^4 CFU/g in Tilsiter cheese during 90 days of ripening at 10-13°C. The above studies show that *L. monocytogenes* can survive during manufacture and ripening of smear-ripened cheeses due to the increase in pH to that occurs as a result of bacterial growth on the cheese surface.

Similar studies have been done on other cheese varieties (*Margolles et al. 1997, Papageorgiou and Marth 1989a, 1989b, Razavilar 1997, Rodriguez et al. 1998, Ryser and Marth 1987b, Stecchini et al. 1995*); the results of which are summarized in Table 4. Growth and survival of *L. monocytogenes* also was investigated in market cheeses that were purchased, inoculated and then stored at 4 to 30°C (*Genigorgis et al. 1991*). Results from this study are summarized in Table 5.

All of the aforementioned studies except those for Swiss and Parmesan show that *L. monocytogenes* can persist beyond the mandatory 60-day ripening period for cheeses

that can be legally prepared from raw or heat-treated milk. These studies point out the need to re-evaluate the safety of current cheesemaking practices. If found inadequate, appropriate changes in current regulations or alternative technologies should be introduced so that safety of these cheeses can be reassured.

FEASIBILITY OF RAW MILK CHEESE

According to current FDA regulations (*Anon. 1949*), milk pasteurization or use of a similar heat treatment during cheesemaking is required for the manufacture of 16 cheese varieties including Brie, cottage, cream, Neufchatel, Monterey, mozzarella, Scamorza, Muenster, Gammelost, Koch Kaese, and Sapsago (*Johnson et al. 1990b*). Seven varieties of manufacturing cheese (i.e., for use in pasteurized processed cheese, cheese foods, cheese spreads) require neither pasteurization of the cheese-milk nor a 60-day minimum ripening period at >1.7°C (35°F); whereas the 34 remaining cheese varieties (mentioned previously) recognized under current standards of identity must either be manufactured from pasteurized milk or held a minimum of 60 days at >1.7°C (35°F) to eliminate pathogenic microorganisms. Although statistics on milk pasteurization for cheesemaking are scarce, available evidence indicates that ~10% of all cheese produced in the United States (~646 million lb/month for 1999) is prepared from raw or heat-treated (subpasteurized) milk (~65 million lb/month) (*Dairy Marketing Fundamentals, Groves 2000b*).

Table 4: Behavior of *L. monocytogenes* During Cheese Ripening as Affected by Cheese Composition:

Cheese	Moisture (%)	Estimated a_w	Estimated salt in water phase (%)	pH		Ripening temp. (°C)	Log_{10} of *L. monocytogenes* CFU/g			Survival (days)
				Initial [a]	Final		Initial [a]	Maximum	Final	
Camembert	54.4	0.975	4.72	4.6	7.5	15/6	3.1-3.6	6.7-7.5	6.7-7.5	65
Blue	38.9	0.950	11.52	4.6	6.3	9-12/4	4.0-5.0	4.0-5.0	1.0-2.3	120
Brick	43.0	0.990	1.89	5.3	7.3	15/10	3.0-4.7	4.6-6.7	2.7-6.1	168
Feta	54.7	0.975	4.57	4.7	4.4	22/4	5.2-6.2	5.7-6.2	2.8-4.6	90
Cheddar	37.2	0.975	4.61	5.1	5.1	6	2.5-3.2	2.6-3.8	0-1.5	70-434
Cheddar	37.2	0.975	4.61	5.1	5.1	13	2.6-3.4	3.0-3.7	0	70-224
Colby	40.0	0.975	3.91	5.1	5.1	4	3.5-4.5	3.6-4.6	2.3-4.1	112-140
Parmesan	32.0	0.935	4.96	5.1	5.1	13	3.3-4.3	3.3-4.3	1.0-1.3	21-112
Hard Italian	NR [c]	0.950	2.12 d	5.3	5.7	4	4.5-5.1	4.5-5.6	2.0	35
Cold-pack cheese food [b]	41.4	0.975	4.90	5.3	5.1	4	2.4-2.8	2.4-2.8	1.1-2.0	180

[a] Approximately 24 h after the start of cheesemaking.

[b] Prepared without preservatives or acidifying agents.

[c] Not reported.

[d] Percentage of salt in solid and water phase.

Source: Reprint from (*Ryser 1999a*)

Table 5: Growth and Inactivation of *L. monocytogenes* in Surface-Inoculated Retail Cheeses During Storage at 4-30°C:

Cheese category and type	pH	% NaCl in moisture phase	Growth
Soft mold ripened			
Brie	6.0-7.7	2.5-3.6	+
Camembert	7.3	2.5	+
Blue	5.1	6.1	-
Bacterial surface ripened			
Limburger	7.2	4.8	-
Muenster	5.5	3.8	-
Soft Italian			
Provolone	5.6	4.6	-
String cheese	5.5	4.4	-
Semisoft and hard ripened			
Monterey Jack	5.0-5.2	1.0-3.0	-
Colby	5.5	4.9	-
Cheddar	4.9-5.6	2.6-5.4	-
Swiss	5.5	2.7	-
Hispanic			
Queso Fresco	6.5-6.6	4.5-6.6	+/-
Queso Ranchero	6.2	4.1	+
Queso Panelia	6.2-6.7	2.5-3.9	+
Cotija	5.5-5.6	9.6-12.5	-
Pickled cheese			
Feta	4.2-4.3	2.2-7.5	-
Ewe's milk cheese			
Kasseri	4.8-5.3	5.5-5.8	-
Soft unripened			
Cottage Cheese	4.9-5.1	1.0-1.2	+/-
Cream Cheese	4.8	<0.9	-
Whey cheeses			
Ricotta	5.9-6.1	<0.7	+
Processed cheese			
American	5.7	2.1	-
Monterey Jack	5.7	4.4	-
Piedmont	6.4	5.1	-

Source: Adapted from (*Genigorgis et al. 1991, Ryser 1999a*).

Research on the use of pasteurized milk for cheesemaking began in 1907 as a joint effort between the United States Department of Agriculture and the University of Wisconsin Agriculture Experiment Station. Although the primary goal was improved quality, product safety was also a concern. During the World War II, many cheesemakers were called into service. Those who took their place were less experienced and had to meet government demands for huge amounts of cheese to fuel the war effort. Thus, quality and safety of the cheese were sometimes compromised. By 1949, pasteurization of milk and dairy products was adopted nationwide (*American Cheese Society*). The mandatory 60-day holding period at >1.7°C (35°F) for cheeses manufactured from raw or heat-treated milk was also adopted at that time (*Anon. 1949, Johnson et al. 1990b*) after researchers demonstrated that *Brucella abortus*, the causative agent of brucellosis, was eliminated from cheese by such an aging process.

Based on epidemiological evidence and outbreak information, the current 60-day holding period generally has been deemed adequate to eliminate most foodborne pathogens. However, considering the results from the aforementioned challenge studies, it appears that organisms such as *Listeria* and *Salmonella* can survive well beyond the 60-day ripening process (*Goepfert et al. 1968, Hargrove et. al 1969, Park et al. 1970, Ryser and Marth 1987a*). Consequently, the adequacy of the 60 day hold at > 1.7°C (35°F) still remains very much in question with safety concerns regarding such cheeses recently voiced by the FDA as well as the Australian Dairy Industry, the Government of Canada (*Farber et al. 1996*) and the International Dairy Federation. In keeping with the grave nature of listeriosis as compared to most other foodborne illnesses, the FDA has continued to maintain a policy of "zero tolerance" for *L. monocytogenes* in all ready-to-

eat foods. Thus far, no well-documented cases of listeriosis have been associated with consumption of cheeses that were legally prepared from raw milk and held a minimum of 60 days at a temperature of ≥1.7°C (35°F) before sale. At FDA's request, the Cheese Subcommittee of the National Advisory Committee for the Microbiological Criteria of Foods reviewed the current data and concluded that the 60 day holding period at ≥ 1.7°C may be insufficient to eliminate all foodborne pathogens (*Anon. 1997*). The Cheese Subcommittee also recommended that the FDA re-examine its current policy regarding the 60-day aging period for hard cheeses prepared from raw and heat-treated milk.

Since ~4% of the raw milk supply can be expected to contain *L. monocytogenes*, it would be prudent to manufacture cheeses from pasteurized milk whenever possible. Although Yousef and Marth (*1989*) demonstrated that ripening Parmesan cheese for 10 months, as legally required, is sufficient to produce a high-quality, *Listeria*-free product, desirable flavor and texture characteristics are not easily attainable in sharp Cheddar and Swiss cheese prepared from pasteurized milk. Hence, alternative means should be developed to enhance the safety of these products. Methods used could include cold sterilization of the milk via microfiltration, sublethal heat treatment (short of pasteurization) or addition of various flavor- and texture-enhancing enzymes (or microorganisms) to pasteurized milk, which would allow the cheesemaker to obtain a higher quality product (*Johnson et al. 1990c*).

POLITICAL CLIMATE SURROUNDING RAW MILK CHEESE

On the international front, the Codex Committee on Food Hygiene, at its meeting in October 2000, considered a proposed code of hygienic practices for milk and milk products that stops short of requiring mandatory pasteurization of milk. The proposal will

be considered at Step 3 in the eight-step Codex process. Several years may be needed until this policy is officially adopted by Codex. Codex is not in favor of mandating pasteurization, but will leave the decision to individual countries. These countries also will be able to determine their own level of public health protection concerning imported dairy products (*Groves 2000a*).

On the domestic front, current participants include government officials along with various dairy industry representatives and consumer groups, all of which have diverse opinions. In 1999, FDA announced that it was rethinking the 60-day aging rule. On July 28, 2000, the front page headline in the Cheese Reporter (*Anon. 2000b*) read: "60-Day aging may be inadequate to eliminate *E. coli* in cheese: Research." This article discussed some studies being conducted at the Illinois Institute of Technology and FDA's National Center for Food Safety and Technology in Summit-Argo, IL to confirm prior work suggesting that 60-day aging of hard cheese made from unpasteurized milk is inadequate to protect public health. Based on preliminary findings, *E. coli* O157:H7 decreased 1 log in raw milk cheese (initial inoculum of 10^5 cells/ml in raw milk) after 60 days of ripening with *E. coli* still detected after 360 days (*Anon. 2000b*). This decision by FDA to review the 60-day aging rule is part of the Food Safety Initiative Program developed by President Clinton. Whether his successor shares the same zeal for the safety of raw milk remains to be seen.

On the industry front, there is certainly nothing resembling unanimity on this issue. The National Cheese Institute's proposed general standard, which is somewhat misunderstood, calls for pasteurization or an equivalent process for dairy ingredients used in cheese. "Equivalent process" is not defined. The Cheese of Choice Coalition was

formed recently by the American Cheese Society and Old-Ways Preservation Trust to advocate continued use of raw milk in cheesemaking. The American Dairy Products Institute's Cheese Division also supports traditional "curing" methods for cheeses made from unpasteurized milk, including the 60-day aging period (*Groves 2000a*).

Consumer groups also are divided on this issue. The Center for Science in the Public Interest seems likely to support mandatory pasteurization. However, Consumer Alert prefers consumer choice in this matter. On July 21st, 2000, Digby Anderson, director of the Social Affairs Unit, decried FDA's possible ban on unpasteurized cheeses in an editorial column of the Wall Street Journal. His column prompted three letters to the editor of that paper, one from Consumer Alert's executive director, all supporting his views (*Groves 2000a*).

Based on the preliminary *E. coli* findings and earlier work with *Listeria*, FDA may eventually propose mandatory pasteurization, or an equivalent, which would force opponents of mandatory pasteurization to come up with an acceptable alternative. The annexes in Codex's proposed milk hygiene code could play a key role since alternatives to pasteurization are outlined that can help ensure the same level of public health protection. Barring an outcome that satisfies all parties, Congress might eventually ask to "referee" this issue, making it more political than it should be (*Groves 2000a*).

RAW MILK VERSUS PASTEURIZED MILK CHEESE

Beneficial effects of milk heat treatment

The proportion of casein and milk fat converted to cheese primarily dictates potential cheese yield. Casein (in micellar form) is in colloidal suspension while fat (triglycerides) is in an oil-in-water emulsion. Enzymatic degradation can increase the

solubility of casein and milk fat. Casein can become more water soluble via chemical changes that do not require enzymatic catalysis. Researchers (*Ali et. al 1980a, 1980b, Pierre and Brule 1981*) have reported that cold storage of raw milk causes solubilization of colloidal calcium phosphate (casein bound) and a concomitant shift in caseins from the micellar to soluble state. Ali et al. (*1980a*) showed that these events caused an increase in rennet clotting time, reduction in firmness of rennet clot and reduced cheese yield. They also demonstrated that solubilization during cold storage could be reversed by heating at 60°C (140°F) for 30 min or 72°C (161.6°F) for 30 to 60 sec, although the milk equilibrium system never fully regained its initial state. Qvist (*Qvist 1979*) reported that pasteurization at 72°C (161.6°F) for 15 sec after cold storage at 5°C (41°F) caused the dissociated casein micelle components to return to micellar form. Additionally, pasteurization shortened the secondary (ionic) phase of coagulation to the level of uncooled milk, but did not reestablish the original rennet clotting time because the primary (enzymatic) phase was not shortened. In some cases, pasteurization further prolonged the primary phase during cold storage. Johnston et al. (*1981*) provided evidence that pasteurization after cold storage resulted in the recovery of soluble casein and calcium, but showed that both pasteurization at 72°C (161.6°F) for 15 sec and heat-treatment at 60, 65 or 70°C (140, 149 or 158°F), for 10 sec and repeated for 15 sec, resulted in significantly prolonged primary and secondary stages of coagulation relative to an unstored unpasteurized control. However, heat-treatment did not cause serious changes in cheesemaking performance. Dzurec and Zall (*1985*) showed that soluble ß-casein decreased with severity of the heat-treatment and subsequent cold storage of milk.

Several investigators (*Banks et. al 1986, Price 1927, Price and Call 1969, Wilson et. al 1945*) demonstrated that cheese made from pasteurized milk exhibited better overall quality and fewer flavor defects than raw milk cheese. Proliferation of psychrotrophic bacteria in either raw or pasteurized milk before cheesemaking, can lead to development of off flavors, gassiness and poor cheese quality (*Cousin 1982, Law 1979*). These proteolytic enzymes produced by psychrotrophic bacteria are not destroyed by pasteurization or thermization (*Stadhouders 1982*). Studies reported that on-the-farm thermization kept the bacterial numbers low and improved the quality of cheese when compared to cheese made from unthermized milk (*Banks et. al 1986, Coghill et. al 1982*). Wilster (*Wilster 1980*) stated that pasteurizing milk for cheesemaking afforded much easier control of the cheesemaking process, especially in regard to control of acid development, which is almost solely due to the starter culture with little influence from microorganisms present in raw milk. The cheesemaking process, and consequently the cheese, would be more uniform from day to day using pasteurized milk.

Detrimental effects of milk heat-treatment

When milk is heated sufficiently, ß-lactoglobulin reacts with K-casein on the casein micelles resulting in denaturation. Depending on the severity of the heat-treatment given to milk and consequent denaturation of ß-lactoglobulin, heated milk may show poor rennetability (increased clotting and hardening time, reduction in firmness of the coagulum) and less spontaneous whey drainage from the coagulum (syneresis) compared to untreated milk (*Hermier and Cerf 1986, Hooydonk et. al 1987*). These effects could result in lost yield, high moisture, and body/textural defects. Ustunol and Brown (*1985*) stated that milk used for cheesemaking should not be heated more than required to meet

current pasteurization requirements since it could impair the enzyme-catalyzed clotting of milk. However, other researchers suggested that pasteurization [72°C (161.6°F)/16 s] would not have any appreciable effect on enzymatic clotting of milk (*Morr 1987, Wilson and Wheelock 1972*). Marshall (*Marshall 1986*) was able to make Cheshire cheese from milk heated at 97°C (206.6°F) for 15 sec, but Cheddar cheese from similarly treated milk was excessively crumbly. Even by changing the manufacturing steps, a satisfactory Cheddar cheese could not be produced. Amantea et al. (*1986*) showed that cheese made from heat-treated milk [63°C (145.4°F)/16 s] was firmer than cheese produced from pasteurized milk, although the cheeses were similar in moisture, salt, pH, and age. The difference in firmness reportedly resulted from irreversible protein denaturation. Over-pasteurization can also lead to a cheese with a "short" or "brittle" body (*O'Keeffe et. al 1982, Price and Call 1969*).

According to Reinhold (*Reinhold 1972*), Swiss cheese can be routinely made from fully pasteurized milk without harmful effects on eye development. However, the impact of pasteurization on flavor development during curing was not described. Ginzinger et al. (*1999*) manufactured Bergkaese, a Swiss-type hard cheese, to examine the effect of raw milk flora on cheese quality. Milk pasteurization had no significant effect on physical properties of the cheese. However, pasteurization adversely affected aroma intensity and bitterness with cheese produced from pasteurized milk having lower flavor intensity and increased bitterness compared to raw milk cheese. They concluded that it would be inappropriate to pasteurize milk intended for making Bergkaese, even for elimination of indigenous milk microflora, due to adverse effects on sensory quality.

Several researchers reported that flavor develops slower in pasteurized as compared to raw milk cheeses (*Banks et. al 1986, Franklin and Sharpe 1962, Hanrahan et. al 1963, Kristoffersen 1985, Melachouris and Tuckey 1966, Price 1927, Scarpallino and Kosikowski 1962, Wilson et. al 1945*). Price and Call (*1969*), and Melachouris and Tuckey (*1966*) observed that cheese made from excessively heated milk was of inferior quality compared to that made from pasteurized milk. Among the enzymes in milk thought to function in cheese curing are plasmin and lipase. Alichanidis et al. (*1986*) indicated that plasmin is largely unaffected by pasteurization. A 30-40% increase in milk protease activity was reported in pasteurized milk compared to raw milk, with this change possibly due to inactivation of a protease inhibitor (*Noomen 1975*). In contrast, milk lipase, is heat sensitive but not completely destroyed by pasteurization. Pasteurization at 72°C (161.6°F)/15 s will decrease milk lipase activity greater than 90%, while heating at 60-67°C (140-152.6°F) for 15 sec results in more than a 60% loss in activity (*Johnson 1974*). Loss of milk lipase and other enzyme activity may adversely affect typical flavor development in Swiss and hard Italian cheeses such as Romano, Parmesan, and Asiago. The contribution of other enzymes present in milk such as acid phosphatase, lactoperoxidase and xanthine oxidase, all of which are not appreciably inactivated by standard pasteurization, to the curing of cheese is unknown (*Andrews 1974, Johnson 1974*). Some lactobacilli and pediococci remaining after pasteurization increased the rate and extent of flavor development (*Law 1984*). Franklin and Sharpe (*1962*) observed a decrease in flavor development in Cheddar cheese made from milk heat-treated at 62.8°C (145°F) for 17 s. As a result of pasteurization, flavor scores also decreased as the number of lactobacilli in cheese milk decreased.

In

cheesemak

Pasteurize

raw milk

control a

of whey

problem

as food

and pro

has ma

cheese

Italian

native

for ch

EFFE

rise

paste

outb

paste

foun

Staj

that

In conclusion, heat treatment or pasteurization does not adversely affect the cheesemaking process or the resulting physical properties of the cheese to a great extent. Pasteurized milk will yield a cheese of more consistent quality than cheese made from raw milk. Pasteurization and other heat-treatments enable improved uniform process control and quality during cheesemaking. However, heating results in some denaturation of whey protein (with pasteurization) as well as some body/texture and moisture control problems. Whey proteins can also lose functionality which could affect their usefulness as food ingredients. Moreover, cheeses made from pasteurized milk ripen more slowly and probably not to the same flavor intensity as do cheeses prepared from raw milk. This has major adverse implications for manufacturers of processed cheese which require cheese with accelerated body breakdown and intense, sharp flavors. Swiss and hard Italian type cheeses, the traditional flavor of which is strongly related to the activity of native milk enzymes and microflora, also would be adversely affected if pasteurized milk for cheesemaking became mandatory.

EFFECT OF HEAT-TREATMENT ON *L. MONOCYTOGENES*

The established association of *L. monocytogenes* with raw milk in the 1950s gave rise to several early studies dealing with the possible resistance of this organism to pasteurization. In 1983, interest in this topic was revived as a result of a listeriosis outbreak in Massachusetts that was epidemiologically linked to consumption of pasteurized milk. Reports of unusual heat resistance of *L. monocytogenes* in milk can be found in the early literature (*Ikonomov and Todorov 1967, Ozgen 1952, Potel 1951, Stajner et al. 1979, Stenberg and Hammainen 1955*). In 1951, Potel (*1951*) demonstrated that *L. monocytogenes* died rapidly in milk held at 80°C. However, the following year,

Ozgen (*1952*) reported that *L. monocytogenes* survived 15 s at 100°C. These early findings indicated that *L. monocytogenes* could survive HTST pasteurization at 71.6°C/15 s, including a study by Bearns and Girard (*1958*) using the open-tube method. However, later studies proved that these early studies were flawed. Donnelly et al. (*1987*) showed that the open-tube method used by Bearns and Girard (*1958*) was unreliable to determine thermal death time. Using a "sealed-tube" method, they demonstrated that *L. monocytogenes* was rapidly inactivated in milk at 62°C. Thermal-inactivation profiles obtained by the sealed-tube method were linear for three strains of *L. monocytogenes* during the entire inactivation period and gave rise to $D_{62°C}$ values between 0.1 and 0.4 min depending on the strain of bacteria. The capillary tube method (a standard method now widely accepted) was used by several investigators to determine thermal resistance of *L. monocytogenes* in liquid media and foods (*El-Shenawy et al. 1989, Lou and Yousef 1997a*). Thermal inactivation rates for *L. monocytogenes* were linear throughout the entire course of heating in the range of 50-75°C. All these studies were conducted using suspended cells. Results from investigations on resistance of intracellular *L. monocytogenes* (cells present in leukocytes) are in conflict as some have shown increased heat resistance of internalized cells (*Bunning et al. 1988, Doyle et. al 1987, Knabel et al. 1990*). Knabel et al. (*1990*) compared heat resistance data of *L. monocytogenes* when the heat-treated cells were recovered from sterile, whole, and homogenized milk by incubation under aerobic and anaerobic conditions. When grown at 43°C and recovered by anaerobic incubation after heating, *L. monocytogenes* had $D_{62.8°C}$ of 243 s compared to 36 s for *Listeria* grown at 37°C and plated aerobically after thermal inactivation at 62.8°C. The FDA (*Bradshaw et. al 1987, Bunning et al. 1992, Lovett et al. 1990*), Centers

for Disease Control and Prevention (CDC) (*Anon. 1988a, 1988c, 1989*), and the World Health Organization (WHO) (*WHO Working Group 1988*) support HTST pasteurization as a safe process. In their review, Lou and Yousef (*Lou and Yousef 1999*) also concluded that "pasteurization is a safe process which reduces the number of *L. monocytogenes* occurring in raw milk to levels that do not pose an appreciable risk to human health."

EFFECT OF ACID/ACIDITY

Although HTST pasteurization is sufficient to destroy *L. monocytogenes* in milk, a growing concern in thermal inactivation is the survival of sublethally injured cells. Garazyabal et al. (*1987*) reported that *L. monocytogenes* was not recoverable from raw milk immediately after heating at 60 to 73°C but grew in the product during extended incubation. Such repair requires an optimal pH near 7.0. According to Bergey's Manual of Systematic Bacteriology (1986) (*Seeliger and Jones 1986*), *L. monocytogenes* can only grow at pH values from 5.6 to 9.6, with optimal growth occurring at neutral to slightly alkaline values; the latter was verified by Petran and Zottola (*1989*). The minimum pH value for growth was based on the work of Seeliger (*Seeliger 1961*), who, in 1961, reported that *L. monocytogenes* failed to grow in dextrose (glucose) broth at pH <5.6 after 2-3 days of incubation at 37°C. In addition, subcultures from the medium were no longer routinely successful. Subsequent investigations have shown that *L. monocytogenes* can proliferate in laboratory media adjusted to far lower pH values. Results from these studies (*Borovian 1989, George et al. 1988, Parish and Higgins 1989, Sorrells et al. 1989*) confirm the ability of *L. monocytogenes* to multiply in similar laboratory media adjusted to pH 4.4-4.6 with hydrochloric, citric, or malic acid. Farber et al. (*1989*) observed growth of *L. monocytogenes* at 30°C in double-strength brain heart infusion (BHI) broth

acidified with hydrochloric acid to a pH value as low as 4.3. Furthermore, *L. innocua*, *L. seeligeri*, and *L. ivanovii* also were reported to grow in BHI broth acidified with hydrochloric acid to pH values as low as 4.53, 4.88, and 5.16, respectively. Thus, the minimum pH at which *L. monocytogenes* and most other *Listeria* spp. can grow is well below pH 5.0 provided that these organisms are incubated at near-optimum temperatures and allowed sufficient time to overcome an extended lag phase.

Fermentation is an age-old method of food preservation which has an inhibitory effect on the growth and survival of pathogenic bacteria. However, proper acid development is critical to the safety and quality of fermented foods. Behavior of *L. monocytogenes* in these foods depends on numerous extrinsic and intrinsic factors, including pH. Camembert (*Ryser and Marth 1987b*) (a mold-ripened cheese), Brick cheese (*Ryser and Marth 1989*), and white pickled cheese (*Abdalla et al. 1993*) supported growth of *L. monocytogenes*, with the pH of these cheeses being 5.9-7.2, 6.9-7.3, and >6.0, respectively. In contrast, the bacterium was inactivated rapidly in cottage (*Ryser and Marth 1985*), Parmesan (*Yousef and Marth 1989*), mozzarella (*Buazzi et al. 1992b*), and water-buffalo mozzarella cheese (*Villani et al. 1996*), having final pH values of 5.0-5.1, 5.2-5.3, and 4.0, respectively. Various degrees of survival have been reported in most other cheeses. *L. monocytogenes* persisted 70 to ≥434 days in Cheddar cheese at pH 5.0-5.15 (*Ryser and Marth 1987a*), ≥ 115 days in Colby cheese at pH 5.0-5.18 (*Yousef and Marth 1988*), 270 days in semihard Manchego-type cheese at pH 5.10-5.80 (*Dominquez et al. 1987*), ~90 days in Trappist cheese at pH 4.70-5.42 (*Kovincic et al. 1991*) and feta cheese at pH 4.6 (*Papageorgiou and Marth 1989a*), <66-80 days in Swiss cheese (*Buazzi*

et al. 1992a), >50 days in blue cheese (*Papageorgiou and Marth 1989b*), and ~ 180 days in cold-pack cheese food without preservatives at pH 5.21-5.45 (*Ryser and Marth 1988*).

SUBLETHAL THERMAL/ACID INJURY

In nature, *L. monocytogenes* may be subjected to various environmental stresses, such as high/low temperature, acidic/alkaline conditions and starvation (*Foster and Spector 1995, Miller 1992*). Environmental stresses can induce stress-adaptive or stress-protective responses e.g., incubating a microorganism such as *L. monocytogenes* at a high but sublethal temperature will induce a heat-shock response. Resistance of *L. monocytogenes* to heat or other lethal factors can be greatly increased by heat-shock or adaptation to other stresses. Bacteria respond to heat shock by synthesizing new proteins, termed heat-shock proteins (HSP) (*Agard 1993, Craig et al. 1993*). Induction of the heat-shock response or HSP usually increases the thermotolerance of microorganisms. As opposed to the intrinsic thermotolerance of microorganisms, heat-shock-induced thermotolerance is transient and non-inheritable and therefore an acquired or adaptive response (*Watson 1990*). Temperatures at which microorganisms are heat-shocked affect the magnitude of the acquired thermotolerance. Optimal heat-shock temperatures for maximal thermotolerance are ~10-15°C above the microbe's optimal growth temperature. *Listeria monocytogenes* has optimal heat-shock temperatures in this range (*Farber and Brown 1990, Lou and Yousef 1999*). The magnitude of heat-shock-related thermotolerance is also affected by the length of exposure to heat, the heating menstruum, heating rates, physiological state of the cells, and the method used to recover injured cells.

processi

thermal

establish

handling

minimal

may occu

cheese cu

time and

increased

Quintava

resistant

investig

tryptic

found

the th

station

increa

to pH

acidic

at pH

Conditions similar to heat-shock can develop in some foods during thermal processing or hot-holding. Slow heating or cooking, preheating, hot water washing, mild thermal processes, and holding food in warm trays (as occurs in food service establishments) are examples of heat-shock that may occur during food processing and handling. Farber and Brown (*1990*) suggested that heat-shock may result when foods are minimally processed or when the food is too bulky to allow rapid heating. Heat-shock may occur during vat pasteurization of dairy products (*Linton et al. 1990*) or cooking of cheese curds during the make process (e.g. Swiss cheese), which involves a long come-up time and low-temperature heating/cooking. Thermotolerance of *L. monocytogenes* is increased by low heating rates (*Quintavalla and Campanini 1991, Stephens et al. 1994*). Quintavalla and Campanini (*1991*) found that *L. monocytogenes* became more heat resistant during slow (0.5°C/min) rather than fast heating. Stephens et al. (*1994*) investigated thermal inactivation of a 17-h-old culture of *L. monocytogenes* (Scott A) in tryptic phosphate broth at 50-64°C by both instantaneous heating and slow heating and found that slow heating significantly increased heat resistance of *L. monocytogenes*.

Besides heat shock, adaptation to other environmental stresses may also increase the thermotolerance of pathogens. Farber and Pagotto (*1992*) found that exposing a stationary-phase culture of *L. monocytogenes* to a laboratory broth at pH 4.0 for 1 h increased the $D_{58°C}$-value in sterile whole milk from 2.75 to 3.90 min. A gradual decrease to pH 4.0 during 4 or 24 h also significantly increased heat resistance (acid adaptation).

Acid adaptation can enhance survival of *L. monocytogenes* when exposed to lethal acidic conditions. Kroll and Patchett (*1992*) found that acid shocking *L. monocytogenes* at pH 3.0 for 20 min prolonged the lag-phase when the organism was subsequently grown

at pH 7.0. Prior incubation at pH 5.0 rather than pH 7.0 increased survival of *L. monocytogenes* by 3 logs during acid shock at pH 3.0 for 40 min. Synthesis of "acid stress proteins" is presumably required for induction of the acid-tolerance response (*O'Driscoll et al. 1996*). Lou and Yousef (*1997b*) found that acid resistance in *L. monocytogenes* was significantly greater after adaptation to mild acidic conditions or after a stepwise increase to high acid-conditions. They suggested that food fermentations, which involve a gradual lowering of pH, could lead to acid adaptation in *L. monocytogenes*.

Acid adaptation also cross protects *L. monocytogenes* against a variety of deleterious factors such as lethal doses of hydrogen peroxide, heat, NaCl, ethanol, and certain surface active hydrophobic compounds (*Lou and Yousef 1999*). Since acid adaptation increases general resistance, including acid tolerance, acid-adapted cells of *L. monocytogenes* may survive better in both acidic and fermented foods (e.g. cheese) than unadapted cells (*Guhan et al. 1996*).

When present in a sublethally injured state in food, *L. monocytogenes* cannot be enumerated directly since the recovery media contains various *Listeria* selective agents, some of which are inhibitory to the repair process while others are toxic and cause death of these injured cells. In order to successfully detect and accurately enumerate sublethally injured cells, an environment favorable for repair of sublethally injured cells must be provided. Current detection procedures for *L. monocytogenes* (FDA, USDA-FSIS, IDF), with the exception of cold enrichment (which is very time consuming and laborious) rely on highly selective enrichment and/or plating media. Therefore, these methods frequently underestimate the true incidence of *Listeria*. Busch and Donnelly (*1992*) developed

Listeria Repair Broth (LRB) which permits complete repair of injured *Listeria* within 5 h at 37°C after which various selective agents can be added to inhibit the growth of competing microflora upon incubation. Considerable research has been conducted to evaluate the efficacy of LRB, University of Vermont Broth as well as LRB modified by adding certain components, to resuscitate heat-, acid-, sanitizer- and freeze-injured *L. monocytogenes* cells (*Donnelly 1999*). Based on the earlier study by Knabel et al. (*1990*), Teo and Knabel (*2000*) developed modified Penn State University (mPSU) Broth for anaerobic recovery of heat-injured *L. monocytogenes* from pasteurized milk. Heat-injured cells of *L. monocytogenes* that were added to various commercial brands of pasteurized whole milk were detected using mPSU broth. Use of a suitable recovery-enrichment medium is necessary if all *L. monocytogenes* (healthy and injured) cells are to be detected in foods.

To summarize, survival and growth of healthy *L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7 in Cheddar, Colby and most other aged cheeses generally decreases during storage (*Hargrove et. al 1969, Park et al. 1970, Reitsma and Henning 1996, Ryser and Marth 1987a, 1985*). Although milk pasteurization is sufficient to destroy pathogens, a growing concern is the survival and recovery of sublethally injured cells (*Garayzabal et. al 1987*). Since such repair requires an optimal pH near 7.0, the harsh nature of the cheese environment (acid + salt) should limit survival of sublethally injured cells in a product such as Cheddar cheese.

GOALS OF THE STUDY

The goal of this study was to investigate the relationship between the heat treatment milk receives prior to cheesemaking and the ability of *L. monocytogenes* to

survive

cheese

subjecti

cheese

investi

monoc

culture

Cheddo

treated

pasteu

can be

Cheddo

indust

survive similar conditions to those encountered during the early stages of a Cheddar cheese fermentation. The potential for *L. monocytogenes* to become inactivated and/or sublethally heat/acid injured during sub-pasteurization heating of the milk before cheesemaking as well as during a simulated Cheddar cheese fermentation was investigated. Procedures were developed for obtaining heat-injured cells of *L. monocytogenes* based on an earlier study by Busch and Donnelly (*1992*). These injured cultures were used to study the influence of a lactic starter culture typical of those used in Cheddar cheese manufacture on growth and survival of the pathogen in raw, low heat-treated (LHT), high heat-treated (HHT), pasteurized and ultra high temperature (UHT) pasteurized milk. The underlying hypothesis was that a sub-pasteurization heat treatment can be identified which will sufficiently injure *L. monocytogenes* to prevent its survival in Cheddar cheese beyond 60 days of ripening and thereby preserve the raw milk cheese industry.

Running Head: Competitive Inhibition of Sublethally Injured *Listeria monocytogenes*

Competition of Thermally Injured *Listeria monocytogenes* with a Mesophilic Lactic Acid Starter Culture during Milk Fermentation

Finny P. Mathew, and Elliot T. Ryser*

Department of Food Science and Human Nutrition

Michigan State University, East Lansing, MI 48824-1224, USA.

Running Head/Keywords: *Listeria monocytogenes*, thermal injury, competitive inhibition, mesophilic milk fermentation.

***Corresponding Author: Elliot T. Ryser**

Phone: (517) 353 9734

Fax: (517) 353 1676

E-mail: ryser@msu.edu

ABSTRACT

The relationship between heat treatment of milk and the ability of sublethally injured *Listeria monocytogenes* cells to survive mesophilic fermentation in milk was investigated. Overnight tryptose broth cultures of three *L. monocytogenes* strains were centrifuged, suspended in 200 ml of tryptose phosphate broth and heated at 56°C/20 min and 64°C/2 min to obtain low heat-injured (LHI) and high heat-injured (HHI) cells, respectively, showing >99 % injury. Flasks containing 200 ml of raw, low heat-treated (56°C/20 min), high heat-treated (64°C/2min), pasteurized or UHT milk were tempered to 31.1°C, inoculated to contain 10^4-10^6 LHI, HHI or healthy *L. monocytogenes* cells and a *Lactococcus lactis* subsp. *lactis*/*Lactococcus lactis* subsp. *cremoris* starter culture at levels of 0.5, 1.0 or 2.0%. Numbers of healthy and injured *L. monocytogenes* cells were determined using tryptose phosphate agar with or without 4.0% NaCl at selected intervals during the 24h fermentation period, numbers of starter organisms were also measured. Presence of *L. monocytogenes* did not adversely affect the growth of the starter culture at any inoculation level. Overall, *L. monocytogenes* survived the 24 h fermentation process and grew to some extent. In starter-free controls, ~76-81% and 59-69% of LHI and HHI cells, respectively, were repaired after 8 hours of incubation, with lowest repair rates observed in raw rather than heat-treated or pasteurized milk. Increased injury was observed for healthy *L. monocytogenes* cells at 1.0 and 2.0% starter levels, with less injury seen for LHI and HHI cells. The extent of sublethal injury for all *L. monocytogenes* was inversely related to severity of the milk heat treatment.

Present-day laws regarding use of pasteurized, heat-treated (sub-pasteurized), and raw milk for cheesemaking date back to World War II (*Anon. 1949*). Options provided to cheese manufacturers were to either (a) pasteurize the milk [71.6°C (161°F)/15 sec] or (b) hold the cheese for a minimum of 60 days at ≥1.7°C (35°F). Thus, any cheese prepared from heat-treated milk was required to be held at least 60 days. Subsequent reports have shown that three important foodborne pathogens, namely, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 can survive up to 434 days (*Ryser and Marth 1987a*), 210 days (*Goepfert et al. 1968, Hargrove et. al 1969, Park et al. 1970*) and 138 days (*Reitsma and Henning 1996*), respectively, in Cheddar cheese produced from pasteurized milk inoculated with these pathogens. Consequently, the adequacy of the 60 day hold at ≥ 1.7°C still remains very much in question.

The United States Food and Drug Administration (FDA) as well as the Australian Dairy Industry, the Government of Canada (*Farber et al. 1996*) and the International Dairy Federation recently voiced concerns regarding safety of cheeses made from raw and heat-treated milk. At FDA's request, the Cheese Subcommittee of the National Advisory Committee for the Microbiological Criteria of Foods reviewed the data and concluded that the 60 day holding period at ≥ 1.7°C may be insufficient to eliminate all foodborne pathogens; the Subcommittee recommended that the FDA re-examine its current policy (*Anon. 1997*). The Codex Committee on Food Hygiene is considering a proposed draft code of hygienic practice for milk and milk products that stops short of requiring mandatory pasteurization of milk (*Groves 2000a*). However, given the superior flavor characteristics of raw milk Cheddar Cheese that result from non-starter lactic acid bacteria and enzymes naturally present in the milk (*Kristoffersen 1985, Melachouris and*

Tuckey 1966, Price 1927, Scarpallino and Kosikowski 1962), cheese manufacturers are reluctant to any change in the current aging policy. The American Cheese Society and the American Dairy Products Institute's Cheese Division support traditional "curing" methods for cheeses made from unpasteurized milk, including the 60-day aging requirement (*Groves 2000a*).

Listeria monocytogenes is one foodborne pathogen of particular concern because it can cause abortion in pregnant women and meningitis in immunocompromised adults (*Gray and Killinger 1966, Seeliger 1961*). Sporadic cases of bovine mastitis and abortion in which *L. monocytogenes* was intermittently shed in milk over several lactation periods have been recorded for more than 50 years. Dairy cows that appear healthy also can serve as reservoirs for *L. monocytogenes* (*Ryser 1999b*) with this pathogen reportedly present in 1.6-12.0%, 1.3-5.4%, and 2.5-6.0% of the raw milk produced in the United States, Canada and Western Europe, respectively (*Donnelly et al. 1988, Hayes et. al 1986, Liewen and Plantz 1988, Lovett and Hunt 1987, Ryser 1999a*).

In 1983, pasteurized milk was epidemiologically implicated as the vehicle of infection in a listeriosis outbreak in Massachusetts that resulted in the death of 14 of 49 individuals (*Fleming et. al 1985*). After 85 fatal cases of listeriosis were traced to consumption of Jalisco-brand Mexican-style cheese in 1985, surveillance efforts were intensified under the Dairy Safety Initiative Program (*Kozak 1986*). FDA reports in 1986 indicated that an average of 2.5 % of all dairy products manufactured from pasteurized milk was contaminated with *L. monocytogenes* (*Anon. 1986*). A subsequent report in February 1987 indicated that 2.6% of all dairy-processing facilities contained *L. monocytogenes* (*Anon. 1987c*). In the United States, this pathogen has been responsible

for at least 46 class I recalls involving domestically produced cheese, 3 of which were prepared from raw milk (*Ryser 1999a*). Thus, the current "zero tolerance" policy for *L. monocytogenes* has extracted a particularly heavy toll on the dairy industry.

Although high temperature-short time pasteurization is sufficient to destroy *L. monocytogenes* in fluid milk (*Bradshaw et. al 1987, Farber 1989, Mackey and Bratchell 1989*), incomplete pasteurization can lead to the survival and recovery of sublethally injured cells. Garazyabol et al. (*1987*) reported that *L. monocytogenes* was not recoverable from raw milk immediately after heating at 60 to 73°C but grew in the product during extended incubation. Such repair requires an optimal pH near 7.0 and is reportedly enhanced under anaerobic conditions (*Knabel et al. 1990*).

Fermentation is an age-old food preservation method used to inhibit the growth and survival of pathogenic bacteria. Studies showed that healthy *L. monocytogenes* cells survived and grew to some extent in samples of sterile skim milk that were fermented with mesophilic and thermophilic starter cultures (*Schaack and Marth 1988a, 1988b*). Studies on survival and growth of healthy *L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7 cells in Cheddar, Colby and other aged cheese indicate that their numbers slowly decrease during cheese ripening (*Hargrove et. al 1969, Park et al. 1970, Reitsma and Henning 1996, Ryser and Marth 1987a*). Demise of these pathogens during aging is principally due to acid development by the starter culture. Given the low pH of Cheddar cheese (~pH 5.0) combined with high levels of salt in the moisture phase, survival of sublethally injured should be far less than that for healthy cells.

The purpose of the study was to assess the ability of low heat-injured (LHI), high heat-injured (HHI) and healthy cells of *L. monocytogenes* to compete with different

levels of a mesophilic lactic acid starter culture in milks that have undergone various degrees of thermal processing.

MATERIALS AND METHODS

Culture preparation:

Three strains of *L. monocytogenes* (CWD 95 and CWD 246 from silage, and CWD 17 from raw milk) were obtained from C. W. Donnelly (Dept. of Nutrition and Food Sciences, University of Vermont, Burlington, VT). The cultures were maintained at -70°C in trypticase soy broth (Becton Dickinson and Co., Cockeysville, MD) containing 10% (v/v) glycerol (J. T. Baker, Phillipsburg, NJ) and subjected to two consecutive overnight transfers (18-24 h/35°C) in 9 ml of tryptose phosphate broth (TPB) (Difco Laboratories, Detroit, MI) containing 0.6% (w/v) yeast extract (Difco). A 3-strain cocktail suitable for sublethal injury work was then prepared by combining equal volumes of these cultures in a sterile 50 ml centrifuge tube (Clear Propylene, Plug Seal Cap, Corning Inc., Corning, NY), centrifuging at 10,000 rpm at 4°C/15 min (Super T21, Sorvall® Products, Newtown, CT), and resuspending the pellet in 9 ml of phosphate buffered saline (PBS) to obtain a suitable culture for injury.

Sublethal Injury:

Heat-injured cells were obtained using the procedure of Busch and Donnelly (*1992*) (Figure 2). In this method, 200 ml of TPB [in a 2800 ml wide bottom Fernbach flask] was tempered to 56°C/64°C in a shaking water bath [50 rpm] (Reciprocal Shaking Bath, Precision Scientific, Winchester, VA), inoculated to contain 10^8-10^9 *L. monocytogenes* CFU/ml and heated at 56°C/up to 30 min and 64°C/up to 5 min to obtain LHI and HHI cells, respectively, showing >99.0%. Samples were appropriately diluted in

PBS and spiral plated (Autoplate® 4000, Spiral Biotech, Inc., Bethesda, MD) on tryptose phosphate agar (Difco) + 0.6 % (w/v) Yeast Extract (non-selective medium, TPA) and TPA + 4.0% (w/v) NaCl (selective medium, TPNA) and incubated at 35°C/48 h to determine numbers of healthy and injured *L. monocytogenes* cells, respectively. Percent injury was determined from the following equation:

$$\% \text{ Injury} = \left\{ 1 - \frac{\text{Count on selective medium}}{\text{Count on non-selective medium}} \right\} \text{ X } 100$$

These heat-injured cultures were then centrifuged at 10,000 rpm at 4°C/15 min, resuspended in PBS and appropriately diluted for inoculation into milk. The heat-injury trials also were repeated in UHT milk to investigate the influence of the heating medium on sublethal injury.

Experimental Design:

A 5 x 3 x 4 factorial design was used to assess the effect of milk type [raw, low heat-treated (LHT), high heat-treated (HHT), pasteurized, and ultra high temperature (UHT) pasteurized] on the ability of *L. monocytogenes* cells in different physiological states [healthy, LHI, and HHI] to compete with different inoculum levels (0%, 0.5%, 1.0% and 2.0%) of a *Lactococcus lactis* subsp. *lactis/L. lactis* subsp. *cremoris* (LLLC) starter culture normally used to manufacture Cheddar cheese. Each trial was carried out in triplicate.

Fresh raw milk (chilled ~4°C) was obtained from the Michigan State University (MSU) Dairy Farm in sterile 2-liter flasks (autoclaved 121°C/15 min), divided into 200 ml aliquots and heated to 56°C and 64°C in a shaking water bath (Precision Scientific) for the same times in the sublethal injury trials (to obtain 99.0% injury) to obtain LHT and HHT milk, respectively. Freshly pasteurized (72°C/25 s) milk was obtained from the

MSU Dairy Plant in sterile 2-liter flasks (autoclaved 121°C/15 min). UHT pasteurized milk (Parmalat whole milk, Parmalat USA, Teaneck, NJ) was purchased locally.

Cans of frozen (-70°C) LLLC starter culture (Blue Label, Direct Vat Set, Chr. Hansen, Milwaukee, WI) were thawed by submersion in deionized water containing 100 ppm available chlorine for 30 min after which 2-ml aliquots were transferred to sterile freezer vials and frozen at –70°C. Working LLLC cultures were prepared by thawing a vial of culture and transferring 0.5ml of the contents to a flask containing 100 ml of sterile (autoclaved at 121°C/15 min) skim milk. Following 4-6 h of incubation at 30°C, the working LLLC starter culture was ready for use in trials.

Milk Inoculation:

Three sets of flasks containing 250 ml of raw, LHT, HHT, pasteurized and UHT pasteurized milk were tempered to 31.1°C in a water bath (Microprocessor Controlled 280 Series water Bath, Precision Scientific, Winchester, VA) (Figure 3). A 1-ml sample was withdrawn to determine the numbers of indigenous bacteria in the milk. One set each was inoculated with healthy, LHI (56°C/10-30 min), or HHI (64°C/1-5 min) cells of *L. monocytogenes* at a level of 10^4-10^6 CFU/ml. Thereafter, a working LLLC starter culture was added at a level of 0.5%, 1.0% or 2.0%. Additional flasks containing the LLLC starter culture alone, and the pathogen alone served as controls for assessing the impact of starter on the pathogen and pathogen on the starter, respectively.

Inoculate

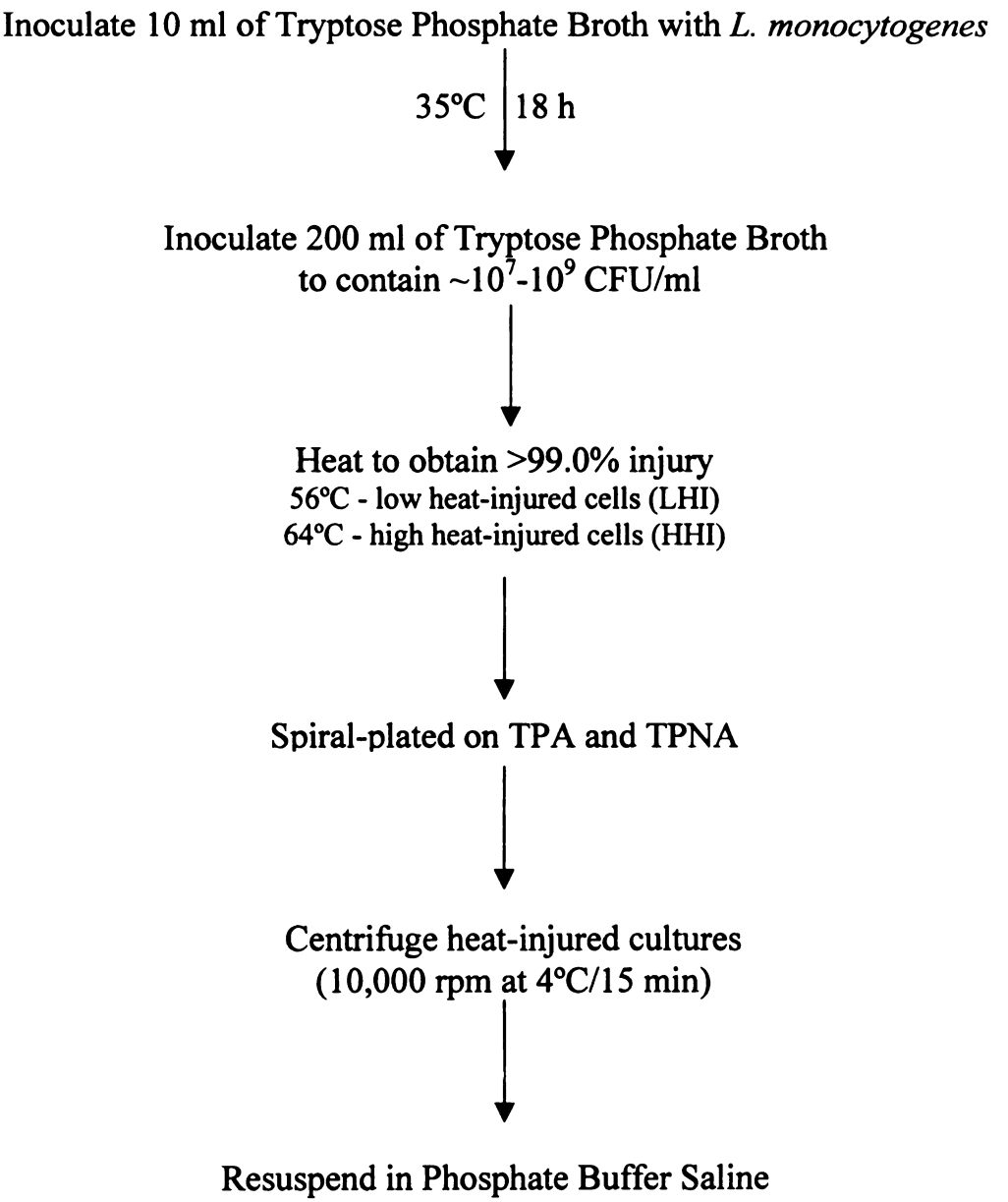

Figure 2: Preparation of heat-injured *L. monocytogenes*

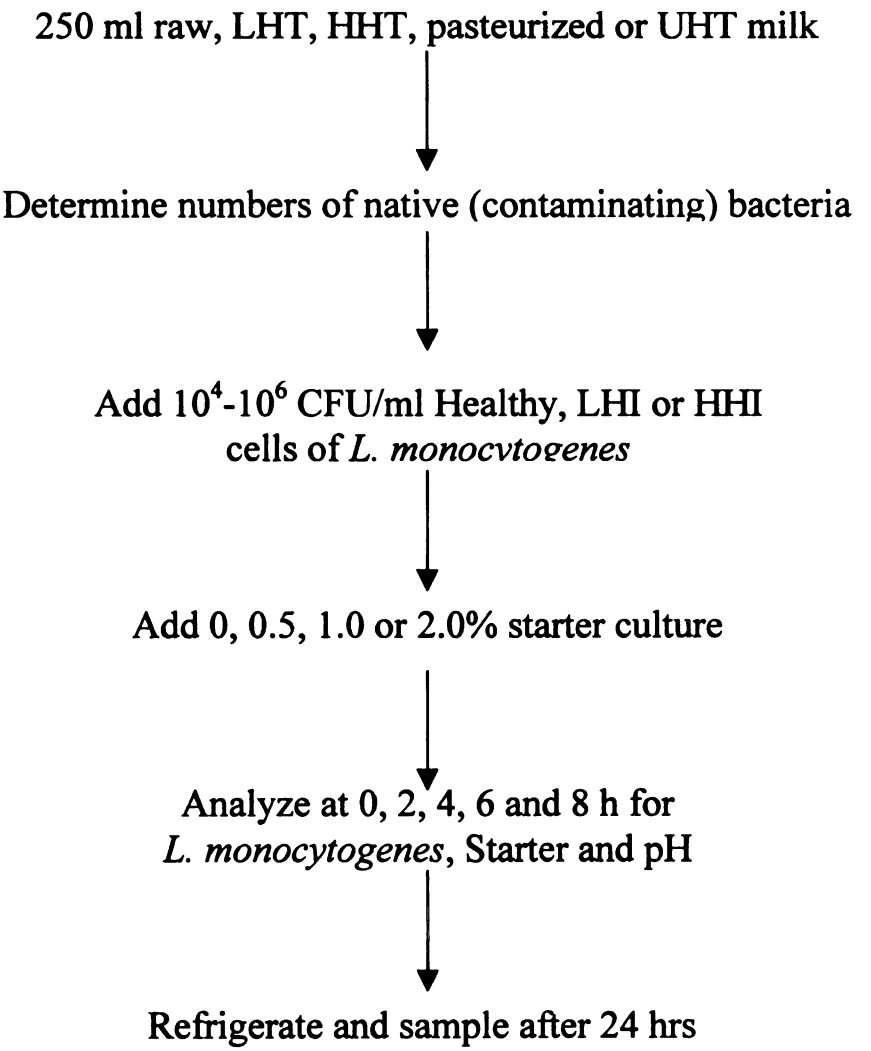

Figure 3: Fermentation of Milk at 31.1°C

Numbers of both healthy and injured cells of *L. monocytogenes* as well as the starter culture were determined from 1-ml samples, which were taken initially and thereafter at 2-h intervals during a fermentation period of 8 h. The pH was also monitored at the time of sampling using a pH meter (ORION model 620, Thermo Orion, Beverly, MA) equipped with a standard combination electrode (ORION model 6157 Solid State pHuture Probe, Thermo Orion). One additional sample was taken after 24 h for analysis.

Microbiological Analysis:

Numbers of indigenous microflora and total (healthy + sublethally injured) *L. monocytogenes* cells in UHT milk were determined by spiral plating samples appropriately diluted in PBS on TPA, while populations of healthy *L. monocytogenes* cells were determined by spiral plating samples on TPNA followed by 48 h incubation at 35°C. Modified tryptose phosphate agar (MTPA) containing esculin (0.1% w/v) (Sigma Chemical Co., St. Louis, MO) and ferric ammonium citrate (0.05% w/v) (Sigma) [non-selective] and MTPA + 4% NaCl [selective] (MTPNA) were used to examine other milk types. Numbers of LLLC starter culture were determined by pour plating appropriately diluted samples in MRS agar. These plates were counted after 48 h of incubation at 35°C.

Statistical Analysis:

Two-way Analysis of Variance (ANOVA) was performed on the data using the Statistical Analysis System (Proc Anova. SAS© Version 8, SAS Institute, Inc., Cary, NC). Arithmetic means were compared using the Duncan grouping test at 95% confidence level (p=0.05). Interactive effects were analyzed using the Autoregressive

Mixed Covariance Model (Proc Mixed Covtest) with the Satterthwaite Degrees of Freedom Method.

RESULTS

Sublethal Injury:

Heating the 3-strain cocktail of *L. monocytogenes* in UHT milk at 56°C/20 min (Figure 4) and 64°C/2 min (Figure 5) produced >99.0% injury. No significant ($p<0.05$) differences were obtained in % injury between trials conducted in TPB and UHT milk at 56°C/20 min as well as 64°C/2 min (Table 6).

Indigenous microflora in milk:

Fresh raw milk samples used for competitive inhibition trials had bacterial populations in the range of $3.0x10^1$- $4.1x10^2$ CFU/ml. Except for one sample ($9.99x10^0$ CFU/ml – LHT milk), no detectable counts were observed when raw milk was subjected to heating at 56°C/20 min (LHT) and 64°C/2 min (HHT). Pasteurized and UHT milks did not yield any detectable bacterial counts. Black colonies of *L. monocytogenes* on the non-selective medium (MTPA) could be easily differentiated from the naturally contaminating bacteria. The catalase test was also used for confirmation.

Growth of L. monocytogenes without starter culture:

When healthy *L. monocytogenes* cells were grown in different types of milk at 31.1°C (typical milk ripening temperature for Cheddar cheesemaking), steady growth was observed during 24 h of incubation. The heat treatment that the milk received before inoculation did not have a significant effect ($p<0.05$) on the growth rate of *L. monocytogenes* during incubation (Table 7).

When LHI and HHI cells of *L. monocytogenes* were grown in different types of milk, populations continually increased in all samples. As the incubation period increased, 78.95-87.74% of the injured cells were repaired after 24 h (Table 8). Repair for LHI cells in raw, LHT and HHT milk was significantly ($p<0.05$) lower than in pasteurized and UHT milk with HHI *L. monocytogenes* cells showing significantly greater ($p<0.05$) repair in UHT milk compared to the other milk types. Maximum repair occurred in pasteurized and UHT milk for LHI (87.57%) and HHI (87.74%) cells, respectively. The extent of repair was generally greater for LHI rather than HHI cells for all time periods up to 8 h, e.g., ~47 % of the LHI cells repaired after 6 h of incubation in raw milk compared to 32% for HHI cells. In UHT milk, ~55% of the LHI cells repaired after 6 h of incubation compared to 40% for HHI cells. However, after 24 h of incubation, differences in the % repair were not significant ($p<0.05$) (Table 9).

Growth of L. monocytogenes in the Presence of Starter Culture: Initial LLLC populations of $4.6x10^6$ to $5x10^7$ CFU/ml increased to about 10^9 CFU/ml after the 24 h fermentation period in all types of milk. Final pH values ranged from 3.85 to 4.4 after fermentation depending on the level of LLLC. Populations of LLLC as well as the pH drop in control (inoculated only with LLLC) and competitive inhibition samples (inoculated with both LLLC starter culture and *L. monocytogenes*) were comparable (raw data in Appendix); therefore, most attention will be given to the behavior of *L. monocytogenes* in competition with LLLC.

When healthy, LHI or HHI *L. monocytogenes* cells (initial level of ~10^4-$5x10^6$ CFU/ml, representing moderate to severe contamination of the milk) were grown in different types of milk in competition with 0.5, 1.0 and 2.0% starter culture, a steady

increase in the total population of *Listeria* was observed in all cases irrespective of the starter inoculum, type of milk or physiological state of *L. monocytogenes*. *Listeria* attained final populations of ~10^8 to 5x10^9 CFU/ml with growth affected by the physiological state of *Listeria* and LLLC level (Table 10). Overall, growth of sublethally injured *L. monocytogenes* was greater than that of healthy cells at all LLLC levels, e.g. 3.09 and 3.46 log increase (significant, $p<0.05$) for healthy and HHI cells, respectively, in pasteurized milk containing 0.5% LLLC after 24 h. A greater increase in total populations of HHI was observed as compared to LHI cells at each LLLC inoculum level, e.g., 3.21 log increase versus 3.12 log for HHI and LHI cells, respectively, after 24 h of incubation in LHT milk containing 1% LLLC. Growth of healthy as well as sublethally injured *L. monocytogenes* cells was inhibited as the LLLC inoculum level increased, e.g., 3.17 and 3.45 log increase for LHI and HHI cells, respectively, in pasteurized milk containing 0.5% LLLC compared to 2.82 and 2.93 log in the same milk containing 2.0% LLLC after 24 h ($p<0.05$).

Injury of healthy *L. monocytogenes* cells increased as the fermentation process progressed (counts on selective MTPNA decreased steadily compared to non-selective MTPA). At the end of the 24-h fermentation period, >90% of the healthy *L. monocytogenes* cells were injured, with slightly higher injury observed at higher LLLC inoculum levels of 1.0% and 2.0%. For LHI and HHI *L. monocytogenes* cells, >99.0% of the initial population was injured, and no repair or significant change was observed in percent injury.

The primary interest of this study was to assess the behavior of sublethally injured cells during fermentation. Analysis of the percent increase in the number of injured cells

showed a significant effect of the type of milk, LLLC inoculum level as well as physiological state of *Listeria*, interactive effects of these factors were also found to be significant (Table 11). A significant increase ($p<0.05$) in the percentage of healthy *L. monocytogenes* cells that became injured was generally observed as the LLLC inoculum increased from 0.5% to 2.0% (Table 12-16), e.g., injured cells increased by 51.63% and 64.93% in UHT milk at LLLC inoculum levels of 0.5% and 2.0%, respectively, after 8 h of fermentation. For LHI and HHI *L. monocytogenes* cells, a reverse trend was observed for the increase in the population of injured as compared to healthy cells. Where significant differences were observed ($p<0.05$) for different LLLC levels (Tables 12-16), the numbers were generally greater for 0.5% than for 2.0% starter culture. In LHT milk containing 0.5% starter culture, the increase observed for LHI and HHI cells after 6 h of incubation was 47.44% and 54.18%, respectively, while at 1.0%, these numbers increased to 46.32% and 50.00%, respectively (Table 13). The increase in percentage of injured cells also was greater for HHI as compared to LHI *L. monocytogenes* cells, although the trend was not always significant ($p<0.05$), e.g. in LHT milk containing 0.5% starter culture, the increase observed for LHI and HHI cells after 6 h of incubation was 47.44% and 54.18%, respectively, while at 1.0%, these numbers increased to 46.32% and 50.00%, respectively (Table 13).

The extent of increase in the number of injured cells was dependent on the type of milk in which *L. monocytogenes* was grown, e.g., in the case of healthy cells, significantly greater percentages of cells became injured in raw milk than in heat treated milks (LHT, HHT, pasteurized and UHT) for all fermentation periods (Tables 17-19).

Similar trends were observed for LHI and HHI cells at all LLLC inoculum levels (Tables 17-19).

Analysis of the raw data to investigate the interactive effects of LLLC inoculum level and milk type confirmed the results obtained from the individual analyses. In general, repair of sublethally injured *L. monocytogenes* cells (in absence of LLLC) increased as the milk heat treatment became more severe. After 6 h of incubation, 32.16% and 40.00% of HHI cells repaired in raw and UHT milk, respectively. The increase in the number of healthy cells that became injured was greater for less severely heated milk and higher LLLC inoculum levels, e.g. 74.03, 69.36, 68.07, 65.89, and 62.48% in raw, LHT, HHT, pasteurized, and UHT milk, respectively, containing 2.0% LLLC after 6 h compared to 59.49, 57.72, 59.62, 53.42, and 47.26% respectively, using an LLLC inoculum level of 0.5%. Conversely, the increase in percent injury for LHI and HHI *L. monocytogenes* cells was greater for less severely heat-treated milk containing lower levels of LLLC, e.g., 56.63, 54.18, and 54.11% for raw, LHI and HHT milk, respectively, containing 0.5% LLLC compared to 52.52, 50.00, and 48.09% for a starter inoculum of 1.0% (for HHI cells).

DISCUSSION

Listeria monocytogenes was sublethally injured (>99.0%) in both UHT milk and TPB when heated at 56°C/20 min and 64°C/2 min. This shows that the heating medium did not have a significant effect on sublethal injury of *L. monocytogenes* at the temperatures studied. These findings were similar to those observed by others (*Busch and Donnelly 1992, Meyer and Donnelly 1992*).

In general, *L. monocytogenes* (in all physiological states) grew steadily in the absence of starter culture in all types of milk at 31.1°C (typical milk ripening temperature for Cheddar cheesemaking) during 24 h of incubation. Similar growth trends were also observed in control samples from other studies (*El-Gazzar et al. 1992, Schaack and Marth 1988a, 1988b, Wenzel and Marth 1991*). Greater repair for LHI and HHI *L. monocytogenes* cells in more severely heat-treated milk (e.g. UHT milk) as compared to raw milk could be explained by the presence of native enzymes and microflora in raw milk that inhibit repair by providing a more hostile environment to the pathogen than heat-treated milk. The extent of repair was generally greater for LHI rather than HHI cells. The more severe heat treatment received by HHI cells compared to LHI cells could be responsible for slower repair. At the end of the 24-h fermentation period, the repair was similar.

Meyer and Donnelly (*1992*) observed that the lag time for heat-injured (at 55°C) cells was inversely proportional to the incubation temperature between 4°C (8 days) and 37°C (none detectable). In our trials, *L. monocytogenes* in all physiological states showed some growth within the first 2 h of incubation. Thus, our results concur with their study since no detectable lag phase was observed at an incubation temperature of 31.1°C.

Steady growth was observed for healthy, LHI and HHI *L. monocytogenes* cells in all types of milk (as in controls) when grown in competition with 0.5, 1.0 and 2.0% starter culture. Sublethally injured *L. monocytogenes* cells grew to a greater extent than healthy cells at all LLLC levels. This may be due to greater susceptibility of healthy cells to acid injury from acid produced by the starter culture, while stress-adaptive responses (e.g. production of heat shock proteins) induced by sublethal heating, resulting in cross

protection against other lethal factors such as acid production, could be responsible for enhanced growth of sublethally injured cells (*Craig et al. 1993*).

Several studies were conducted to assess competitive inhibition of healthy *L. monocytogenes* in sterile skim milk. El-Gazzar et al. (*1992*) observed that *L. monocytogenes* survived during a mesophilic fermentation process (starter culture containing a 4 strain mixture of *Lactococcus lactis* subsp. *cremoris*) in skim milk as well as further storage for 4-6 weeks at 4°C. Schaack and Marth (*1988a*) reported variable growth of *L. monocytogenes* in the presence of *Lactococcus lactis* subsp. *lactis* or *Lactococcus lactis* subsp. *cremoris* depending on inoculum level, with highest populations observed in starter-free controls. Greatest inhibition was observed using a 5.0% starter culture inoculum and an incubation temperature of 30°C. While these results agree with our findings, neither of these studies examined sublethal injury of healthy *L. monocytogenes* cells that may result from competitive inhibition and/or acid production. In our study, more than 90% of the healthy *L. monocytogenes* cells were injured after 24 h fermentation period in all types of milk. The extent of increase in the number of injured cells was inversely related to severity of the heat-treatment that the milk received. As mentioned previously, this could again be due to the more hostile environment of raw as compared to heat-treated milk.

For LHI and HHI *L. monocytogenes* cells, increasing LLLC inoculum levels from 0.5% to 2.0% resulted in a lesser increase in the number of injured cells. This is likely due to increased inhibition of LHI and HHI *L. monocytogenes* cells by higher inoculum levels of LLLC, causing the total population and consequently the percentage of injured *L. monocytogenes* cells to decrease, thus explaining the reverse trend. Schaack and Marth

(*1988a*) also showed that inhibition of healthy *L. monocytogenes* cells increased with increasing levels of starter culture. Our results show the same trend for healthy as well as sublethally injured *L. monocytogenes* cells.

A greater increase in the percentage of injured cells was observed for HHI *L. monocytogenes* as compared to LHI cells. Increased growth (total population) of HHI cells during the 24 h fermentation period as compared to LHI cells (resulting in concomitant increase in injured cells) could possibly explain this trend. Williams and Golden (*1998*) observed that acid injury of *L. monocytogenes* was enhanced by prior heat stress. The extent of sublethal injury could also influence recovery on selective media. Thus, the above results show that *L. monocytogenes* (irrespective of initial physiological state) can survive the 24-h fermentation period at 31.1°C. In all instances, the pathogen exhibited some growth in the presence of LLLC, albeit less than in the controls. Some inhibition was observed at higher LLLC inoculum levels for *L. monocytogenes* in all physiological states. Higher levels of LLLC increased acid production, resulting in a concomitant increase in the number of healthy *L. monocytogenes* cells that became injured. Growth and activity of the starter culture was not affected by the presence of *L. monocytogenes* as observed from comparable values obtained for the LLLC population as well as the pH drop in controls and test samples.

Most studies investigating the behavior of *L. monocytogenes* during cheesemaking and curing have used pasteurized milk inoculated with the pathogen (*Ryser and Marth 1987a, 1989, Yousef and Marth 1988*). Factors affecting the fate of pathogens during cheesemaking and subsequent aging include the characteristics of the pathogen (heat, acid and salt tolerance, physiological state), temperature/time profile of the milk

from silo storage to completion of cheesemaking, pH profile, generation of metabolites (volatile compounds, inhibitors and bacteriocins produced by the starter culture), native milk enzymes and added enzymes. In addition, raw milk contains various antibacterial factors including antibodies, complement and non-antibody proteins such as lysozyme, lactoferrin and lactoperoxidase as well as macrophages, polymorphonuclear leukocytes, and lymphocytes (*Johnson et al. 1990a*). Their presence will influence the survival of intracellular pathogens such as *L. monocytogenes* in fermented dairy products.

In our study, raw and subpasteurized milk allowed less repair of sublethally injured cells and also showed higher numbers of injured cells compared to pasteurized milk. Given the low pH and high salt content of cheese, complete inactivation of sublethally injured *L. monocytogenes* in cheese (even if it survives the cheesemaking/fermentation process) during the 60-day storage period may be possible. The various heat treatments given to milk for cheesemaking should be investigated to better define conditions that will minimize pathogen survival in cheeses that are subject to the mandatory 60-day ripening rule.

ACKNOWLEDGMENTS

We would like to thank the National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI for funding this research. We would also like to thank Mr. Kadir Kizilkaya and Ms. Arzu Cagri for helping with programming in SAS.

TABLES

Table 6: Percent Heat-injury of *L. monocytogenes* in TPB and UHT Milk

Heating Medium	56°C/20 min	64°C/2 min
Tryptose Phosphate Broth	99.71±0.36[a]	99.74±0.18[a]
UHT Milk	99.44±0.31[a]	99.41±0.36[a]

Means±standard deviations (n=3). Means in the same column with different superscript are significantly different ($p<0.05$).

Table 7: Mixed Covariance Procedure Table for Growth of Healthy *L. monocytogenes* Cells

Effect	Num DF	Den DF	F Value	Pr > F
Milk	4	12.5	17	<.0001
Time	5	48.7	554.44	<.0001
Milk * Time	**20**	**46.9**	**1.59**	**0.0974**

Note: Pr > F value less than 0.05 indicates significant effect of the particular interaction

Table 8: Repair of *L. monocytogenes* in Different Types of Milk without Starter Culture

Incubation period (h)	Type of Milk	Low Heat Injured %	High Heat injured %
2	Raw	5.18±3.54^{a}	0.24±0.13^{a}
	LHT	1.75±0.39^{b}	0.18±0.08^{a}
	HHT	0.39±0.12^{b}	0.32±0.13^{a}
	Pasteurized	2.68±0.74^{ab}	0.25±0.11^{a}
	UHT	2.25±1.05^{ab}	2.56±2.88^{a}
4	Raw	17.25±3.67^{ab}	2.60±0.72^{c}
	LHT	15.37±3.14^{b}	3.71±1.77^{bc}
	HHT	20.82±6.48^{ab}	6.88±0.26^{a}
	Pasteurized	21.32±2.94^{ab}	4.42±1.03^{bc}
	UHT	25.15±2.91^{a}	5.62±1.43^{ab}
6	Raw	47.12±1.23^{c}	32.16±1.53^{c}
	LHT	51.19±1.54^{b}	33.72±1.24^{bc}
	HHT	51.27±0.92^{b}	33.79±1.53^{bc}
	Pasteurized	54.15±1.48^{a}	35.74±0.93^{b}
	UHT	55.36±1.42^{a}	40.00±1.26^{a}
8	Raw	76.43±1.87^{b}	59.54±0.92^{d}
	LHT	78.58±1.42^{ab}	68.29±1.47^{ab}
	HHT	81.32±2.24^{a}	65.01±2.72^{bc}
	Pasteurized	79.91±3.04^{ab}	63.03±2.66^{cd}
	UHT	80.17±1.32^{ab}	69.03±1.68^{a}
24	Raw	78.95±0.74^{b}	80.93±1.57^{b}
	LHT	84.28±4.60^{ab}	82.59±2.71^{b}
	HHT	83.63±3.98^{ab}	82.04±2.67^{b}
	Pasteurized	87.57±1.62^{a}	80.17±1.10^{b}
	UHT	87.29±3.30^{a}	87.74±3.88^{a}

Means±standard deviations (n=3). Means in the same column and fermentation period with different superscript are significantly different ($p<0.05$).

Table 9: Mixed Covariance Procedure Table for Repair of Sublethally Injured *L. monocytogenes* Cells After 24 h

Effect	Num DF	Den DF	F Value	Pr > F
Milk	4	14.3	5.64	0.0062
Phys. State	**1**	**6.52**	**1.99**	**0.2046**
Milk * Phys. State	**4**	**13.4**	**2.12**	**0.1352**

Note: Pr > F value less than 0.05 indicates significant effect of the particular interaction

Table 10: Mixed Covariance Procedure Table for Log Increase of total *L. monocytogenes* after 24 h

Effect	Num DF	Den DF	F Value	Pr > F
Milk	4	60.2000	6.3300	0.0003
Starter	3	91.3000	35.2800	<.0001
Milk * Starter	12	88.1000	3.2400	0.0007
Phys. State	2	97.7000	10.2000	<.0001
Milk* Phys. State	8	93.3000	0.9100	0.5101
Starter * Phys. State	6	63.2000	4.2600	0.0011
Milk* Starter * Phys. State	24	71.7000	0.7500	0.7849

Note: Pr > F value less than 0.05 indicates significant effect of the particular interaction

Table 11: Mixed Covariance Procedure Table for Percent Increase in Injured *L. monocytogenes* Cells

Effect	Num DF	Den DF	F value	Pr >F
Milk	4	84	4.48	0.0025
Starter	3	67.4	11.38	<.0001
Milk * Starter	12	83	0.94	0.5129
Phys. State	2	258	259.70	<.0001
Milk * Phys. State	8	183	0.80	0.6035
Starter * Phys. State	6	238	28.84	<.0001
Milk * Starter * Phys. State	**24**	**185**	**3.43**	**<.0001**

Note: Pr > F value less than 0.05 indicates significant effect of the particular interaction

Table 12: Percent Increase in Injured *L. monocytogenes* Cells in Raw Milk at Different Starter Culture Levels

Fermentation Period (h)	Starter Culture (%)	Healthy (%)	Low Heat-Injured (%)	High Heat-Injured (%)
2	0.5	32.30±1.98^{b}	23.29±4.47^{a}	36.92±6.48^{a}
	1.0	39.17±6.86^{ab}	22.48±6.33^{a}	25.20±6.57^{b}
	2.0	48.09±5.06^{a}	21.83±3.17^{a}	18.10±3.46^{b}
4	0.5	48.26±3.22^{b}	38.00±2.55^{a}	49.18±4.05^{a}
	1.0	48.24±5.89^{b}	36.03±2.08^{a}	40.20±1.56^{b}
	2.0	62.81±3.78^{a}	36.80±4.25^{a}	33.04±2.72^{c}
6	0.5	59.59±1.78^{b}	48.60±0.65^{a}	56.63±1.54^{a}
	1.0	60.58±1.33^{b}	46.76±1.39^{ab}	52.52±0.66^{b}
	2.0	74.03±1.62^{a}	44.71±0.95^{b}	45.61±0.47^{c}
8	0.5	64.23±2.88^{b}	49.63±0.48^{a}	58.63±0.38^{a}
	1.0	64.16±2.46^{b}	49.45±4.14^{a}	55.87±0.40^{b}
	2.0	74.42±2.26^{a}	47.04±0.85^{a}	47.51±1.71^{c}
24	0.5	66.27±5.32^{a}	48.99±3.08^{a}	60.58±1.87^{a}
	1.0	67.28±3.59^{a}	51.53±7.08^{a}	60.51±0.94^{a}
	2.0	74.33±4.27^{a}	47.41±1.62^{a}	47.97±2.24^{b}

Means±standard deviations (n=3). Means in the same column and fermentation period with different superscript are significantly different ($p<0.05$).

Table 13: Percent Increase in Injured *L. monocytogenes* Cells in Low Heat-Treated Milk at Different Starter Culture Levels

Fermentation Period (h)	Starter Culture (%)	Healthy (%)	Low Heat-Injured (%)	High Heat-Injured (%)
2	0.5	24.69±3.18^{b}	22.19±1.27^{a}	27.18±2.58^{a}
	1.0	24.65±2.59^{b}	21.84±2.86^{a}	23.43±4.48^{a}
	2.0	39.62±3.59^{a}	23.04±3.28^{a}	22.44±6.96^{a}
4	0.5	43.80±1.46^{b}	38.96±1.88^{a}	42.68±1.20^{a}
	1.0	39.72±4.16^{b}	34.56±1.58^{ab}	39.61±4.88^{ab}
	2.0	54.59±7.67^{a}	33.68±3.43^{b}	34.77±5.16^{b}
6	0.5	57.72±0.88^{b}	47.44±1.30^{a}	54.18±0.63^{a}
	1.0	56.38±0.62^{b}	46.32±0.98^{a}	50.00±1.04^{b}
	2.0	69.33±0.58^{a}	44.13±0.41^{b}	44.82±0.84^{c}
8	0.5	65.34±1.70^{b}	51.73±1.13^{a}	55.50±1.75^{a}
	1.0	58.62±1.47^{c}	49.03±1.31^{b}	50.77±1.03^{b}
	2.0	70.66±0.44^{a}	45.96±1.00^{c}	47.98±1.17^{c}
24	0.5	67.86±3.72^{b}	53.47±1.38^{a}	57.13±2.50^{a}
	1.0	61.58±2.63^{ab}	50.19±2.48^{a}	52.00±2.26^{b}
	2.0	74.35±2.78^{a}	49.54±1.73^{a}	50.50±0.51^{b}

Means±standard deviations (n=3). Means in the same column and fermentation period with different superscript are significantly different ($p<0.05$).

Table 14: Percent Increase in Injured *L. monocytogenes* Cells in High Heat-Treated Milk at Different Starter Culture Levels

Fermentation Period (h)	Starter Culture (%)	Healthy (%)	Low Heat-Injured (%)	High Heat-Injured (%)
2	0.5	24.57±2.14^{a}	25.95±0.96^{a}	26.65±0.99^{a}
	1.0	30.89±2.63^{a}	24.56±1.15^{a}	21.11±5.37^{a}
	2.0	34.83±5.56^{a}	19.17±4.25^{b}	22.26±2.65^{a}
4	0.5	44.77±1.83^{ab}	41.04±1.34^{a}	43.05±0.90^{a}
	1.0	42.87±4.27^{b}	35.49±1.32^{ab}	34.29±3.40^{b}
	2.0	53.36±7.93^{a}	31.42±2.17^{b}	36.03±0.85^{b}
6	0.5	59.62±1.38^{b}	47.50±0.55^{a}	54.11±0.72^{a}
	1.0	55.31±1.15^{b}	46.10±0.80^{b}	48.09±0.60^{b}
	2.0	68.08±0.73^{a}	43.36±0.27^{c}	44.91±0.60^{c}
8	0.5	65.62±0.69^{ab}	52.25±1.34^{a}	56.73±0.89^{a}
	1.0	61.15±1.50^{b}	48.91±0.91^{b}	48.94±0.43^{b}
	2.0	71.26±1.18^{a}	44.82±0.46^{c}	50.14±1.00^{b}
24	0.5	70.56±3.63^{ab}	53.81±1.13^{a}	57.72±1.68^{a}
	1.0	65.64±2.02^{b}	51.48±0.74^{a}	50.33±0.64^{b}
	2.0	74.24±2.85^{a}	46.63±0.60^{b}	51.86±0.84^{b}

Means±standard deviations (n=3). Means in the same column and fermentation period with different superscript are significantly different ($p<0.05$).

Table 15: Percent Increase in Injured *L. monocytogenes* Cells in Pasteurized Milk at Different Starter Culture Levels

Fermentation Period (h)	Starter Culture (%)	Healthy (%)	Low Heat-Injured (%)	High Heat-Injured (%)
2	0.5	22.72±3.47^{a}	24.31±2.84^{a}	29.59±3.04^{a}
	1.0	27.67±5.11^{a}	22.68±2.66^{ab}	22.07±5.65^{ab}
	2.0	31.50±0.53^{a}	18.87±2.67^{b}	20.29±5.34^{b}
4	0.5	41.10±3.37^{a}	40.28±1.68^{a}	44.27±2.06^{a}
	1.0	42.82±4.07^{a}	36.89±2.00^{a}	36.16±2.77^{b}
	2.0	45.19±4.76^{a}	30.57±0.58^{b}	36.08±2.12^{b}
6	0.5	53.42±0.52^{b}	47.43±1.38^{a}	53.76±0.88^{a}
	1.0	53.82±1.95^{b}	45.92±1.49^{a}	47.39±1.37^{b}
	2.0	65.89±1.46^{a}	43.01±1.63^{b}	43.18±1.28^{c}
8	0.5	64.09±5.10^{ab}	51.41±2.17^{a}	58.16±1.91^{a}
	1.0	59.98±1.59^{b}	47.59±1.68^{ab}	49.05±0.77^{b}
	2.0	69.51±2.19^{a}	45.08±1.34^{b}	47.24±3.26^{b}
24	0.5	67.49±6.45^{a}	53.29±3.78^{a}	60.89±2.99^{a}
	1.0	62.71±0.80^{a}	49.65±2.55^{ab}	50.43±1.14^{b}
	2.0	70.55±3.59^{a}	45.90±1.86^{b}	48.53±4.60^{b}

Means±standard deviations (n=3). Means in the same column and fermentation period with different superscript are significantly different ($p<0.05$).

Table 16: Percent Increase in Injured *L. monocytogenes* Cells in UHT–Pasteurized Milk at Different Starter Culture Levels

Fermentation Period (h)	Starter Culture (%)	Healthy (%)	Low Heat-Injured (%)	High Heat-Injured (%)
2	0.5	18.00±7.02^{b}	25.76±0.67^{a}	31.95±1.15^{a}
	1.0	24.94±4.79^{b}	22.83±6.09^{a}	14.87±2.33^{b}
	2.0	41.37±4.21^{a}	31.07±6.61^{a}	18.91±2.83^{b}
4	0.5	34.94±2.00^{b}	33.79±2.99^{a}	45.43±1.52^{a}
	1.0	36.16±5.45^{b}	32.67±5.79^{ab}	32.34±2.35^{b}
	2.0	57.19±5.37^{a}	35.84±4.92^{a}	31.52±8.68^{b}
6	0.5	47.26±0.58^{b}	47.41±0.82^{a}	53.46±0.93^{a}
	1.0	48.00±1.47^{b}	45.01±1.00^{b}	45.72±0.81^{b}
	2.0	62.48±1.27^{a}	42.67±1.14^{c}	42.64±0.88^{c}
8	0.5	51.63±3.71^{b}	49.40±2.74^{a}	54.89±1.94^{a}
	1.0	53.92±2.01^{b}	44.78±1.14^{b}	47.26±1.14^{b}
	2.0	64.93±3.51^{a}	46.09±1.70^{ab}	43.03±0.64^{c}
24	0.5	51.68±4.22^{b}	50.52±4.67^{a}	53.89±4.35^{a}
	1.0	56.83±3.03^{b}	42.77±3.29^{b}	49.41±1.18^{ab}
	2.0	71.43±3.06^{a}	49.73±3.19^{a}	45.64±1.64^{b}

Means±standard deviations (n=3). Means in the same column and fermentation period with different superscript are significantly different ($p<0.05$).

Table 17: Percent Increase in Injured *L. monocytogenes* Cells in Different Types of Milk Fermented with 0.5% LLLC

Fermentation Period (h)	Type of Milk	Healthy (%)	Low Heat-Injured (%)	High Heat-Injured (%)
2	Raw	32.30±1.98^{a}	23.29±4.47^{a}	36.92±6.48^{a}
	LHT	24.69±3.18^{b}	22.19±1.27^{a}	27.18±2.58^{b}
	HHT	24.57±2.14^{b}	25.95±1.27^{a}	26.65±0.99^{b}
	Pasteurized	22.72±3.47^{b}	24.31±2.84^{a}	29.59±3.04^{b}
	UHT	18.00±7.01^{b}	25.76±0.67^{a}	31.95±1.15^{ab}
4	Raw	48.26±3.22^{a}	38.00±2.55^{a}	49.17±4.05a
	LHT	43.80±1.46^{ab}	38.96±1.88^{a}	42.68±1.20^{b}
	HHT	44.77±1.83^{ab}	41.04±1.34^{a}	43.05±0.90^{b}
	Pasteurized	41.10±3.37^{b}	40.28±1.68^{a}	44.27±2.06^{b}
	UHT	34.94±2.00^{c}	33.79±2.99^{b}	45.42±1.52^{ab}
6	Raw	59.49±1.96^{a}	48.60±0.65^{a}	56.63±1.54^{a}
	LHT	57.72±0.88^{a}	47.44±1.30^{a}	54.18±0.63^{b}
	HHT	59.62±1.38^{a}	47.50±0.55^{a}	54.11±0.73^{b}
	Pasteurized	53.42±0.52^{b}	47.43±1.38^{a}	53.76±0.88^{b}
	UHT	47.26±0.58^{c}	47.41±0.82^{a}	53.46±0.93^{b}
8	Raw	64.23±2.88^{a}	49.63±0.48^{a}	58.63±0.38^{a}
	LHT	65.34±1.70^{a}	51.73±1.13^{a}	55.50±1.75^{bc}
	HHT	65.62±0.69^{a}	52.25±1.34^{a}	56.73±0.89^{abc}
	Pasteurized	64.09±5.11^{a}	51.47±2.17^{a}	58.16±1.91^{ab}
	UHT	51.63±3.71^{b}	49.40±2.74^{a}	54.89±1.94^{c}
24	Raw	66.27±5.33^{a}	48.99±3.08^{a}	60.58±1.87^{a}
	LHT	67.86±3.72^{a}	53.47±1.38^{a}	57.13±2.50^{ab}
	HHT	70.55±3.63^{a}	53.81±1.13^{a}	57.72±1.68^{ab}
	Pasteurized	67.49±6.45^{a}	53.29±3.78^{a}	60.89±2.99^{a}
	UHT	51.68±4.22^{b}	50.52±4.67^{a}	53.89±4.35^{b}

Means±standard deviations (n=3). Means in the same column and fermentation period with different superscript are significantly different ($p<0.05$).

Table 18: Percent Increase in Injured *L. monocytogenes* Cells in Different Types of Milk Fermented with 1.0% LLLC

Fermentation Period (h)	Type of Milk	Healthy (%)	Low Heat-Injured (%)	High Heat-Injured (%)
2	Raw	39.17±6.86^{a}	22.47±6.33^{a}	25.20±6.57^{a}
	LHT	24.65±2.59^{b}	21.85±2.86^{a}	23.43±4.48^{ab}
	HHT	30.89±2.63^{ab}	24.56±1.14^{a}	21.11±5.37^{ab}
	Pasteurized	27.67±5.11^{b}	22.68±2.66^{a}	22.07±5.65^{ab}
	UHT	24.94±4.79^{b}	22.83±6.09^{a}	14.87±2.33^{b}
4	Raw	48.24±5.89^{a}	36.03±2.08^{a}	40.20±1.59^{a}
	LHT	39.72±4.16^{ab}	34.56±1.58^{a}	39.60±4.88^{a}
	HHT	42.87±4.27^{ab}	35.49±1.32^{a}	34.29±3.40^{ab}
	Pasteurized	42.82±4.07^{ab}	36.88±2.00^{a}	36.16±2.77^{ab}
	UHT	36.16±5.45^{b}	32.66±5.79^{a}	32.34±2.35^{b}
6	Raw	60.58±1.33^{a}	46.76±1.39^{a}	52.52±0.66^{a}
	LHT	56.38±0.63^{b}	46.32±0.98^{a}	50.00±1.04^{b}
	HHT	55.31±1.15^{b}	46.10±0.80^{a}	48.09±0.60^{c}
	Pasteurized	53.82±1.95^{b}	45.92±1.49^{a}	47.39±1.37^{c}
	UHT	48.00±1.47^{c}	45.01±1.00^{a}	45.38±0.26d
8	Raw	64.16±2.46^{a}	49.45±4.14^{a}	55.87±0.40^{a}
	LHT	58.62±1.47^{b}	49.03±1.31^{ab}	50.77±1.03^{b}
	HHT	61.14±1.50^{ab}	48.91±0.91^{ab}	48.94±0.43^{c}
	Pasteurized	59.98±1.59^{b}	47.59±1.68^{ab}	49.05±0.77^{c}
	UHT	53.92±2.01^{c}	44.78±1.14^{b}	47.26±1.14^{d}
24	Raw	67.28±3.59^{a}	51.53±7.08^{a}	58.51±0.94^{a}
	LHT	61.58±2.63^{b}	50.19±2.47^{a}	52.00±2.26^{b}
	HHT	65.63±2.02^{ab}	51.48±0.74^{a}	50.33±0.64^{b}
	Pasteurized	62.71±0.80^{ab}	49.65±2.54^{ab}	50.43±1.14^{b}
	UHT	56.83±3.03^{c}	42.77±3.29^{b}	49.41±1.18^{b}

Means±standard deviations (n=3). Means in the same column and fermentation period with different superscript are significantly different ($p<0.05$).

Table 19: Percent Increase in Injured *L. monocytogenes* Cells in Different Types of Milk Fermented with 2.0% LLLC

Fermentation Period (h)	Type of Milk	Healthy (%)	Low Heat-Injured (%)	High Heat-Injured (%)
2	Raw	48.09±5.06^{a}	21.83±3.17^{b}	18.10±3.46^{a}
	LHT	39.62±3.59^{b}	23.04±3.28^{b}	22.44±6.96^{a}
	HHT	34.83±5.56^{b}	19.17±4.25^{b}	22.26±2.65^{a}
	Pasteurized	31.50±0.53^{c}	18.87±2.67^{b}	20.29±5.34^{a}
	UHT	41.37±4.21^{ab}	31.07±6.61^{a}	18.90±2.83^{a}
4	Raw	62.81±3.78^{a}	36.80±4.25^{a}	33.04±2.72^{a}
	LHT	54.59±7.67^{ab}	3368±3.43^{a}	34.77±5.16^{a}
	HHT	53.36±7.93^{ab}	31.42±2.17^{a}	36.03±0.85^{a}
	Pasteurized	45.19±4.76^{b}	30.57±0.58^{a}	36.08±2.12^{a}
	UHT	57.19±5.37^{a}	35.84±4.92^{a}	31.52±8.68^{a}
6	Raw	74.03±1.62^{a}	44.71±0.95^{a}	45.61±0.46^{a}
	LHT	69.36±0.55^{b}	44.13±0.41^{ab}	44.82±0.84^{a}
	HHT	68.07±0.73^{b}	43.36±0.27^{ab}	44.91±0.56^{a}
	Pasteurized	65.89±1.46^{c}	43.01±1.63^{ab}	43.18±1.28^{b}
	UHT	62.48±1.27^{d}	42.67±1.14^{b}	42.64±0.88^{b}
8	Raw	74.42±2.26^{a}	47.04±0.85^{a}	47.51±1.71^{a}
	LHT	70.66±0.44^{ab}	45.96±1.00^{a}	47.98±1.17^{a}
	HHT	71.26±1.18^{ab}	44.82±0.46^{a}	50.14±1.00^{a}
	Pasteurized	69.51±2.19^{b}	45.08±1.34^{a}	47.24±3.26^{a}
	UHT	64.93±3.51^{c}	46.09±1.70^{a}	43.03±0.64^{b}
24	Raw	74.33±4.27^{a}	47.41±1.62^{a}	47.97±2.36^{ab}
	LHT	74.35±2.78^{a}	49.54±1.73^{a}	50.50±0.51^{a}
	HHT	74.24±2.84^{a}	46.63±0.60^{a}	51.86±0.84^{a}
	Pasteurized	70.55±3.59^{a}	45.90±1.86^{a}	48.53±4.60^{ab}
	UHT	71.43±3.06^{a}	49.73±3.19^{a}	45.64±1.64^{b}

Means±standard deviations (n=3). Means in the same column and fermentation period with different superscript are significantly different ($p<0.05$).

FIGURE LEGENDS

Figure 4: Sublethal heat injury of *L. monocytogenes* in UHT milk at 56°C.

Key: TPA represents the total population of both healthy as well as sublethally injured cells, TPNA represents the population of healthy cells.

Figure 5: Sublethal heat injury of *L. monocytogenes* in UHT milk at 64°C.

Key: same as that for Figure 1.

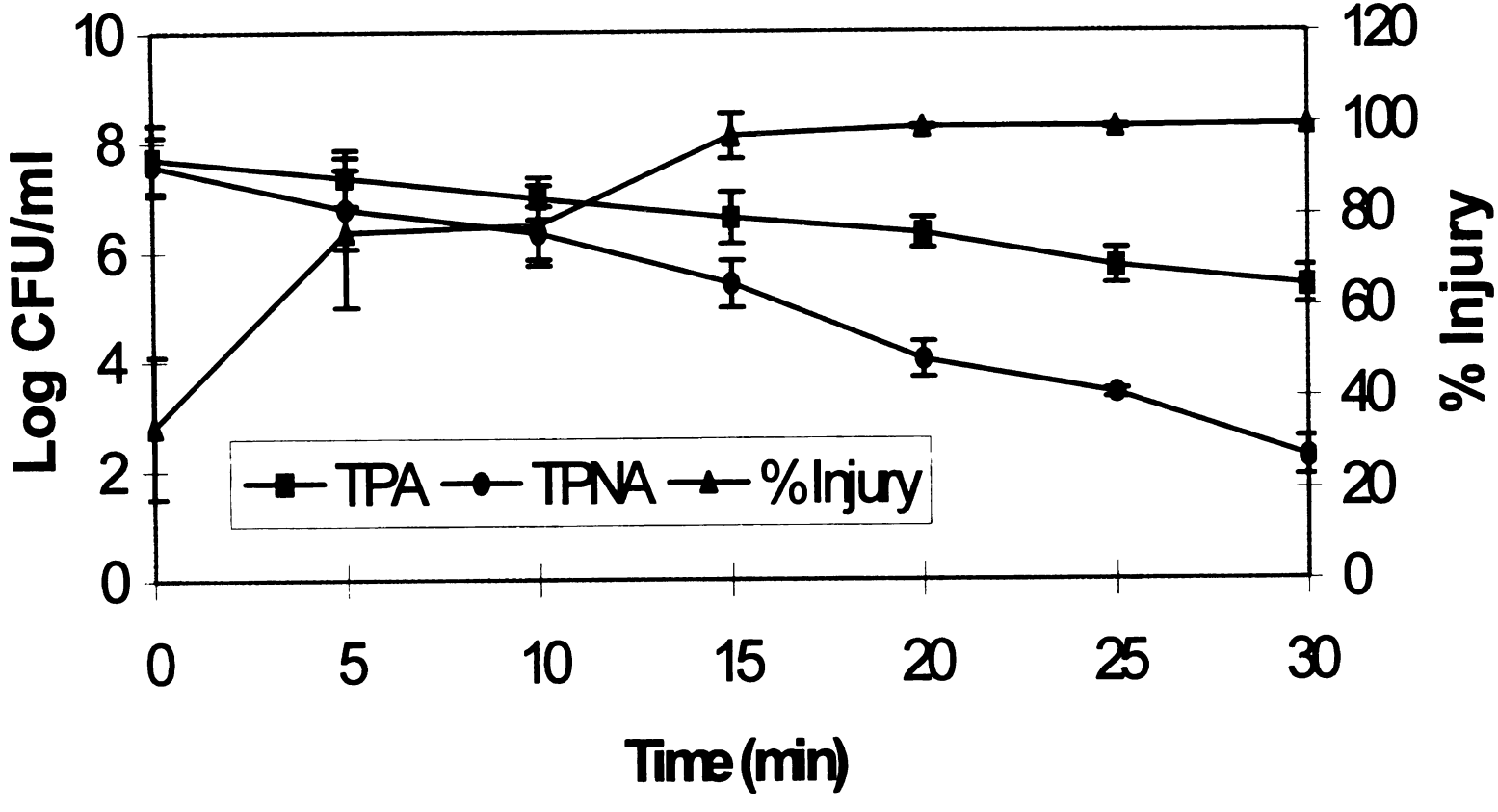

Figure 4: Heat injury of *L. monocytogenes* in UHT milk at 56°C.

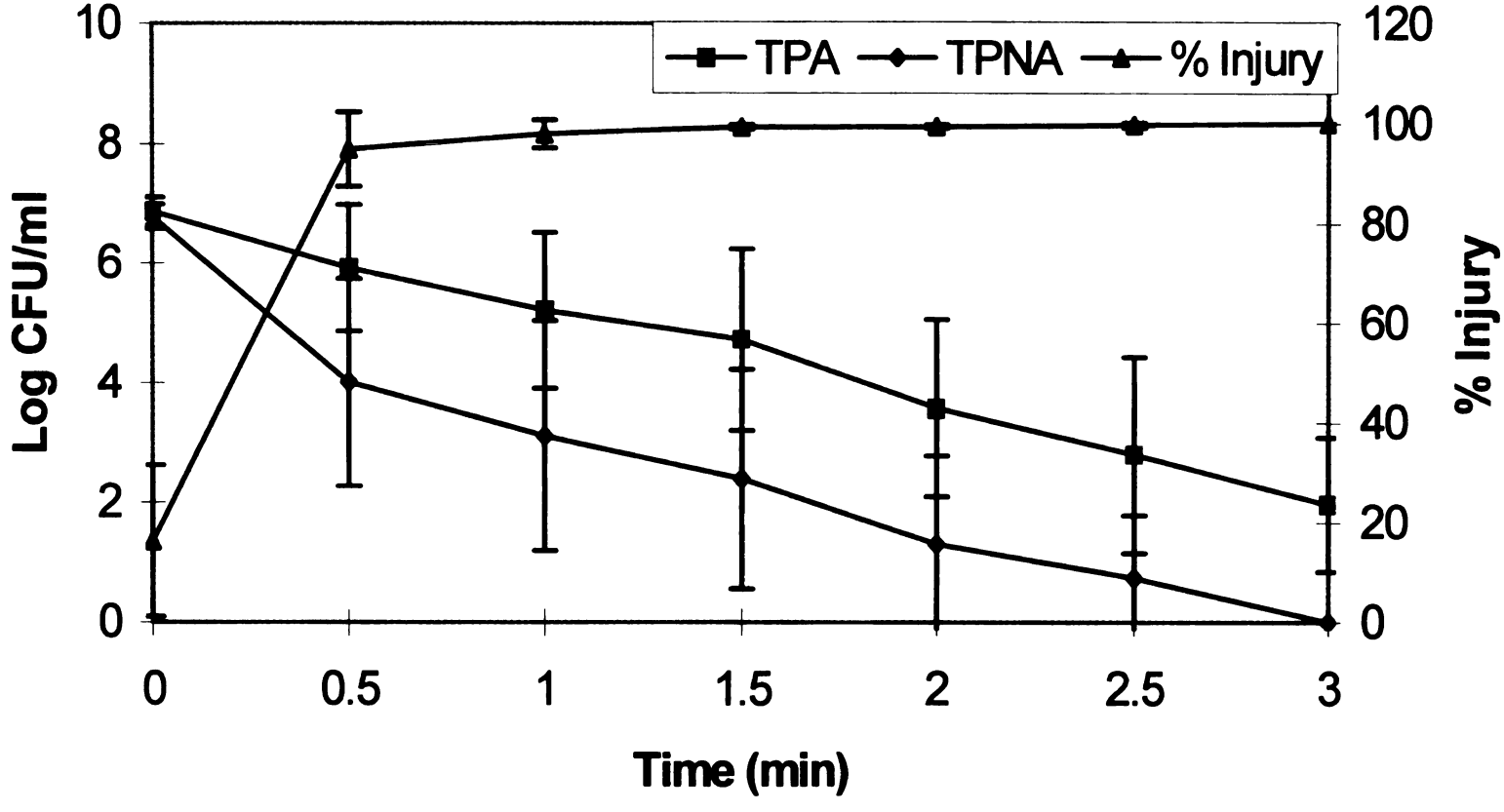

Figure 5: Heat injury of *L. monocytogenes* in UHT milk at 64°C.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Abdalla, O. M., G. L. Christen, and P. M. Davidson. 1993. Chemical composition of and *Listeria monocytogenes* survival in white pickled cheese. J. Food Prot. 56:841-846.

2. Agard, D. A. 1993. To fold or not to fold. Science 260:1903-1904.

3. Ali, A. E., A. T. Andres, and G. C. Cheeseman. 1980a. Influence of storage of milk on casein distribution between the micellular and soluble phase and its relationship to cheesemaking paramenters. J. Dairy Res. 47:371-382.

4. Ali, A. E., A. T. Andrews, and G. C. Cheeseman. 1980b. Factors influencing casein distribution in cold-stored milk and their effects on cheesemaking parameters. J. Dairy Res. 47:383-391.

5. Alichanidis, E., J. H. M. Wrathall, and A. T. Andrews. 1986. Heat stability of plasmin (milk proteinase) and plasminogen. J. Dairy Res. 53:259-269.

6. Amantea, G. F., B. J. Skura, and S. Nakai. 1986. Culture effect on ripening characteristics and rheological behavior of Cheddar cheese. J. Food Sci. 51:912-918.

7. American CheeseSociety. Preserving raw milk cheese industry. [Online] Available http://www.cheesesociety.org/raw-milk.htm, May 2000.

8. Andrews, A. T. 1974. Bovine milk acid phosphatase. II. binding to casein substrates and heat-inactivation studies. J. Dairy Res. 41:229- 237.

9. Anonymous. 1949. Cheeses, processed cheeses, cheese foods, cheese spreads, and related foods, definitions, and standards of identity. Federal Register. April 22, 1949, 1960-1992.

10. Anonymous. 1985. Soft cheese manufacturers to be inspected under FDA priority program. Food Chem. News 27(18):22.

11. Anonymous. 1986. Food and Drug Administration Dairy Product Safety Initiatives - Preliminary Status Report. FDA Center for Food Safety and Applied Nutrition - Milk Safety Branch. Washington, DC, Sep. 22.

12. Anonymous. 1987a. Hard cheese from Italian firm blocklisted. Food Chem. News 29(23):19.

13. Anonymous. 1987b. FDA sets separate program for cheese from unpasteurized milk. Food Chem. News. April. 27, 1987, pp. 17-18.

14. Anonymous. 1987c. FDA continues to find *Listeria* during dairy plant operations. Food Chem. News 29(1):47-48.

15. Anonymous. 1988a. Update - Listeriosis and pasteurized milk. M. M. W. R. 37:764-765.

16. Anonymous. 1988b. FDA finds *Listeria* in foreign soft cheeses. Food Chem. News 29(49):36-37.

17. Anonymous. 1988c. Proper pasteurization procedures should control *Listeria*, CDC says. Food Chem. News 30(4):10.

18. Anonymous. 1989. Update - Listeriosis and pasteurized milk. Dairy Food Environ. Sanitat. 9:150.

19. Anonymous. 1990. USDA, FDA officials report apparent decrease in *Listeria* isolations. Food Chem. News 32(1):12-15.

20. Anonymous. 1997. Letter from NACMF - Cheese Subcommittee, April 3.

21. Anonymous. 1998a. Blue cheese recalled United Press International Release, April 11.

22. Anonymous. 1998b. Quesos Puebla Mexican style fresh white cheese recalled FDA Enforcement Report, November 11.

23. Anonymous. 1998c. Queseria Zurita Spanish white cheese recalled FDA Enforcement Report, November 11.

24. Anonymous. 1998d. Blue cheese recalled FDA Enforcement Report, July 22.

25. Anonymous. 1999a. Queso Fresco cheese recalled FDA Enforcement Reports, November 17.

26. Anonymous. 1999b. Ricotta Salata cheese recalled FDA Enforcement Report, October 20.

27. Anonymous. 1999c. Mild Cheddar cheese recalled FDA Enforcement Report, July 14.

28. Anonymous. 1999d. Raw milk "Chives Colby" cheese recalled FDA Enforcement Report, June 30.

29. Anonymous. 1999e. Shredded Monterey Jack cheese recalled FDA Enforcement Report, May 26.

30. Anonymous. 1999f. Pimento cheese spread recalled FDA Enforcement Report, March 3.

31. Anonymous. 2000a. Organic Medium Cheddar cheese recalled. FDA Enforcement Report, September 20.

32. Anonymous. 2000b. 60-Day aging may be inadequate to eliminate *E. coli* in cheese: Research. Cheese Reporter 125(3):1,10.

33. Anonymous. 2000c. Pimento spread recalled FDA Enforcement Report, June 21.

34. Archer, D. L. 1988. Review of the latest FDA information on the presence of *Listeria* in foods WHO Working Group on Foodborne Listeriosis, Geneva, Switzerland, February 15-19.

35. Azadian, B. S., G. T. Finnerty, and A. D. Pearson. 1989. Cheese-borne *Listeria* meningitis in immunocompetent patient. Lancet, February 11, 322-323.

36. Bachmann, H. P., and U. Spahr. 1995. The fate of potentially pathogenic bacteria in Swiss hard and semihard cheeses made from raw milk. J. Dairy Sci. 78:476-483.

37. Banks, J. M., M. W. Griffiths, J. D. Phillips, and D. D. Muir. 1986. The yield and quality of Cheddar cheese produced from thermized milk. Dairy Ind. Int. 51(7):31-32, 34-35.

38. Bannister, B. A. 1987. *Listeria monocytogenes* meningitis associated with eating soft cheese. J. Infect. 15:165-168.

39. Barrett, N. J. 1986. Communicable disease associated with milk and dairy products in England and Wales: 1983-1984. J. Infect. 12:265- 272.

40. Barza, M. 1985. Listeriosis and milk. New Engl. J. Med. 312:438-440.

41. Bearns, R. E., and K. F. Girard. 1958. The effect of pasteurization on *Listeria monocytogenes*. Can. J. Microbiol 4:55-61.

42. Borovian, G. E. 1989. Control of *Listeria monocytogenes* in comparison to other food pathogens using food preservatives. Proceedings of Annual Meeting of the American Society of Microbiology, New Orleans, May 14-18. Abstr. 167.

43. Bradshaw, J. G., J. T. Peeler, 3. J. Corwin, J. M. Hunt, J. T. Tierney, E. P. Larkin, and R. M. Twedt. 1985. Thermal resistance of *Listeria monocytogenes* in milk. J. Food Prot. 48:743-745.

44. Bradshaw, J. G., J. T. Peeler, J. J. Corwin, J. M. Hunt, and R. M. Twedt. 1987. Thermal resistance of *Listeria monocytogenes* in dairy products. J. Food Prot. 50:543-544, 556.

45. Breer, C. 1987. *Listeria* in cheese, p. 106-109. *In* A. Schoenberg (ed.) Listeriosis-Joint WHO/ROI Consultation on Prevention and Control. Berlin, West Germany, Dec. 10-12.

46. Bryan, F. L. 1983. Epidemiology of milk-borne diseases. J. Food Prot. 46:637-649.

47. Bryan, F. L. 1988. Risks associated with vehicles of foodborne pathogens and toxins. J. Food Prot. 51:498-508.

48. Buazzi, M. M., M. E. Johnson, and E. H. Marth. 1992a. Survival of *Listeria monocytogenes* during manufacture and ripening of Swizz cheese. J. Dairy Sci. 75:380-386.

49. Buazzi, M. M., M. E. Johnson, and E. H. Marth. 1992b. Fate of *Listeria monocytogenes* during the manufacture of Mozzarella cheese. J. Food Prot. 55:80-83.

50. Bula, Ch., J. Bille, F. Mean, M. P. Glauser, and The Group "*Listeria* Vaud". 1988. Epidemic food-borne listeriosis in Western Switzerland. I. Description of the 58 adult cases. Interscience Conference on Antmicorbial Agents and Chemotherapy, Los Angeles, CA, Oct. 23-26.

51. Bunning, V. K., R. G. Crawford, J. T. Tierney, and J. T. Peeler. 1992. Thermotolerance of heat-shocked *Listeria monocytogenes* in milk exposed to high-temperature, short time pasteurization. Appl. Environ. Microbiol. 58:2096-2098.

52. Bunning, V. K., C. W. Donnelly, J. T. Peeler, E. H. Briggs, J. G. Bradshaw, R. G. Crawford, C. M. Beliveau, and J. T. Tierney. 1988. Thermal inactivation of *Listeria monocytogenes* within bovine milk phagocytes. Appl. Environ. Microbiol. 54:364-370.

53. Busch, S. V., and C. W. Donnelly. 1992. Development of a repair/enrichment broth for resuscitation of heat-injured *Listeria monocytogenes* and *Listeria innocua*. Appl. Environ. Microbiol. 58:14-20.

54. CDC. 1985. Annual Summary 1982. Food-borne disease surveillance, CDC, Atlanta.

55. Cody, S. H., S. L. Abbott, A. A. Marfin, B. Schulz, P. Wagner, K. Robbins, J. C. Mohle-Boetani, D. J. Vugia. 1999. Two outbreaks of multidrug-resistant *Salmonella* serotype Typhimurium DT104 infections linked to raw-milk cheese in northern California J. Am. Med. Assoc. 281:1805-1810.

56. Coghill, D. M., I. D. Mutzelburgh, and S. J. Birch. 1982. Effect of thermization on the bacteriological and chemical quality of milk. Austral. J. Dairy Technol. 37:48-50.

57. Cousin, M. A. 1982. Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review. J. Food Prot. 45:172-207.

58. Craig, E. A., B. D. Gambill, and R. J. Nelson. 1993. Heat shock proteins: molecular chaperones of protein biogenesis. Microbiol. Rev. 57:402-414.

59. Dairy Marketing Fundamentals. Cheese production for 1999. [Online] Available http://www.canr.msu.edu/msue_thumb/dairy_mktg/mktg_html/prod_prdn/total_cheese_prdn_99.htm, November 1, 2000.

60. Dalton, C. B., C. C. Austin, J. Sobel, P. S. Hayes, W. F. Bibb, L. M. Graves, B. Swaminathan, M. E. Proctor, and P. M. Griffin. 1997. Listeriosis from chocolate milk: linking of an outbreak of febrile gastroenteritis and "sporadic" invasive disease. N. Engl. J. Med. 336:100-105.

61. D'Aoust, J.-Y. 1985a. Infective dose of *Salmonella typhimurium* in Cheddar cheese. Am. J. Epidemiol. 122:717-720.

62. D'Aoust, J.-Y., D. W. Warburton, and A. M. Sewell. 1985b. *Salmonella typhimurium* phage-Type 10 from Cheddar cheese implicated in a major Canadian foodborne outbreak. J. Food Prot. 48:1062-1066.

63. Dominquez, L., J. F. F. Garayzabal, J. A. Vazquez, J. L. Blanco, and G. Suarez. 1987. Fate of *Listeria monocytogenes* during manufacture and ripening of semi-hard cheese. Letters Appl. Microbiol. 4:125-127.

64. Donnelly, C. W. 1999. Conventional methods to detect and isolate *Listeria monocytogenes*, p. 225-260. *In* E. T. Ryser, and E. H. Marth (eds.), *Listeria*, Listeriosis, and Food Safety, 2nd. edition. Marcel Dekker, New York.

65. Donnelly, C. W., E. H. Briggs, and L. S. Donnelly. 1987. Comparison of heat resistance of *Listeria monocytogenes* by neutrophils and macrophages of bovine origin. Proceedings of Annual Meeting of the American Society of Microbiology, Atlanta, March 1-6.

66. Donnelly, C. W., G. J. Baignet, and E. H. Briggs. 1988. Flow cytometry for automated analysis of milk containing *Listeria monocytogenes*. J. Assoc. Off. Anal. Chem. 71:655-658.

67. Doyle, M. P., and K. A. Glass, J. T. Beery, G. A. Garcia, D. J. Pollard, and R. D. Schultz. 1987. Survival of *Listeria monocytogenes* in milk during high-temperature, short-time pasteurization. Appl. Environ. Microbiol. 53:1433-1438.

68. Dzurec, D. J., and R. R. Zall. 1985. Effect of heating cooling and storing milk on casein and whey proteins. J. Dairy Sci. 68:273-280.

69. El-Gazzar, F. E., H. F. Bohner, and E. H. Marth. 1992. Antagonism between *Listeria monocytogenes* and Lactococci during fermentation of products from ultrafiltered skim milk. J. Dairy Sci. 75(1):43-50.

70. El-Shenawy, M. A., A. E. Yousef, and E. H. Marth. 1989. Heat injury and inactivation of *Listeria monocytogenes* in reconstituted nonfat dry milk. Milchwissenschaft 44:741-745.

71. Ennahar, S., D. Aoude-Werner, O. Sorokine, A. Van-Dorsselaer, F. Bringel, J.-C. Hubert, and C. Hasselman. 1996. Production of pediocin AcH by *Lactobacillus plantarum* WHE 92 isolated from cheese. Appl. Environ. Microbiol. 62:4381-4387.

72. Farber, J. M. 1989. Thermal resistance of *Listeria monocytogenes* in foods. Int. J. Food Microbiol. 8:285-291.

73. Farber, J. M., and B. E. Brown. 1990. Effect of prior heat shock on heat resistance of *Listeria monocytogenes* in meat. Appl. Environ. Microbiol. 56:1584-1587.

74. Farber, J. M., and F. Pagotto. 1992. The effect of acid shock on the heat resistance of *Listeria monocytogenes*. Letters Appl. Microbiol. 15:197-201.

75. Farber, J. M., G. W. Sanders, and S. A. Malcolm. 1988. The presence of *Listeria* spp. in raw milk in Ontario. Can. J. Microbiol. 34:95-100.

76. Farber, J. M., W. H. Ross, and J. Harwig. 1996. Health risk assessment of *Listeria monocytogenes* in Canada. Int. J. Food Microbiol. 30:145-156.

77. Farber, J. M., G. W. Sanders, S. Dunfield, and R. Prescott. 1989. The effect of various acidulants on the growth of *Listeria monocytogenes*. Letters Appl. Microbiol. 9:181-183.

78. FDA/CFSAN. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook - *Listeria monocytogenes*. [Online] Available http://vm.cfsan.fda.gov/~mow/chap6.html, March 8, 2000.

79. FDA News Relsease. Colby Jack and Mexican blend cheese products recalled. [Online] Available http://www.fda.gov/oc/po/firmrecalls/schreiber11_00.html, November 8, 2000.

80. Fenlon, D. R. 1999. *Listeria monocytogenes* in the natural environment, p. 21. *In* E. T. Ryser, and E. H. Marth (eds.), *Listeria*, Listeriosis, and Food Safety, 2nd. edition. Marcel Dekker, Inc., New York.

81. Finch, M. J., and P. A. Blake. 1985. Foodborne outbreaks of campylobacteriosis: the United States experience, 1980-1982. Am. J. Epidemiol. 122:262-268.

82. Fleming, D. W., S. L. Cochi, K. MacDonald, J. Brodum, P. Hayes, B. Plikaytis, M. Holmes, A. Audier, C. Broome, and A. Reingoid. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. New Engl. J. Med. 312:404-407.

83. Fontaine, R. E., M. L. Cohen, W. T. Martin, and T. M. Vernon. 1980. Epidemic salmonellosis from Cheddar cheese: surveillance and prevention. Am. J. Epidemiol. 111:247-253.

84. Foster, J. W., and M. P. Spector. 1995. How *Salmonella* survives against the odds. Ann. Rev. Microbiol. 49:145-174.

85. Franklin, J. G., and M. E. Sharpe. 1962. The bacterial content of milk used for making Cheddar cheese and its effect on the flora and quality of the cheeses produced. Proceedings 16th Int. Dairy Congress 3:305-312.

86. Galbraith, N. S., P. Forbes, and C. Clifford. 1982. Communicable disease associated with milk and dairy products in England and Wales 1951-80. Br. Med. J. 284:1761-1765.

87. Garayzabal, J. F. F., L. D. Rodriguez, J. A. V. Boland, E. F. R. Ferri, B. B. Dieste, J. L. Cancelo, and G. S. Fernandez. 1987. Survival of *Listeria monocytogenes* in raw milk treated in a pilot plant size pasteurizer. J. Appl. Bacteriol. 63:533-537.

88. Gellin, B. G., and C. V. Broome. 1989. Listeriosis. JAMA 261:1313-1320.

89. Genigorgis, C., M. Carniciu, D. Dutulescu, and T. B. Flower. 1991. Growth and survival of *Listeria monocytogenes* in market cheeses stored at 4 to 30°C. J. Food Prot. 54:662-668.

90. George, S. M., B. M. Lund, and T. F. Brocklehurst. 1988. The effect of pH and temperature on initiation of growth of *Listeria monocytogenes*. Letters Appl. Microbiol. 15:49-52.

91. Ginzinger, W., D. Jaros, P. Lavanchy, and H. Rohm. 1999. Raw milk flora affects composition and quality of Bergkaese. III. Physical and sensory properties, and conclusions. Lait 79:411-421.

92. Goepfert, J. M., N. F. Olson, and E. H. Marth. 1968. Behavior of *Salmonella typhimurium* during manufacture and curing of Cheddar cheese. Appl. Microbiol. 16:862-866.

93. Gravani, R. 1999. Incidence and Control of Listeria in Food-Processing Facilities, p. 657-659. *In* E. T. Ryser, and E. H. Marth (eds.), *Listeria*, Listeriosis, and Food Safety. Marcel Dekker, Inc., New York.

94. Gray, M. L., and A. H. Killinger. 1966. *Listeria monocytogenes* and listeric infections. Bacteriol. Rev. 30:309-382.

95. Groves, D. 2000a. Pasteurization of cheese milk issue shaping up as Epic Battle. Cheese Reporter 125(5):2.

96. Groves, D. 2000b. Personal Communication.

97. Guhan, C. G. M., B. O'Driscoll, and C. Hill. 1996. Acid adaptation of *Listeria monocytogenes* can enhance survival in acidic foods and during milk fermentation. Appl. Environ. Microbiol. 62:3128-3132.

98. Hanrahan, F. P., H. E. Walter, A. M. Sadler, and R. P. Tittsler. 1963. Modified equipment for pasteurizing and deodorizing market milk and for pasteurizing, deodorizing, and slightly concentrating cheese milk. J. Dairy Sci. 46:69-72.

99. Hargrove, R. E., F. E. McDonough, and W. A. Mattingly. 1969. Factors affecting survival of *Salmonella* in Cheddar and Colby cheese. J. Milk Food Technol. 32:480-484.

100. Hayes, P. S., J. C. Feeley, L. M. Graves, G. W. Ajello, and D. W. Fleming. 1986. Isolation of *Listeria monocytogenes* from raw milk. Appl. Environ. Microbiol. 51:438-440.

101. Hermier, J., and O. Cerf. 1986. Methods of heat treatment, p. 149-156. *In* A. Eck (ed.) Cheesemaking Science and Technology. Lavoisier Publishing Inc., New York, NY.

102. Hooydonk, A. C. M., P. G. van de Koster, and I. J. Boerrigter. 1987. The renneting properties of heated milk. Neth. Milk Dairy J. 41:3- 18.

103. Ikonomov, L., and D. Todorov. 1964. Studies of the viability of *Listieria monocytogenes* in ewe's milk and dairy products. Vet. Med. Nauki, Sofiya 7:23-29.

104. Ikonomov, L., and D. Todorov. 1967. Microbiological studies on the pasteurization of ewe's milk. III. Resistance of some pathogenic bacteria. Vet. Med. Nauki, Sofiya 4:99-108.

105. Johnson, A. H. 1974. The composition of milk. *In* B. H. Webb, A. H. Johnson, and J. A. Alford (eds.), Fundamentals of Dairy Chemistry, 2nd. edition. AVI Publishing Co., Inc., Westport, Connecticut.

106. Johnson, E. A., J. H. Nelson, and M. Johnson. 1990a. Microbiological safety of cheese made from heat-treated milk, Part II. Microbiology J. Food Prot. 53:519-540.

107. Johnson, E. A., J. H. Nelson, and M. Johnson. 1990b. Microbiological safety of cheese made from heat-treated milk, Part I. Executive Summary, introduction and history. J. Food Prot. 53:441-452.

108. Johnson, E. A., J. H. Nelson, and M. Johnson. 1990c. Microbiological safety of cheese made from heat-treated milk, Part III. Technology, discussion, recommendations, bibliography. J. Food Prot. 53:610-623.

109. Johnston, D. E., R. J. Murphy, A. Gilmour, and R. G. Macelhinney. 1981. Thermization of milk. Chemical Aspects. Milchwissenschaft. 36:748-750.

110. Kaplan, M. M., M. Abdussalam, and G. Bijlanga. 1962. Diseases transmitted through milk, p. 11-74. *In* Milk Hygiene. World Health Monograph Series, No. 48. World Health Organization, Geneva, Switzerland.

111. Kaufmann, U. 1990. Behavior of *Listeria monocytogenes* in raw milk hard cheeses. Revue Suisse Agric. 22:5-9.

112. Kleeburg, H. H. 1975. Tuberculosis and other mycobacterioses. *In* W. T. Hubbert, W. F. McCullock, and P. R. Schnurrenburger (eds.), Diseases transmitted from animals to man, 6th edition. Charles C. Thomas, Springfield, IL.

113. Knabel, S. J., H. W. Walker, P. A. Hartman, and A. F. Mendonca. 1990. Effects of growth termperature and strictly anaerobic recovery on survival of *Listeria monocytogenes* during pasteurization. Appl. Environ. Microbiol. 56:370-376.

114. Kornacki, J. L. 1982. Foodborne illness caused by *Escherichia coli*: a review. J. Food Prot. 45:1051-1067.

115. Kovincic, I., I. F. Vujicic, M. Svabic-Vlahovic, M. Vuluc, M. Gagic, and I. V. Wesley. 1991. Survival of *Listeria monocytogenes* during the manufacture and ripening of Trappist cheese. J. Food Prot. 54:418-420.

116. Kozak, J. J. 1986. FDA's dairy program initiatives. Dairy Food Sanit. 6:184-185.

117. Kristoffersen, T. 1985. Development of flavor in cheese. Milchwissenschaft 40:187-199.

118. Kroll, R. G., and R. A. Patchett. 1992. Induced acid tolderance in *Listeria monocytogenes*. Letters Appl. Microbiol. 14:224-227.

119. Law, B. A. 1979. Reviews of the progress of dairy science: enzymes of psychrotrophic bacteria and their effects on milk and milk products. J. Dairy Res. 46:573-588.

120. Law, B. A. 1984. Microorganisms and their enzymes in the maturation of cheeses, p. 245-283. *In* M. E. Bushnell (ed.) Progr. in Ind. Microbiol., vol. 19. Elsevier, New York, NY.

121. Liewen, M. B., and M. W. Plantz. 1988. Occurrence of *Listeria monocytogenes* in raw milk in Nebraska. J. Food Prot. 51:840-841.

122. Linnan, M. J., L. Mascola, X. O. Lou, V. Goulet, S. May, C. Saiminen, D. W. Hird, L. Yonekura, P. Hayes, R. Weaver, A. Andurier, B. D. Plikaytis, S. L. Fannin, A. Kleks, and C. V. Broome. 1988. Epidemic listeriosis associated with Mexican-style cheese. New Engl. J. Med. 319:823-828.

123. Linton, R. H., M. D. Pierson, and J. R. Bishop. 1990. Increase in heat restistance of *Listeria monocytogenes* during pasteurization. J. Food Prot. 55:84-87.

124. Lou, Y., and A. E. Yousef. 1997a. Resistance of *Listeria monocytognes* to heat after adaptation to environmental stresses. J. Food Prot. 59:465-471.

125. Lou, Y., and A. E. Yousef. 1997b. Adaptation to sublethal environmental stresses protects *Listeria monocytogenes* against lethal preservation factors. Appl. Environ. Microbiol. 63:1252-1255.

126. Lou, Y., and A. E. Yousef. 1999. Characteristics of *Listeria monocytogenes important to food processors*, p. 131-224. *In* E. T. Ryser, and E. H. Marth (eds.), Listeria, Listeriosis, and Food Safety, 2nd. edition. Marcel Dekker, Inc., New York.

127. Lovett, J., I. V. Wesley, M. J. Vandermaaten, J. G. Bradshaw, D. W. Francis, R. G. Crawford, C. W. Donelly, and J. W. Wesser. 1990. High-temperature, short time pasteurization inactivates *Listeria monocytogenes*. J. Food Prot. 53:734-738.

128. Lovett, J., D. W. Francis, and J, M. Hunt. 1987. *Listeria monocytogenes* in raw milk: detection, incidence, and pathogenicity. J. Food Prot. 50:188-192.

129. MacDonald, K. L., M. Eidson, C. Strohmeyer, M. E. Levy, J. G. Wells, N. D. Puhr, K. Wachsmuth, N. T. Nargett, and M. L. Cohen. 1985. A multistate outbreak of gastrointestinal illness caused by enterotoxigenic *Escherichia coli* in imported semisoft cheese. J. Infect. Dis. 151:716-720.

130. Mackey, B. M., and N. Bratchell. 1989. A review. The heat resistance of *Listeria monocytogenes*. Letters Appl. Microbiol. 9:89-94.

131. Malinverni, R., J. Bille, C. Perret, F. Regli, F. Tanner, and M. P. Glauser. 1985. Listeriose epdemique. Schweitz Med. Woschr. 115:2-10.

132. Margolles, A., A. Rodriguez, and Reyes-Gavilan-CG-de-los. 1997. Behavior of *Listeria monocytogenes* during the manufacture, ripening, and cold storage of Afuega'l Pitu cheese. J. Food Prot. 60:689-693.

133. Marier, R., J. G. Wells, R. C. Swanson, and I. J. Mehiman. 1973. An outbreak of enteropathogenic *Escherichia coli* foodborne disease traced to imported French cheese. Lancet ii:1376-1378.

134. Marshall, R. J. 1986. Increasing cheese yields by high heat treatment of milk. J. Dairy Res. 53:313-322.

135. McBean, L. D. 1988. A perspective on food safety concerns. Dairy, Food Environ. Sanit. 8:112-118.

136. Melachouris, N. P., and S. L. Tuckey. 1966. Changes of the proteins in Cheddar cheese made from milk heated at different temperatures. J. Dairy Sci. 49:800-805.

137. Meyer, D. H., and C. W. Donnelly. 1992. Effect of incubation temperature on repair of heat-injured *Listeria* in milk. J. Food Prot. 55:579-582.

138. Miller, A. J. 1992. Combined water activity and solute effects on growth and survival of *Listeria monocytogenes*. J. Food Prot. 55:414-418.

139. Morr, C. V. 1987. Effect of HTST pasteurization of milk, cheese whey, and cheese whey UF retentate upon the composition, physicochemical and functional properties of whey protein concentrates. J. Food Sci. 52:312-317.

140. Mossel, D. A. A. 1987. Microbial recontamination of dairy products. Mechanisms, health impact, management, and monitoring. Microbiol. Aliment. Nutrition. 5:267-282.

141. Noomen, A. 1975. Proteolytic activity of milk protease in raw and pasteurized cows milk. Neth. Milk Dairy J. 28:153-161.

142. O'Driscoll, B., C. G. M. Gahan, and C. Hill. 1996. Adaptive acid tolerance reponse in *Listeria monocytogenes*: isolation of an acid-tolerant mutant which demonstrates increased virulence. Appl. Environ. Microbiol. 62:1693-1698.

143. O'Keeffe, A. M., J. A. Phelan, K. Keogh, and P. Kelly. 1982. Studies of milk composition and its relationship to some processing criteria. IV. Factors influencing for renneting properties of a seasonal milk supply. Ir. J. Food Sci. Technol. 6:39-49.

144. Ozgen, H. 1952. Zur serologic der *Listeria monocytogenes*. Z. Tropenmed. 4:40-45.

145. Papageorgiou, D. K., and E. H. Marth. 1989a. Fate of *Listeria monocytogenes* during the manufacture and ripening of Feta cheese. J. Food Prot. 52:82-87.

146. Papageorgiou, D. K., and E. H. Marth. 1989b. Fate of *Listeria monocytogenes* during the manufacture and ripening of blue cheese. J. Food Prot. 52:459-465.

147. Parish, M. E., and D. P. Higgins. 1989. Survival of *Listeria monocytogenes* in low pH model broth systems. J. Food Prot. 52:144-147.

148. Park, H. S., E. H. Marth, and J. M. Goepfert. 1970. The fate of *Salmonella typhimurium* in the manufacture and ripening of low-acid Cheddar cheese. J. Milk Food Technol. 33:280-287.

149. Parry, W. H. 1966. Milk-borne diseases. An epidemiological review. Lancet July 23, p. 216-219.

150. Petran, R. L., and E. A. Zottola. 1989. A study of factors affecting growth and recovery of *Listeria monocyotgenes* Scott A. J. Food Sci. 54:616-618.

151. Pierre, A., and G. Brule. 1981. Mineral and protein equilibria between the colloidal and soluble phases of milk at low temperature. J. Dairy Res. 49:417-428.

152. Potel, J. 1951. The morphology, culture and pathogenicity of *C. infantisepticum.* Zbl. Bakteriol. Parasitol. 156:490-496.

153. Potter, M. E. 1984. Unpasteurized milk. The hazards of a health fetish. J. Amer. Med. Assoc. 252:2048-2052.

154. Prentice, G. A., and P. Neaves. 1987. *Listeria monocytogenes* in food: its significance and methods for its detection. IDF, Commission E, Draft Doc. 302. International Dairy Federation Brussels, Belgium.

155. Price, W. V. 1927. Cheddar cheese from pasteurized milk. J. Dairy Sci. 10:155-168.

156. Price, W. V., and A. O. Call. 1969. Cheddar cheese: comparison of effects of raw and heated milk on quality and ripening. J. Milk Food Technol. 32:304-311.

157. Quintavalla, S., and M. Campanini. 1991. Effect of rising temperature on the heat resistance of *Listeria monocytogenes* in meat emulsion. Letters Appl. Microbiol. 12:184-187.

158. Qvist, K. B. 1979. Reestablishment of the original rennetability of milk after cooling. 1. The effect of cooling and LTST pasteurization of milk on renneting. Milchwissenschaft 34:467-470.

159. Ratnam, S., and S. B. March. 1986. Laboratory studies on *Salmonella*-contaminated cheese involved in a major outbreak of gastroenteritis. J. Appl. Bacteriol. 61:51-56.

160. Razavilar, V. 1997. Behavior of *Listeria monocytogenes* in a soft fresh type cheese without lactic starter affected by serotype, temperature and storage time. J. Faculty Vet. Med. 52(1):83-93.

161. Reinhold, G. W. 1972. Swiss cheese varieties. Pfizer Cheese Monographs. Vol. V. Pfizer, Inc., New York, NY.

162. Reitsma, C. J., and D. R. Henning. 1996. Survival of enteropathogenic *Escherichia coli* O157:H7 during the manufacture and curing of Cheddar cheese. J. Food Prot. 59:460-465.

163. Rocourt, J. 1999. The genus *Listeria* and *Listeria monocytogenes*: phylogenetic position, taxonomy, and identification, p. 8-9. *In* E. T. Ryser, and E. H. Marth (eds.), *Listeria*, Listeriosis, and Food Safety, 2nd. edition. Marcel Dekker, Inc., New York.

164. Rodriguez, E., P. Gaya, M. Nunez, and M. Medina. 1998. Inhibitory activity of a nisin-producing starter culture on *Listeria innocua* in raw ewes milk Manchego cheese. Int. J. Food Microbiol. 39(1/2):129-132.

165. Ryser, E. T. 1999a. Incidence and behavior of *Listeria monocytogenes* in cheese and other fermented products, p. 411-503. *In* E. T. Ryser, and E. H. Marth (eds.), *Listeria*, Listeriosis, and Food Safety. Marcel Dekker, Inc., New York.

166. Ryser, E. T. 1999b. Foodborne Listeriosis, p. 302-304. *In* E. T. Ryser, and E. H. Marth (eds.), *Listeria*, Listeriosis and Food Safety, 2nd. edition. Marcel Dekker, New York.

167. Ryser, E. T. 1999c. Incidence and behavior of *Listeria monocytogenes* in unfermented dairy products, p. 359-409. *In* E. T. Ryser, and E. H. Marth (eds.), *Listeria*, Listeriosis, and Food Safety, 2nd. edition. Marcel Dekker, New York.

168. Ryser, E. T., S. Maisner-Patin, J. J. Gratadoux, and J. Richard. 1994. Isolation and identification of cheese-smear bacteria inhibitory to *Listeria* spp. Int. J. Food Microbiol. 21:237-246.

169. Ryser, E. T., and E. H. Marth. 1987a. Behavior of *Listeria monocytogenes* during the manufacture and ripening of Cheddar cheese. J. Food Prot. 50:7-13.

170. Ryser, E. T., and E. H. Marth. 1987b. Fate of *Listeria monocytogenes* during the manufacture and ripening of Camembert cheese. J. Food Prot. 50:372-378.

171. Ryser, E. T., and E. H. Marth. 1988. Survival of *Listeria monocytogenes* in cold-pack cheese food during refrigerated storage. J. Food Prot. 51:615-621.

172. Ryser, E. T., and E. H. Marth. 1989. Behavior of *Listeria monocytogenes* during manufacture and ripening of brick cheese. J. Dairy Sci. 72:838-853.

173. Ryser, E. T., E. H. Marth, and M. P. Doyle. 1985. Survival of *Listeria monocytogenes* during manufacture and storage of Cottage cheese. J. Food Prot. 48:746-750, 753.

174. Scarpallino, R. J., and F. V. Kosikowski. 1962. Evolution of volatile compounds in ripening raw and pasteurized milk Cheddar cheese observed by gas chromatography. J. Dairy Sci. 45:343-348.

175. Schaack, M. M., and E. H. Marth. 1988a. Behavior of *Listeria monocytogenes* in skim milk during fermentation with mesophilic lactic starter culture. J. Food Prot. 51:600-606.

176. Schaack, M. M., and E. H. Marth. 1988b. Behavior of *Listeria monocytogenes* in skim milk and in yogurt mix during fermentation by thermophilic lactic acid bacteria. J. Food Prot. 51:607-614.

177. Seeliger, H. P. R. 1961. Listeriosis. Hafner Publishing Co. Inc., New York.

178. Seeliger, H. P. R., and D. Jones. 1986. *Listeria*, p. 1235-1245. *In* Bergey's Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore.

179. Sharp, J. C. M. 1985. Pasteurisation and the control of milkborne infection in Britain. Br. Med. J. 291:463-464.

180. Sharp, J. C. M. 1987. Infections associated with milk and dairy products in Europe and North American, 1980-85. Bull. WHO 65(3):397-406.

181. Skinner, K. J. 1989. *Listeria* - Battling back against one "tough bug". Dairy, Food Environ. Sanit. 9:23-24.

182. Slutsker, L., and A. Schuchat. 1999. Listeriosis in humans. *In* E. T. Ryser, and E. H. Marth (eds.), *Listeria*, Listeriosis, and Food Safety, 2nd. edition. Marcel Dekker, Inc., New York.

183. Sorrells, K. M., D. C. Enigl, and J. R. Hatfield. 1989. Effect of pH, acidulant, time and temperature on the growth of *Listeria monocytogenes*. J. Food Prot. 52:571-573.

184. Stadhouders, J. 1982. Cooling and thermization as a mean to extend the keeping quality of raw milk. Kiel. Mitchwirtsch. Forschungsber. 34(l):19-28.

185. Stadhouders, J., M. M. Cordes, and A. W. J. van Schouwenberg-van Focken. 1978. The effect of manufacturing conditions on the development of staphylococci in cheese: their inhibition by starter bacteria. Neth. Milk Dairy J. 32:193-203.

186. Stajner, B., S. Zakula, I. Kovincic, and M. Galic. 1979. Heat resistance of *Listeria monoctogenes* and its survival in raw milk products. Vet. Glasnik 33:109-112.

187. Stecchini, M. L., V. Aquili, and I. Sarais. 1995. Behavior of *Listeria monocytogenes* in Mozzarella cheese in presence of *Lactococcus lactis*. Int. J. Food Microbiol. 25:301-310.

188. Stenberg, H., and T. Hammainen. 1955. On determination of *in vitro* of the resistance of *Listeria monocytogenes* to sodium chloride and heat and on experimental monocytosis in albino mice. Nord. Vet. Med. 7:853-868.

189. Stephens, P. J., M. B. Cole, and M. V. Jones. 1994. Effect of heating rate on the thermal inactivation of *Listeria monocytogenes*. J. Appl. Bacteriol. 77:702-708.

190. Tatini, S. R., J. J. Jezeski, J. C. Olson, and E. P. Casman. 1971. Factors influencing the production of staphylococcal enterotoxin A in milk. J. Dairy Sci. 54:312-320.

191. Tatini, S. R., W. D. Wesala, J. J. Jezeski, and H. A. Morris. 1973. Production of staphylococcal enterotoxin A in Blue, Brick, Mozzarella, and Swiss cheeses. J. Dairy Sci. 56:429-435.

192. Teo, A. Y. L., and S. J. Knabel. 2000. Development of a simple recovery-enrichment system for enhanced detection of heat-injured *Listeria monocytogenes* in pasteurized milk. J. Food Prot. 63:462-472.

193. Tuckey, S. L., M. E. Stiles, Z. J. Ordal, and L. D. Witter. 1964. Relation of cheese-making operations to survival and growth of *Staphylococcus aureus* in different varieties of cheese. J. Dairy Sci. 47:604-611.

194. Ustunol, Z., and R. J. Brown. 1985. Effects of heat treatment and post treatment holding time on rennet clotting of milk. J. Dairy Sci. 68:526-530.

195. Valk, H. de, A. E. Delarocque, G. Colomb, S. Ple, E. Godard, V. Vaillant, S. Haeghebaert, P. Bouvet, F. Grimont, and J. C. Desenclos. 2000. A community-wide outbreak of *Salmonella enterica* serotype Typhimurium infection associated with eating a raw milk soft cheese in France. Epidemiol. Inf. 124:1-7.

196. Villani, F., O. Pepe, G. Mauriello, G. Moschetti, L. Sannino, and S. Coppola. 1996. Behavior of *Listeria monocytogenes* during traditional manufacture of water-buffalo Mozzarella cheese. Letters Appl. Microbiol. 22:357-360.

197. Villar, R. G., M. D. Macek, S. Simons, P. S. Hayes, M. J. Goldoft, J. H. Lewis, L. L. Rowan, D. Hursh, M. Patnode, and P. S. Mead. 1999. Investigation of multidrug-resistant *Salmonella* serotype Typhimurium DT104 infections linked to raw-milk cheese in Washington State J. Am. Med. Assoc. 281(19):1811-1816.

198. Wang, L.-L., and E. A. Johnson. 1992. Inhibition of *Listeria monocytogenes* by fatty acids and monoglycerides. Appl. Environ. Microbiol. 58:624-629.

199. Watson, K. 1990. Microbial stress proteins. Adv. Microb. Physiol. 31:184-223.

200. Wenzel, J. M., and E. H. Marth. 1991. Behavior of *Listeria monocytogenes* in the presence of lactic acid bacteria in an agitated medium with internal pH control. J. Food Prot. 54:183-188.

201. Wesley, I. V. 1999. Listeriosis in animals, p. 49. *In* E. T. Ryser, and E. H. Marth (eds.), *Listeria*, Listeriosis, and Food Safety, 2nd. edition. Marcel Dekker, Inc., New York.

202. WHO Working Group. 1988. Foodborne listeriosis. Bull. WHO 66:421-428.

203. Williams, R. C., and D. A. Golden. 1998. Suitability of selective media for recovery and enumeration of sublethally heat- and acid-injured *Listeria monocytogenes*. J. Rapid Methods Automation Microbiol. 6:307-316.

204. Wilson, G. A., and J. V. Wheelock. 1972. Factors affecting the action of rennin in heated milk. J. Dairy Res. 39:412-419.

205. Wilson, H. L., S. A. Hall, and L. A. Rogers. 1945. The manufacture of Cheddar cheese from pasteurized milk. J. Dairy Sci. 28:187-200.

206. Wilster, G. H. 1980. Practical cheesemaking. O.S.U. Book Stores, Inc., Corvallis, OR.

207. Wood, D. S., D. L. Collins-Thompson, D. M. Irvine, and A. N. Myhr. 1984. Source and persistence of *Salmonella muenster* in naturally contaminated Cheddar cheese. J. Food Prot. 47:20-22.

208. Yousef, A. E., and E. H. Marth. 1988. Behavior of *Listeria monocytogenes* during the manufacture and storage of Colby cheese. J. Food Prot. 51:12-15.

209. Yousef, A. E., and E. H. Marth. 1989. Fate of *Listeria monocytogenes* during manufacture and ripening of Parmesan cheese. J. Dairy Sci. 73:3351-3356.

210. Zehren, V. L., and V. F. Zehren. 1968. Relation of acid development during cheesemaking to development of staphylococcal entertoxin A. J. Dairy Sci. 51:645-649.

APPENDICES

Appendix A

COMPETITIVE INHIBITION OF ACID-INJURED *LISTERIA MONOCYTOGENES*

INTRODUCTION

Acid-injury trials for *Listeria monocytogenes* were performed to study the behavior of acid-injured *L. monocytogenes* in competition with different levels of *Lactococcus lactis* subsp. *lactis*/ *Lactococcus lactis* subsp. *cremoris* as was done for heat-injured *L. monocytogenes*.

MATERIALS AND METHODS

Culture preparation:

Listeria monocytogenes cultures were prepared in the same manner as described previously for heat-injury trials (page 59).

Sublethal Injury:

Tryptose phosphate broth (TPB) [200 ml] was adjusted to pH 3.5 with 10% lactic acid (prepared from lactic acid, 85%, J. T. Baker), inoculated to contain 10^8-10^9 *L. monocytogenes* CFU/ml and held at room temperature (~21°C) up to 1 h in an attempt to obtain acid-injured cells, showing 99.0% injury. Samples were spiral plated (Autoplate® 4000, Sprial Biotech, Inc., Bethsada, MD) on tryptose phosphate agar (Difco) + 0.6% (w/v) yeast extract (non-selective medium, TPA) and TPA + 4.0% (w/v) NaCl (selective medium, TPNA) and incubated at 35°C/48 h to determine numbers of healthy and injured *L. monocytogenes* cells, respectively. Percent injury was determined from the following equation:

$$\% \text{ Injury} = \left\{ 1 - \frac{\text{Counts on selective medium}}{\text{Counts on non-selective medium}} \right\} \text{ X } 100$$

These acid-injured cultures were then centrifuged at 10,000 rpm at 4°C/15 min, resuspended in PBS and appropriately diluted for inoculation into milk.

RESULTS

Efforts to obtain acid-injured cells proved unsuccessful as only 50.32% (mean of 4 trials) of the cells were injured after a 1-h exposure to acidified broth (pH 3.5). Consequently, investigations on acid-injury were not pursued further.

Appendix B

Research Data Used for Manuscript:

NOTE: [1]Values in all tables are arithmetic mean±standard deviation for n=3.

[2]All bacterial populations are expressed in Log CFU/ml

Table 20: Heat Injury of *L. monocytogenes* in Tryptose Phosphate Broth and UHT Milk at 56°C

Time	Tryptose Phosphate Broth			UHT Milk		
(min)	TPA	TPNA	% Injury	TPA	TPNA	% Injury
0	7.57±0.44	7.54±0.44	6.84±1.21	7.71±0.61	7.58±0.53	24.67±15.53
5	6.93±0.31	6.36±0.41	71.62±10.37	7.35±0.52	6.78±0.72	70.45±16.40
10	6.56±0.41	4.57±0.23	98.61±0.05	6.97±0.38	6.35±0.49	74.98±8.81
15	6.38±0.25	3.82±0.74	99.60±0.51	6.61±0.47	5.41±0.43	92.53±4.93
20	6.32±0.25	3.71±0.75	99.71±0.36	6.33±0.27	4.02±0.33	99.44±0.31
25	6.17±0.26	3.09±0.99	99.89±0.15	5.73±0.32	3.40±0.08	99.47±0.33
30	5.87±0.14	2.81±0.89	99.90±0.13	5.38±0.34	2.24±0.35	99.83±0.24

Table 21: Heat Injury of *L. monocytogenes* in Tryptose Phosphate Broth and UHT Milk at 64°C

Time	Tryptose Phosphate Broth			UHT Milk		
(min)	TPA	TPNA	% Injury	TPA	TPNA	% Injury
0	7.53±0.32	7.44±0.38	18.58±10.83	6.86±0.24	6.78±0.21	16.27±15.17
0.5	6.56±0.37	5.80±0.48	81.85±7.27	5.92±1.06	4.00±1.74	94.82±7.50
1.0	6.04±0.60	3.15±1.06	99.81±0.19	5.21±1.30	3.11±1.92	97.84±2.83
1.5	5.37±0.82	2.25±1.23	99.84±0.22	4.72±1.52	2.39±1.83	99.30±0.52
2.0	4.61±1.05	1.90±0.80	99.74±0.18	3.58±1.48	1.30±1.47	99.41±0.36
2.5	4.32±0.82	1.24±1.13	99.89±0.15	2.79±1.64	0.74±1.04	99.65±0.56
3.0	2.88±1.52	ND*	100.00±0.0	1.96±1.12	ND	100.00±0.0

*ND – Not Detectable

Table 22: Fate of Uninjured *L. monocytogenes* in Raw Milk without Starter Culture

Time → (h)	0	2	4	6	8	24
MTPA	5.92±0.30	7.41±0.31	8.10±0.34	8.68±0.08	8.85±0.05	9.13±0.02
MTPNA	5.83±0.30	7.34±0.32	8.02±0.33	8.61±0.09	8.81±0.05	9.06±0.03
Injured	5.15±0.29	6.55±0.26	7.30±0.44	7.80±0.04	7.82±0.08	8.33±0.08
% Injury	17.31±1.4	13.96±2.6	16.39±4.4	13.59±2.8	9.29±0.8	16.07±3.6

(*Native contaminating bacterial count in milk: 2.09±0.48)

Table 23: Fate of LHI *L. monocytogenes* in Raw Milk without Starter Culture

Time → (h)	0	2	4	6	8	24
MTPA	5.81±0.10	6.80±0.02	7.65±0.25	8.61±0.02	8.88±0.04	8.99±0.11
MTPNA	3.05±0.05	5.46±0.31	6.88±0.17	8.29±0.01	8.77±0.03	8.89±0.11
Injured	5.81±0.10	6.78±0.03	7.57±0.27	8.33±0.03	8.25±0.07	8.31±0.10
% Injury	99.82±0.1	94.64±3.6	82.57±3.7	52.70±1.2	23.39±1.9	20.87±0.7

(*Native contaminating bacterial count in milk: 2.05±0.48)

Table 24: Fate of HHI *L. monocytogenes* in Raw Milk without Starter Culture

Time → (h)	0	2	4	6	8	24
MTPA	5.60±0.24	6.79±0.24	7.80±0.29	8.39±0.21	8.93±0.08	9.04±0.07
MTPNA	2.79±0.25	4.37±0.32	6.24±0.20	7.90±0.19	8.70±0.07	8.94±0.06
Injured	5.60±0.24	6.79±0.24	7.79±0.21	8.22±0.21	8.53±0.09	8.31±0.10
% Injury	99.82±0.1	99.58±0.2	97.22±0.7	67.66±1.4	40.28±0.9	18.89±1.5

(*Native contaminating bacterial count in milk: 1.28±0.68)

Table 25: Fate of Uninjured *L. monocytogenes* in Raw Milk at a 0.5% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.86±0.30	7.14±0.46	7.93±0.60	8.41±0.46	8.62±0.44	8.71±0.32
MTPNA	5.74±0.28	6.64±0.17	7.36±0.37	7.50±0.31	7.38±0.33	7.32±0.35
Injured	5.23±0.37	6.93±0.58	7.77±0.69	8.35±0.48	8.59±0.45	8.69±0.32
Starter	6.83±0.64	7.57±0.66	8.33±0.33	8.84±0.19	9.01±0.17	8.88±0.21
pH	6.41±0.03	6.25±0.04	5.98±0.15	5.02±0.42	4.60±0.34	3.75±0.23

(*Native contaminating bacterial count in milk: 2.49±0.21)

Table 26: Fate of LHI *L. monocytogenes* in Raw Milk at a 0.5% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	6.02±0.43	7.41±0.30	8.30±0.60	8.94±0.68	9.00±0.63	8.95±0.45
MTPNA	2.85±0.35	4.18±0.50	5.27±0.26	5.11±0.35	5.28±0.45	5.11±0.10
Injured	6.02±0.43	7.41±0.30	8.30±0.60	8.94±0.68	9.00±0.63	8.95±0.45
Starter	6.87±0.21	7.67±0.59	8.38±0.21	8.77±0.13	8.95±0.17	9.03±0.09
pH	6.43±0.02	6.22±0.05	5.99±0.02	5.25±0.18	4.70±0.22	4.16±0.13

(*Native contaminating bacterial count in milk: 1.69±0.44)

Table 27: Fate of HHI *L. monocytogenes* in Raw Milk at a 0.5% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.60±0.24	7.67±0.51	8.35±0.47	8.77±0.30	8.88±0.39	8.99±0.33
MTPNA	2.92±0.46	3.79±0.20	4.72±0.34	5.21±0.08	5.24±0.15	5.15±0.07
Injured	5.60±0.24	7.67±0.51	8.35±0.47	8.77±0.30	8.88±0.39	8.99±0.33
Starter	6.84±0.31	7.83±0.26	8.55±0.10	8.90±0.15	9.02±0.12	9.13±0.17
pH	6.42±0.03	6.23±0.05	6.02±0.03	5.46±0.20	4.83±0.06	4.26±0.14

(*Native contaminating bacterial count in milk: 1.42±0.80)

Table 28

Time (h)	
MTPA	
MTPNA	
Injured	
Starter	
pH	

(*Native

Table 29

Time (h)	
MTPA	
MTPNA	
Injured	
Starter	
pH	

(*Nati

Table

Tim (h)	
MTPA	
MTPN	
Injure	
Starte	
pH	

(*Na

Table 28: Fate of Uninjured *L. monocytogenes* in Raw Milk at a 1.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.89±0.37	7.54±0.21	8.02±0.46	8.67±0.43	8.86±0.39	9.02±0.31
MTPNA	5.71±0.40	6.34±0.35	6.58±0.30	6.63±0.12	6.67±0.14	6.54±0.07
Injured	5.40±0.31	7.50±0.23	8.00±0.47	8.67±0.43	8.86±0.39	9.02±0.31
Starter	6.55±0.05	7.79±0.21	8.60±0.19	8.83±0.07	9.01±0.04	9.09±0.03
pH	6.42±0.02	6.20±0.02	5.88±0.03	5.11±0.03	4.41±0.08	4.54±1.21

(*Native contaminating bacterial count in milk: 2.02±0.58)

Table 29: Fate of LHI *L. monocytogenes* in Raw Milk at a 1.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	6.02±0.50	7.35±0.24	8.18±0.56	8.83±0.65	8.98±0.49	9.10±0.35
MTPNA	2.96±0.22	4.45±0.29	5.28±0.26	5.50±0.07	5.49±0.06	5.51±0.04
Injured	6.02±0.50	7.35±0.24	8.18±0.56	8.83±0.65	8.98±0.49	9.10±0.35
Starter	6.68±0.28	7.55±0.51	8.38±0.22	8.73±0.25	8.93±0.13	9.04±0.09
pH	6.40±0.02	6.18±0.04	5.93±0.05	5.13±0.08	4.51±0.14	3.98±0.10

(*Native contaminating bacterial count in milk: 1.80±1.12)

Table 30: Fate of HHI *L. monocytogenes* in Raw Milk at a 1.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.61±0.18	7.02±0.52	7.86±0.20	8.55±0.25	8.74±0.26	8.89±0.25
MTPNA	2.82±0.07	4.00±0.09	5.05±0.17	5.36±0.10	5.36±0.07	5.29±0.05
Injured	5.61±0.18	7.02±0.52	7.86±0.20	8.55±0.25	8.74±0.26	8.89±0.25
Starter	6.62±0.32	7.47±0.33	8.15±0.10	8.69±0.17	8.95±0.02	9.01±0.01
pH	6.41±0.03	6.18±0.02	5.93±0.06	5.22±0.04	4.69±0.08	4.05±0.06

(*Native contaminating bacterial count in milk: 1.78±0.42)

Table 31: Fate of Uninjured *L. monocytogenes* in Raw Milk at a 2.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.92±0.42	7.63±0.15	8.33±0.40	8.89±0.37	8.90±0.36	8.89±0.26
MTPNA	5.84±0.44	6.89±0.15	7.10±0.58	7.37±0.19	7.29±0.14	7.33±0.15
Injured	5.10±0.27	7.54±0.15	8.30±0.39	8.87±0.39	8.89±0.37	8.88±0.27
Starter	6.65±0.04	7.63±0.57	8.56±0.36	8.79±0.17	9.01±0.03	9.09±0.02
pH	6.42±0.05	6.22±0.06	5.88±0.04	4.95±0.36	4.40±0.08	3.90±0.10

(*Native contaminating bacterial count in milk: 2.61±0.01)

Table 32: Fate of LHI *L. monocytogenes* in Raw Milk at a 2.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	6.01±0.26	7.32±0.22	8.21±0.42	8.70±0.42	8.84±0.36	8.86±0.29
MTPNA	2.85±0.13	4.21±0.30	5.07±0.30	5.26±0.17	5.32±0.18	5.23±0.13
Injured	6.01±0.26	7.32±0.22	8.21±0.42	8.70±0.42	8.84±0.36	8.86±0.29
Starter	6.61±0.08	7.76±0.31	8.32±0.38	8.73±0.19	8.88±0.14	9.01±0.05
pH	6.42±0.01	6.18±0.04	5.92±0.07	5.22±0.04	4.59±0.06	4.02±0.10

(*Native contaminating bacterial count in milk: 1.75±0.44)

Table 33: Fate of HHI *L. monocytogenes* in Raw Milk at a 2.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	6.05±0.22	7.14±0.23	8.05±0.28	8.81±0.34	8.93±0.23	8.95±0.20
MTPNA	2.67±0.35	3.83±0.06	4.90±0.11	5.24±0.10	5.23±0.13	5.21±0.04
Injured	6.05±0.22	7.14±0.23	8.05±0.28	8.81±0.34	8.93±0.23	8.95±0.20
Starter	6.59±0.63	7.47±0.41	8.42±0.09	8.73±0.26	9.00±0.09	9.01±0.04
pH	6.44±0.03	6.20±0.01	5.93±0.04	5.20±0.09	4.71±0.05	4.08±0.03

(*Native contaminating bacterial count in milk: 1.82±0.50)

Table 3

Time (h)
MTPA
MTPN
Injured
% Injur
(*No na

Table 3

Time (h)
MTPA
MTPN
Injured
% Injur
(*No na

Table 3

Time (h)
MTPA
MTPN
Injurec
% Inju
(*No r

Table 34: Fate of Uninjured *L. monocytogenes* in LHT Milk without Starter Culture

Time (h) →	0	2	4	6	8	24
MTPA	6.01±0.32	7.29±0.21	8.52±0.20	8.86±0.12	9.07±0.02	9.18±0.06
MTPNA	5.93±0.31	7.23±0.21	8.45±0.21	8.80±0.13	8.98±0.03	9.12±0.08
Injured	5.27±0.35	6.42±0.23	7.69±0.18	7.94±0.28	8.34±0.01	8.27±0.16
% Injury	18.09±1.6	13.57±1.8	15.32±5.6	13.64±7.1	18.69±1.4	13.40±6.3

(*No native bacteria detected in milk after heat treatment)

Table 35: Fate of LHI *L. monocytogenes* in LHT Milk without Starter Culture

Time (h) →	0	2	4	6	8	24
MTPA	5.86±0.16	6.94±0.09	7.75±0.07	8.71±0.07	8.92±0.06	9.09±0.16
MTPNA	3.06±0.10	5.21±0.01	6.94±0.06	8.42±0.05	8.82±0.07	9.01±0.18
Injured	5.86±0.16	6.93±0.09	7.68±0.08	8.39±0.08	8.25±0.04	8.27±0.11
% Injury	99.84±0.1	98.09±0.4	84.47±3.2	48.65±1.6	21.27±1.4	15.56±4.6

(*No native bacteria detected in milk after heat treatment)

Table 36: Fate of HHI *L. monocytogenes* in LHT Milk without Starter Culture

Time (h) →	0	2	4	6	8	24
MTPA	5.64±0.23	6.78±0.24	7.76±0.07	8.60±0.11	8.88±0.12	9.06±0.06
MTPNA	2.87±0.11	4.32±0.24	6.32±0.28	8.13±0.11	8.72±0.11	8.97±0.07
Injured	5.64±0.23	6.78±0.24	7.74±0.06	8.42±0.11	8.38±0.14	8.29±0.06
% Injury	99.82±0.1	99.64±0.1	96.11±1.7	66.10±1.3	31.53±1.5	17.23±2.8

(*No native bacteria detected in milk after heat treatment)

Table 37: Fate of Uninjured *L. monocytogenes* in LHT Milk at a 0.5% Starter Inoculum

Time (h) →	0	2	4	6	8	24
MTPA	5.82±0.19	7.03±0.33	7.86±0.16	8.52±0.20	8.77±0.28	8.87±0.16
MTPNA	5.67±0.18	6.82±0.27	7.50±0.14	8.02±0.11	7.72±0.07	7.54±0.06
Injured	5.28±0.22	6.58±0.42	7.59±0.24	8.32±0.31	8.72±0.30	8.85±0.17
Starter	6.90±0.54	7.54±0.66	8.28±0.28	8.78±0.18	8.97±0.10	9.02±0.02
pH	6.45±0.03	6.26±0.03	5.95±0.06	5.42±0.16	4.79±0.19	4.13±0.05

(*No native bacteria detected in milk after heat treatment)

Table 38: Fate of LHI *L. monocytogenes* in LHT Milk at a 0.5% Starter Inoculum

Time (h) →	0	2	4	6	8	24
MTPA	5.85±0.06	7.14±0.11	8.12±0.18	8.62±0.10	8.87±0.08	8.97±0.02
MTPNA	2.84±0.06	4.26±0.33	5.25±0.24	5.38±0.13	5.35±0.05	5.31±0.10
Injured	5.85±0.06	7.14±0.11	8.12±0.18	8.62±0.10	8.87±0.08	8.97±0.02
Starter	6.64±0.07	7.36±0.56	8.23±0.51	8.64±0.43	8.92±0.11	8.95±0.12
pH	6.42±0.04	6.26±0.08	6.01±0.05	5.33±0.26	4.88±0.14	4.39±0.13

(*No native bacteria detected in milk after heat treatment)

Table 39: Fate of HHI *L. monocytogenes* in LHT Milk at a 0.5% Starter Inoculum

Time (h) →	0	2	4	6	8	24
MTPA	5.67±0.23	7.21±0.26	8.09±0.29	8.74±0.32	8.81±0.25	8.90±0.22
MTPNA	2.53±0.21	3.86±0.12	4.91±0.14	5.30±0.11	5.26±0.04	5.19±0.01
Injured	5.67±0.23	7.21±0.26	8.08±0.29	8.74±0.32	8.81±0.25	8.90±0.22
Starter	6.91±0.30	7.47±0.39	8.43±0.06	8.80±0.11	8.92±0.10	9.03±0.05
pH	6.46±0.04	6.26±0.07	6.00±0.08	5.32±0.18	4.79±0.14	4.18±0.05

(*Native bacterial count in milk after heat-treatment: 0.52±0.00)

Table 40: Fate of Uninjured *L. monocytogenes* in LHT Milk at a 1.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.98±0.20	7.24±0.36	7.83±0.31	8.47±0.33	8.57±0.28	8.72±0.23
MTPNA	5.85±0.20	7.09±0.33	7.51±0.21	7.50±0.12	7.43±0.16	7.41±0.09
Injured	5.39±0.23	6.72±0.43	7.53±0.41	8.42±0.35	8.54±0.29	8.70±0.23
Starter	6.71±0.16	7.61±0.47	8.41±0.40	8.75±0.24	8.94±0.10	9.06±0.06
pH	6.43±0.01	6.18±0.03	5.84±0.04	5.08±0.06	4.36±0.06	3.86±0.04

(*No native bacteria detected in milk after heat treatment)

Table 41: Fate of LHI *L. monocytogenes* in LHT Milk at a 1.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.96±0.11	7.27±0.30	8.03±0.23	8.73±0.10	8.89±0.08	8.96±0.01
MTPNA	2.84±0.24	4.09±0.40	4.98±0.31	5.15±0.17	5.20±0.19	5.18±0.15
Injured	5.96±0.11	7.27±0.30	8.03±0.23	8.73±0.10	8.89±0.08	8.96±0.01
Starter	6.76±0.08	7.82±0.14	8.29±0.24	8.72±0.06	8.91±0.08	9.00±0.04
pH	6.45±0.03	6.20±0.03	5.93±0.08	5.23±0.16	4.55±0.19	4.01±0.12

(*No native bacteria detected in milk after heat treatment)

Table 42: Fate of HHI *L. monocytogenes* in LHT Milk at a 1.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.89±0.14	7.27±0.24	8.22±0.25	8.83±0.17	8.88±0.15	8.95±0.08
MTPNA	2.50±0.17	4.36±0.14	4.93±0.03	5.17±0.03	5.21±0.01	5.54±0.57
Injured	5.89±0.14	7.27±0.24	8.22±0.25	8.83±0.17	8.88±0.15	8.95±0.08
Starter	6.45±0.17	7.54±0.43	8.25±0.40	8.77±0.24	8.93±0.10	9.03±0.03
pH	6.43±0.03	6.21±0.04	5.97±0.05	5.28±0.05	4.72±0.07	4.04±0.05

(*No native bacteria detected in milk after heat treatment)

Table 43: Fate of Uninjured *L. monocytogenes* in LHT Milk at a 2.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.64±0.17	7.53±0.19	8.11±0.30	8.72±0.28	8.77±0.30	8.95±0.26
MTPNA	5.47±0.16	7.29±0.23	7.62±0.16	7.53±0.05	7.45±0.05	7.41±0.03
Injured	5.13±0.20	7.15±0.15	7.92±0.38	8.68±0.31	8.75±0.33	8.94±0.27
Starter	6.88±0.35	7.90±0.01	8.55±0.13	8.92±0.05	9.02±0.03	9.09±0.02
pH	6.41±0.01	6.16±0.02	5.90±0.03	5.11±0.08	4.38±0.03	3.89±0.06

(*No native bacteria detected in milk after heat treatment)

Table 44: Fate of LHI *L. monocytogenes* in LHT Milk at a 2.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	6.00±0.11	7.38±0.11	8.02±0.18	8.65±0.13	8.76±0.10	8.98±0.08
MTPNA	3.01±0.24	4.17±0.37	5.17±0.21	5.51±0.17	5.24±0.08	5.19±0.06
Injured	6.00±0.11	7.38±0.11	8.02±0.26	8.65±0.23	8.76±0.14	8.98±0.06
Starter	6.68±0.20	7.75±0.13	8.34±0.26	8.75±0.23	8.94±0.14	9.03±0.06
pH	6.56±0.12	6.19±0.02	5.92±0.08	5.16±0.08	4.62±0.09	4.06±0.05

(*No native bacteria detected in milk after heat treatment)

Table 45: Fate of HHI *L. monocytogenes* in LHT Milk at a 2.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.99±0.03	7.34±0.42	8.08±0.31	8.68±0.04	8.87±0.05	9.02±0.02
MTPNA	2.72±0.22	3.86±0.12	4.57±0.33	5.18±0.01	5.23±0.06	5.15±0.05
Injured	5.99±0.03	7.34±0.42	8.08±0.31	8.68±0.04	8.87±0.05	9.02±0.02
Starter	6.40±0.44	7.68±0.44	8.63±0.28	8.79±0.23	8.98±0.10	9.04±0.06
pH	6.43±0.02	6.21±0.03	5.95±0.03	5.22±0.08	4.66±0.22	4.05±0.09

(*No native bacteria detected in milk after heat treatment)

Table 46: Fate of Uninjured *L. monocytogenes* in HHT Milk without Starter Culture

Time (h) →	0	2	4	6	8	24
MTPA	5.93±0.26	7.17±0.18	8.31±0.15	8.78±0.11	8.97±0.05	9.10±0.03
MTPNA	5.86±0.28	7.12±0.16	8.21±0.15	8.72±0.11	8.91±0.04	9.04±0.03
Injured	5.07±0.12	6.11±0.39	7.60±0.13	7.93±0.10	8.08±0.14	8.23±0.06
% Injury	14.28±4.8	10.15±7.0	19.59±2.6	14.19±1.3	13.00±2.5	13.30±1.3

(*No native bacteria detected in milk after heat treatment)

Table 47: Fate of LHI *L. monocytogenes* in HHT Milk without Starter Culture

Time (h) →	0	2	4	6	8	24
MTPA	5.78±0.18	6.93±0.14	7.70±0.10	8.48±0.23	8.91±0.12	9.10±0.12
MTPNA	3.00±0.04	4.67±0.15	7.00±0.24	8.19±0.22	8.82±0.12	9.03±0.13
Injured	5.78±0.18	6.93±0.14	7.59±0.08	8.16±0.23	8.17±0.11	8.30±0.07
% Injury	99.83±0.1	99.44±0.1	79.01±6.5	48.56±1.0	18.51±2.3	16.20±4.0

Table 48: Fate of HHI *L. monocytogenes* in HHT Milk without Starter Culture

Time (h) →	0	2	4	6	8	24
MTPA	5.58±0.17	6.67±0.19	7.58±0.18	8.56±0.10	8.91±0.11	9.05±0.12
MTPNA	2.74±0.13	4.31±0.12	6.42±0.19	8.09±0.11	8.73±0.10	8.96±0.10
Injured	5.58±0.17	6.66±0.19	7.55±0.18	8.38±0.09	8.45±0.13	8.29±0.18
% Injury	99.85±0.1	99.53±0.2	92.97±0.3	66.09±1.6	34.84±2.7	17.64±2.8

(*No native bacteria detected in milk after heat treatment)

Table 49: Fate of Uninjured *L. monocytogenes* in HHT Milk at a 0.5% Starter Inoculum

Time (h) →	0	2	4	6	8	24
MTPA	5.79±0.25	6.85±0.36	7.70±0.25	8.39±0.23	8.59±0.37	8.79±0.21
MTPNA	5.67±0.26	6.65±0.38	7.34±0.22	7.88±0.06	7.70±0.05	7.61±0.05
Injured	5.14±0.24	6.41±0.36	7.44±0.30	8.20±0.33	8.51±0.41	8.76±0.23
Starter	6.64±0.13	7.54±0.66	8.38±0.46	8.74±0.13	9.04±0.09	9.05±0.03
pH	6.41±0.03	6.28±0.05	5.94±0.15	5.43±0.07	4.64±0.07	4.11±0.07

(*No native bacteria detected in milk after heat treatment)

Table 50: Fate of LHI *L. monocytogenes* in HHT Milk at a 0.5% Starter Inoculum

Time (h) →	0	2	4	6	8	24
MTPA	5.76±0.11	7.25±0.10	8.12±0.22	8.49±0.19	8.77±0.17	8.86±0.15
MTPNA	2.80±0.04	4.27±0.39	5.25±0.23	5.43±0.05	5.41±0.05	5.29±0.13
Injured	5.76±0.11	7.25±0.10	8.12±0.22	8.49±0.19	8.77±0.17	8.86±0.15
Starter	6.84±0.30	7.61±0.55	8.34±0.46	8.79±0.26	8.99±0.13	9.03±0.10
pH	6.46±0.02	6.26±0.01	6.00±0.03	5.29±0.21	4.72±0.24	4.20±0.17

(*No native bacteria detected in milk after heat treatment)

Table 51: Fate of HHI *L. monocytogenes* in HHT Milk at a 0.5% Starter Inoculum

Time (h) →	0	2	4	6	8	24
MTPA	5.62±0.18	7.12±0.19	8.04±0.29	8.67±0.30	8.81±0.25	8.87±0.20
MTPNA	2.58±0.27	3.82±0.24	4.80±0.14	5.11±0.06	5.13±0.11	5.10±0.19
Injured	5.62±0.18	7.12±0.19	8.04±0.29	8.67±0.30	8.81±0.25	8.87±0.20
Starter	6.89±0.33	7.63±0.52	8.36±0.40	8.75±0.26	8.96±0.14	9.06±0.10
pH	6.44±0.03	6.26±0.08	6.01±0.02	5.38±0.11	4.83±0.12	4.26±0.09

(*No native bacteria detected in milk after heat treatment)

Table 52: Fate of Uninjured *L. monocytogenes* in HHT Milk at a 1.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.88±0.19	7.33±0.14	7.92±0.22	8.42±0.21	8.70±0.29	8.92±0.19
MTPNA	5.71±0.20	7.03±0.19	7.55±0.28	7.60±0.16	7.56±0.10	7.47±0.15
Injured	5.38±0.18	7.03±0.11	7.68±0.18	8.35±0.22	8.66±0.30	8.90±0.19
Starter	6.64±0.14	7.69±0.38	8.55±0.18	8.82±0.04	8.94±0.08	9.04±0.05
pH	6.45±0.06	6.19±0.03	5.88±0.04	5.12±0.13	4.45±0.13	3.94±0.05

(*No native bacteria detected in milk after heat treatment)

Table 53: Fate of LHI *L. monocytogenes* in HHT Milk at a 1.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.91±0.08	7.36±0.11	8.01±0.18	8.63±0.15	8.80±0.07	8.95±0.08
MTPNA	2.76±0.15	4.17±0.37	5.17±0.21	5.35±0.03	5.24±0.08	5.19±0.06
Injured	5.91±0.08	7.36±0.11	8.01±0.18	8.63±0.15	8.80±0.07	8.95±0.08
Starter	6.82±0.20	7.75±0.13	8.34±0.26	8.78±0.16	8.93±0.16	9.03±0.07
pH	6.45±0.03	6.22±0.04	5.960.03	5.26±0.06	4.58±0.06	4.08±0.04

(*No native bacteria detected in milk after heat treatment)

Table 54: Fate of HHI *L. monocytogenes* in HHT Milk at a 1.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.97±0.09	7.24±0.42	8.02±0.31	8.85±0.12	8.90±0.11	8.98±0.10
MTPNA	2.65±0.30	3.99±0.27	4.81±0.24	5.25±0.05	5.20±0.04	5.16±0.02
Injured	5.97±0.09	7.24±0.42	8.02±0.31	8.84±0.12	8.90±0.11	8.98±0.10
Starter	6.62±0.12	7.87±0.09	8.44±0.10	8.76±0.03	8.90±0.11	9.00±0.07
pH	6.42±0.05	6.18±0.03	5.87±0.05	5.07±0.05	4.43±0.03	3.99±0.03

(*No native bacteria detected in milk after heat treatment)

Table 55: Fate of Uninjured *L. monocytogenes* in HHT Milk at a 2.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.77±0.33	7.28±0.14	7.96±0.17	8.64±0.32	8.81±0.29	8.94±0.23
MTPNA	5.65±0.36	7.04±0.17	7.33±0.17	7.36±0.07	7.53±0.05	7.46±0.01
Injured	5.13±0.21	6.90±0.16	7.85±0.17	8.61±0.33	8.78±0.31	8.93±0.24
Starter	6.77±0.15	7.95±0.08	8.36±0.10	8.77±0.26	8.97±0.12	9.05±0.08
pH	6.42±0.03	6.18±0.03	5.90±0.09	5.22±0.20	4.58±0.22	4.01±0.09

(*No native bacteria detected in milk after heat treatment)

Table 56: Fate of LHI *L. monocytogenes* in HHT Milk at a 2.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	6.12±0.09	7.29±0.23	8.04±0.16	8.77±0.11	8.86±0.10	8.97±0.10
MTPNA	2.98±0.09	4.12±0.35	4.97±0.21	5.21±0.08	5.24±0.09	5.22±0.08
Injured	6.12±0.09	7.29±0.23	8.04±0.16	8.77±0.11	8.86±0.10	8.97±0.10
Starter	6.48±0.09	7.50±0.36	8.31±0.26	8.77±0.26	8.93±0.04	9.01±0.01
pH	6.44±0.03	6.19±0.01	5.92±0.04	5.19±0.09	4.59±0.04	4.05±0.05

(*No native bacteria detected in milk after heat treatment)

Table 57: Fate of HHI *L. monocytogenes* in HHT Milk at a 2.0% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.91±0.10	7.22±0.21	8.04±0.17	8.56±0.17	8.87±0.15	8.97±0.10
MTPNA	2.64±0.19	4.37±0.14	4.93±0.03	5.08±0.08	5.24±0.15	5.15±0.07
Injured	5.91±0.10	7.22±0.21	8.04±0.17	8.56±0.17	8.87±0.15	8.97±0.10
Starter	6.65±0.34	7.54±0.43	8.22±0.35	8.73±0.19	8.97±0.12	9.02±0.03
pH	6.42±0.02	6.20±0.03	5.92±0.05	5.17±0.01	4.57±0.13	4.00±0.08

(*No native bacteria detected in milk after heat treatment)

Table 58: Fate of Uninjured *L. monocytogenes* in Pasteurized Milk without Starter Culture

Time (h) →	0	2	4	6	8	24
MTPA	5.91±0.26	7.29±0.25	8.51±0.22	8.86±0.14	9.07±0.05	9.18±0.08
MTPNA	5.84±0.29	7.21±0.23	8.44±0.21	8.80±0.15	8.99±0.08	9.11±0.09
Injured	5.03±0.12	6.49±0.35	7.68±0.29	7.93±0.09	8.24±0.10	8.39±0.03
% Injury	14.05±5.3	16.32±4.3	15.51±5.9	12.04±2.5	15.61±5.0	16.05±1.9

(*No native bacteria detected in milk after heat treatment)

Table 59: Fate of LHI *L. monocytogenes* in Pasteurized Milk without Starter Culture

Time (h) →	0	2	4	6	8	24
MTPA	5.83±0.14	6.94±0.08	7.81±0.05	8.66±0.05	8.92±0.08	9.07±0.22
MTPNA	3.06±0.06	5.39±0.11	7.14±0.03	8.40±0.04	8.83±0.08	9.01±0.21
Injured	5.83±0.14	6.93±0.09	7.71±0.07	8.32±0.07	8.22±0.11	8.16±0.27
% Injury	99.83±0.1	97.15±0.8	78.51±3.0	45.68±1.5	19.91±3.0	12.25±1.7

(*No native bacteria detected in milk after heat treatment)

Table 60: Fate of HHI *L. monocytogenes* in Pasteurized Milk without Starter Culture

Time (h) →	0	2	4	6	8	24
MTPA	5.62±0.23	6.78±0.21	7.82±0.14	8.57±0.12	8.93±0.10	9.10±0.07
MTPNA	2.83±0.16	4.38±0.23	00.14	8.13±0.12	8.73±0.11	9.00±0.07
Injured	5.62±0.23	6.78±0.21	7.80±0.14	8.38±0.12	8.49±0.09	8.39±0.09
% Injury	99.83±0.1	99.57±0.2	95.41±1.0	64.09±1.0	36.80±2.6	19.65±1.1

(*No native bacteria detected in milk after heat treatment)

Table 61: Fate of Uninjured *L. monocytogenes* in Pasteurized Milk at a 0.5% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.89±0.29	6.91±0.42	7.74±0.17	8.41±0.26	8.82±0.15	8.98±0.10
MTPNA	5.75±0.31	6.64±0.37	7.28±0.07	7.90±0.04	7.74±0.04	7.64±0.06
Injured	5.36±0.26	6.58±0.47	7.55±0.22	8.22±0.38	8.78±0.16	8.96±0.10
Starter	6.92±0.58	7.54±0.69	8.29±0.20	8.79±0.26	9.01±0.16	8.79±0.23
pH	6.41±0.03	6.27±0.03	6.01±0.14	5.39±0.21	4.76±0.22	4.07±0.09

(*No native bacteria detected in milk after heat treatment)

Table 62: Fate of LHI *L. monocytogenes* in Pasteurized Milk at a 0.5% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.98±0.33	7.42±0.28	8.39±0.56	8.81±0.44	9.05±0.49	9.15±0.28
MTPNA	2.83±0.12	4.22±0.48	5.27±0.25	5.48±0.09	5.55±0.19	5.36±0.18
Injured	5.98±0.33	7.42±0.28	8.39±0.56	8.81±0.44	9.05±0.49	9.15±0.28
Starter	6.60±0.13	7.58±0.62	8.13±0.72	8.73±0.31	8.94±0.13	9.03±0.08
pH	6.43±0.02	6.25±0.07	6.00±0.07	5.26±0.27	4.70±0.24	4.15±0.05

(*No native bacteria detected in milk after heat treatment)

Table 63: Fate of HHI *L. monocytogenes* in Pasteurized Milk at a 0.5% Starter Inoculum

Time → (h)	0	2	4	6	8	24
MTPA	5.68±0.21	7.37±0.39	8.20±0.41	8.74±0.28	8.99±0.25	9.14±0.18
MTPNA	2.63±0.35	3.81±0.20	4.57±0.33	5.18±0.03	5.23±0.06	5.15±0.05
Injured	5.68±0.21	7.37±0.39	8.20±0.41	8.74±0.28	8.99±0.25	9.14±0.18
Starter	6.74±0.03	7.70±0.40	8.65±0.25	8.85±0.07	8.97±0.12	9.05±0.05
pH	6.44±0.03	6.21±0.04	6.01±0.02	5.40±0.05	4.87±0.05	4.10±0.04

(*No native bacteria detected in milk after heat treatment)

Table 64: Fate of Uninjured *L. monocytogenes* in Pasteurized Milk at a 1.0% Starter Inoculum

Time (h) →	0	2	4	6	8	24
MTPA	5.98±0.29	7.33±0.11	7.96±0.18	8.51±0.28	8.81±0.25	8.96±0.30
MTPNA	5.80±0.33	7.04±0.17	7.33±0.18	7.56±0.15	7.57±0.09	7.46±0.01
Injured	5.49±0.21	7.01±0.05	7.84±0.18	8.45±0.30	8.79±0.26	8.94±0.31
Starter	7.14±0.22	7.95±0.07	8.40±0.09	8.95±0.08	9.04±0.04	9.09±0.06
pH	6.43±0.02	6.21±0.02	5.92±0.03	5.12±0.05	4.44±0.04	3.90±0.06

(*No native bacteria detected in milk after heat treatment)

Table 65: Fate of LHI *L. monocytogenes* in Pasteurized Milk at a 1.0% Starter Inoculum

Time (h) →	0	2	4	6	8	24
MTPA	5.94±0.15	7.29±0.23	8.14±0.32	8.67±0.21	8.77±0.14	8.89±0.10
MTPNA	2.94±0.13	4.12±0.35	4.99±0.23	5.23±0.16	5.25±0.11	5.22±0.08
Injured	5.94±0.15	7.29±0.23	8.14±0.32	8.67±0.21	8.77±014	8.89±0.10
Starter	6.65±0.08	7.50±0.36	8.31±0.26	8.75±0.09	8.93±0.04	9.01±0.01
pH	6.45±0.03	6.19±0.02	5.90±0.05	5.14±0.05	4.46±0.08	3.94±0.03

(*No native bacteria detected in milk after heat treatment)

Table 66: Fate of HHI *L. monocytogenes* in Pasteurized Milk at a 1.0% Starter Inoculum

Time (h) →	0	2	4	6	8	24
MTPA	5.97±0.13	7.30±0.49	8.14±0.27	8.80±0.14	8.90±0.15	8.99±0.13
MTPNA	2.54±0.58	3.83±0.15	4.47±0.16	5.22±0.08	5.22±0.08	5.16±0.07
Injured	5.97±0.13	7.30±0.49	8.14±0.27	8.80±0.14	8.90±0.15	8.99±0.13
Starter	6.69±0.09	7.50±0.34	8.19±0.10	8.63±0.12	8.89±0.05	9.03±0.01
pH	6.42±0.01	6.20±0.01	5.95±0.04	5.20±0.06	4.69±0.09	4.09±0.12

(*No native bacteria detected in milk after heat treatment)

Table 6

Inoculu

Time (h)	
MTPA	
MTPN	
Injured	
Starter	
pH	

(*No n

Table

Tim (h)	
MTPA	
MTPY	
Injure	
Start	
pH	

(*No

Tab

T	
MT	
MT	
Inj	
St	
pH	

(*

Table 67: Fate of Uninjured *L. monocytogenes* in Pasteurized Milk at a 2.0% Starter Inoculum

Time (h) →	0	2	4	6	8	24
MTPA	5.82±0.31	7.28±0.15	7.91±0.22	8.67±0.21	8.83±0.20	8.88±0.14
MTPNA	5.69±0.36	7.08±0.14	7.64±0.13	7.64±0.13	7.57±0.09	7.49±0.03
Injured	5.20±0.20	6.83±0.23	7.54±0.34	8.62±0.25	8.81±0.22	8.86±0.15
Starter	6.61±0.05	7.57±0.25	8.46±0.18	8.73±0.01	8.96±0.04	9.03±0.03
pH	6.39±0.03	6.16±0.04	5.92±0.06	5.22±0.07	4.55±0.07	4.05±0.04

(*No native bacteria detected in milk after heat treatment)

Table 68: Fate of LHI *L. monocytogenes* in Pasteurized Milk at a 2.0% Starter Inoculum

Time (h) →	0	2	4	6	8	24
MTPA	6.15±0.09	7.31±0.26	8.03±0.14	8.79±0.09	8.92±0.08	8.97±0.04
MTPNA	2.94±0.19	4.10±0.23	5.03±0.16	5.26±0.12	5.39±0.07	5.38±0.06
Injured	6.15±0.09	7.31±0.26	8.03±0.14	8.79±0.09	8.92±0.08	8.97±0.04
Starter	6.69±0.25	7.75±0.21	8.36±0.11	8.77±0.12	8.97±0.03	9.05±0.04
pH	6.45±0.03	6.17±0.03	5.91±0.09	5.13±0.12	4.50±0.13	3.99±0.07

(*No native bacteria detected in milk after heat treatment)

Table 69: Fate of HHI *L. monocytogenes* in Pasteurized Milk at a 2.0% Starter Inoculum

Time (h) →	0	2	4	6	8	24
MTPA	6.04±0.23	7.27±0.56	8.22±0.39	8.65±0.26	8.89±0.15	8.97±0.08
MTPNA	2.62±0.15	3.95±0.23	5.03±0.15	5.21±0.05	5.26±0.07	5.23±0.04
Injured	6.04±0.24	7.27±0.56	8.22±0.39	8.65±0.26	8.89±0.15	8.97±0.08
Starter	6.75±0.08	7.60±0.41	8.27±0.35	8.73±0.10	8.93±0.10	9.02±0.03
pH	6.45±0.04	6.20±0.03	5.87±0.09	5.26±0.11	4.55±0.06	4.01±0.06

(*No native bacteria detected in milk after heat treatment)

Table 7

Time (h)	
TPA	
TPNA	
Injured	
% Injur	

(*No n

Table 7

Time (h)	
TPA	
TPNA	
Injured	
% Inju	

(*No n

Table

Tim (h)	
TPA	
TPNA	
Injure	
% Inj	

(*No

Table 70: Fate of Uninjured *L. monocytogenes* in UHT Milk without Starter Culture

Time → (h)	0	2	4	6	8	24
TPA	5.17±0.51	6.03±0.57	7.19±0.45	7.86±0.09	8.20±0.32	8.45±0.46
TPNA	5.12±0.50	5.99±0.57	7.15±0.45	7.82±0.09	8.15±0.29	8.41±0.45
Injured	4.27±0.63	4.90±0.61	6.15±0.42	6.77±0.07	7.20±0.64	7.34±0.51
% Injury	12.76±3.5	7.66±2.1	9.30±2.3	8.41±1.8	11.51±6.5	7.96±1.5

(*No native bacteria detected in milk after heat treatment)

Table 71: Fate of LHI *L. monocytogenes* in UHT Milk without Starter Culture

Time → (h)	0	2	4	6	8	24
TPA	4.29±0.59	4.40±0.58	4.62±0.54	5.17±0.36	6.05±0.08	7.53±0.12
TPNA	2.05±0.65	2.83±0.75	4.03±0.58	4.92±0.37	5.96±0.09	7.47±0.14
Injured	4.29±0.59	4.39±0.57	4.49±0.52	4.82±0.34	5.33±0.06	6.60±0.0
% Injury	99.42±0.1	97.17±1.1	74.27±3.0	44.06±1.5	19.28±1.5	12.13±3.3

(*No native bacteria detected in milk after heat treatment)

Table 72: Fate of HHI *L. monocytogenes* in UHT Milk without Starter Culture

Time → (h)	0	2	4	6	8	24
TPA	3.84±0.59	4.64±0.62	5.09±0.42	5.81±0.91	6.24±0.67	7.46±0.16
TPNA	1.41±0.68	2.95±0.13	3.86±0.34	5.42±0.92	6.08±0.68	7.41±0.17
Injured	3.84±0.59	4.63±0.63	5.06±0.42	5.59±0.90	5.72±0.64	6.52±0.07
% Injury	99.59±0.2	97.04±2.7	93.97±1.3	59.60±1.2	30.56±1.7	11.86±4.0

(*No native bacteria detected in milk after heat treatment)

Table 73: Fate of Uninjured *L. monocytogenes* in UHT Milk at a 0.5% Starter Inoculum

Time (h) →	0	2	4	6	8	24
TPA	6.56±0.28	7.45±0.39	8.27±0.43	8.72±0.30	8.92±0.16	8.93±0.12
TPNA	6.46±0.30	7.16±0.36	7.79±0.75	7.89±0.19	7.67±0.42	7.72±0.37
Injured	5.86±0.23	6.93±0.67	7.91±0.26	8.64±0.37	8.89±0.16	8.89±0.15
Starter	7.11±0.41	7.84±0.16	8.51±0.31	8.87±0.01	9.03±0.04	9.01±0.03
pH	6.44±0.02	6.34±0.03	6.13±0.11	5.64±0.19	4.94±0.10	4.14±0.19

(*No native bacteria detected in milk after heat treatment)

Table 74: Fate of LHI *L. monocytogenes* in UHT Milk at a 0.5% Starter Inoculum

Time (h) →	0	2	4	6	8	24
TPA	5.94±0.17	7.47±0.23	7.96±0.13	8.76±0.21	8.87±0.16	8.94±0.06
TPNA	2.98±0.59	4.86±0.13	6.25±0.82	6.61±0.50	6.52±0.54	6.45±0.53
Injured	5.94±0.17	7.47±0.23	7.94±0.11	8.75±0.20	8.87±0.15	8.93±0.06
Starter	7.00±0.43	7.54±0.73	8.37±0.37	8.82±0.26	8.97±0.12	8.98±0.23
pH	6.44±0.03	6.31±0.02	6.13±0.03	5.73±0.17	5.03±0.07	4.02±0.11

(*No native bacteria detected in milk after heat treatment)

Table 75: Fate of HHI *L. monocytogenes* in UHT Milk at a 0.5% Starter Inoculum

Time (h) →	0	2	4	6	8	24
TPA	5.74±0.23	7.57±0.30	8.35±0.38	8.80±0.32	8.88±0.32	8.82±0.12
TPNA	3.07±0.43	5.09±0.08	5.81±0.23	5.99±0.08	6.09±0.07	6.04±0.09
Injured	5.74±0.23	7.57±0.30	8.34±0.38	8.80±0.32	8.88±0.32	8.82±0.12
Starter	6.90±0.29	7.36±0.43	8.19±0.40	8.54±0.49	8.82±0.23	8.99±0.06
pH	6.45±0.04	6.24±0.03	6.03±0.10	5.27±0.08	4.92±0.08	3.96±0.05

(*No native bacteria detected in milk after heat treatment)

Table 76: Fate of Uninjured *L. monocytogenes* in UHT Milk at a 1.0% Starter Inoculum

Time (h) →	0	2	4	6	8	24
TPA	6.04±0.16	7.18±0.11	7.72±0.14	8.38±0.30	8.71±0.38	8.86±0.20
TPNA	5.81±0.15	6.42±0.28	6.61±0.22	6.92±0.10	7.19±0.44	6.64±0.22
Injured	5.65±0.21	7.05±0.25	7.68±0.14	8.36±0.31	8.70±0.37	8.86±0.21
Starter	6.92±0.40	7.57±0.70	8.42±0.47	8.62±0.13	8.89±0.05	9.03±0.10
pH	6.42±0.02	6.25±0.03	5.99±0.05	5.15±0.08	4.56±0.08	3.89±0.02

(*No native bacteria detected in milk after heat treatment)

Table 77: Fate of LHI *L. monocytogenes* in UHT Milk at a 1.0% Starter Inoculum

Time (h) →	0	2	4	6	8	24
TPA	6.32±0.25	7.78±0.68	8.39±0.53	9.17±0.39	9.15±0.30	9.02±0.15
TPNA	3.56±0.49	4.86±0.53	6.00±0.24	6.35±0.11	6.20±0.06	6.09±0.16
Injured	6.32±0.25	7.78±0.68	8.39±0.53	9.17±0.39	9.15±0.30	9.02±0.15
Starter	7.38±0.53	7.99±0.11	8.46±0.39	8.67±0.11	8.89±0.15	8.97±0.06
pH	6.42±0.02	6.24±0.04	5.95±0.08	5.01±0.11	4.52±0.10	3.93±0.10

(*No native bacteria detected in milk after heat treatment)

Table 78: Fate of HHI *L. monocytogenes* in UHT Milk at a 1.0% Starter Inoculum

Time (h) →	0	2	4	6	8	24
TPA	5.96±0.27	6.84±0.19	7.88±0.45	8.68±0.36	8.77±0.37	8.89±0.34
TPNA	2.97±0.66	4.20±0.67	4.63±0.57	5.63±0.21	5.49±0.20	5.44±0.19
Injured	5.95±0.27	6.84±0.19	7.88±0.45	8.68±0.36	8.77±0.37	8.89±0.34
Starter	6.95±0.40	7.35±0.56	8.26±0.20	8.76±0.24	8.96±0.12	8.92±0.18
pH	6.41±0.03	6.24±0.04	6.00±0.09	5.21±0.24	4.64±0.21	3.87±0.17

(*No native bacteria detected in milk after heat treatment)

Table

Tim (h
TPA
TPN
Injur
Start
pH
(*N

Tabl

Ti (h
TPA
TPN
Injur
Star
pH
(*N

Tab

Ti (
TPA
TPN
Inju
Sta
pH
(*N

Table 79: Fate of Uninjured *L. monocytogenes* in UHT Milk at a 2.0% Starter Inoculum

Time (h) →	0	2	4	6	8	24
TPA	6.47±0.37	8.14±0.43	8.45±0.43	8.70±0.20	8.82±0.07	9.15±0.09
TPNA	6.43±0.39	7.87±0.77	7.57±0.29	7.69±0.23	7.61±0.35	7.78±0.42
Injured	5.33±0.14	7.53±0.26	8.38±0.46	8.66±0.20	8.78±0.06	9.13±0.08
Starter	7.12±0.38	8.01±0.18	8.60±0.38	8.85±0.11	9.04±0.02	9.02±0.02
pH	6.44±0.03	6.19±0.04	5.86±0.04	5.05±0.04	4.65±0.12	3.95±0.04

(*No native bacteria detected in milk after heat treatment)

Table 80: Fate of LHI *L. monocytogenes* in UHT Milk at a 2.0% Starter Inoculum

Time (h) →	0	2	4	6	8	24
TPA	5.89±0.28	7.72±0.05	8.01±0.10	8.41±0.33	8.61±0.31	8.82±0.26
TPNA	3.26±0.23	6.06±0.22	6.22±0.50	6.40±0.58	6.31±0.54	6.18±0.52
Injured	5.89±0.29	7.71±0.04	7.99±0.10	8.40±0.34	8.60±0.32	8.82±0.26
Starter	7.08±0.30	8.01±0.24	8.64±0.41	8.91±0.12	9.06±0.04	9.10±0.03
pH	6.44±0.04	6.19±0.04	5.92±0.02	5.13±0.01	4.71±0.07	3.96±0.04

(*No native bacteria detected in milk after heat treatment)

Table 81: Fate of HHI *L. monocytogenes* in UHT Milk at a 2.0% Starter Inoculum

Time (h) →	0	2	4	6	8	24
TPA	6.09±0.13	7.26±0.35	8.01±0.54	8.69±0.15	8.71±0.14	8.87±0.11
TPNA	3.44±0.46	5.52±0.35	5.94±0.22	6.24±0.32	6.28±0.54	5.76±0.20
Injured	6.09±0.13	7.26±0.35	8.01±0.54	8.69±0.15	8.71±0.14	8.87±0.11
Starter	7.08±0.13	7.85±0.49	8.49±0.50	8.65±0.54	8.89±0.28	9.05±0.04
pH	6.47±0.04	6.19±0.06	5.93±0.06	5.15±0.07	4.79±0.04	3.96±0.04

(*No native bacteria detected in milk after heat treatment)

Table 82: Growth of Starter Culture at 0.5% Inoculum Level without *L. monocytogenes*

Time → (h)	0	2	4	6	8	24
Starter	7.01±0.21	7.95±0.14	8.59±0.36	8.93±0.08	9.01±0.02	9.06±0.05
pH	6.43±0.01	6.31±0.02	6.16±0.06	5.78±0.12	5.02±0.07	4.20±0.17

Table 83: Growth of Starter Culture at 1.0% Inoculum Level without *L. monocytogenes*

Time → (h)	0	2	4	6	8	24
Starter	6.91±0.22	7.90±0.07	8.39±0.35	8.92±0.04	9.00±0.02	9.03±0.01
pH	6.42±0.02	6.24±0.03	5.98±0.03	5.19±0.06	4.62±0.08	4.03±0.04

Table 84: Growth of Starter Culture at 2.0% Inoculum Level without *L. monocytogenes*

Time → (h)	0	2	4	6	8	24
Starter	6.84±0.20	8.07±0.07	8.81±0.09	8.95±0.02	9.03±0.04	9.06±0.03
pH	6.43±0.02	6.22±0.03	5.90±0.09	5.06±0.09	4.57±0.06	3.97±0.05

Table 85: Acid-Injury of *L. monocytogenes* in Tryptose Phosphate Broth (pH 3.5)

Time (min)	TPA	TPNA	% Injury
0	7.67±0.24	7.61±0.26	15.13±6.65
15	7.60±0.25	7.50±0.31	24.96±11.06
30	7.50±0.27	7.38±0.30	26.11±5.89
45	7.42±0.27	7.24±0.28	35.55±4.56
60	7.22±0.11	6.91±0.10	50.32±1.71

Mean±standard deviation (n=4)