To the Graduate Council:

I am submitting herewith a dissertation written by Mohamed Osman M. Abdalla entitled "Effect of Processing Conditions on the Microbiological Quality of White Pickled Cheese." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Science and Technology.

Genevieve Christen

Genevieve L. Christen, Major Professor

We have read this dissertation
and recommend its acceptance:

A. Ann Draughon

Accepted for the Council:

C. W. Minkel

Associate Vice Chancellor
and Dean of The Graduate School

EFFECT OF PROCESSING CONDITIONS ON THE MICROBIOLOGICAL QUALITY OF WHITE PICKLED CHEESE

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Mohamed Osman M. Abdalla

December, 1992

AG-VET-MED

THESIS
926
.A2322

DEDICATION

This work is dedicated to Abdel Rahman and his sister Tahany; I hope you achieve more than what I did. It is also dedicated to my parents, to whom I will always be in debt.

ACKNOWLEDGEMENT

With genuine humility, I acknowledge your help, O GOD. Without your guidance and love, this work would not have been possible.

The author wishes to express his sincere appreciation and thanks to Dr. Genevieve L. Christen, major professor and chairperson of the graduate committee, for her patience, invaluable guidance, friendship and encouragement during this study and in preparation of this dissertation. Thanks are extended to committee members, Dr. Hugh O. Jaynes, Dr. Stephen P. Oliver and Dr. F. Ann Draughon for their friendship and advice while serving on the graduate committee. Special regards are extended to Dr. P. M. Davidson, former major professor for his kindness and support during the supervision of this project.

Financial support and the permission for use of facilities from both the University of Khartoum, Sudan and the University of Tennessee are very much appreciated.

Thanks are due to friends and colleagues, faculty and staff in the Department of Food Science and Technology for their friendship during this study. Thanks are due to Mr. Tommy Burch for his help in collecting milk as well as the staff of the University Farm.

Last but not least, the author will be forever in debt to his father Osman M. Abdalla, mother Zahra A. Mohamed, brothers

and sisters and to his wife and children for their love, understanding, support and encouragement.

ABSTRACT

Effects of starter culture, nisin, H_2O_2 and potassium sorbate on *Listeria monocytogenes, Staphylococcus aureus* and *Salmonella typhimurium* in white pickled cheese made from pasteurized milk with 4% salt and preserved in 4% brine solution at 4°C for 60 days was studied. The starter culture inhibited all three pathogens while antimicrobials did not. Beyond day 50 in curd and day 30 in brine solution, *L. monocytogenes* was not detected by direct plating. *S. aureus* was not detected after day 30 in curd and day 20 in brine solution. *S. typhimurium* was not detected after day 30 in cheese curd, and was not detected in brine solution at any time. The pH of brine solution of starter treatment dropped below 4.7 in all experiments, while antimicrobial treatments had always pH above 5.5.

The chemical composition of white pickled cheese made from pasteurized milk with 8% salt and preserved in the whey at 4°C for 60 days was determined, and the cheese was challenged with *L. monocytogenes*. While fat, protein, total solids and ash contents decreased in curd during ripening, the acidity and salt did not change. In cheese whey, fat, protein, acidity and salt showed a slight increase while total solids and ash contents were unchanged. High salt did not affect *L. monocytogenes* and suppressed the activity of lactic acid bacteria such that they were not able to produce acid and

inhibit *L. monocytogenes*. During ripening, the pH never decreased below 6.0.

A single strain of *Lactococcus lactis ssp. cremoris* was isolated from a commercial cheese culture and chemical mutation was attempted to develop salt-resistant mutants for use in high salt white pickled cheese. No salt-resistant mutant was isolated.

TABLE OF CONTENTS

CHAPTER		Page
I	INTRODUCTION	1
II	REVIEW OF LITERATURE	4
	LACTIC ACID BACTERIA	4
	Taxonomy and Classification	4
	Media for Growth and Differentiation	5
	Production of Mutant Strains	8
	LISTERIA MONOCYTOGENES	10
	Characteristics	10
	The Disease	11
	Food-borne Outbreaks	12
	Incidence in Dairy Products	13
	Growth and Survival in Dairy Products	17
	Effect of Antimicrobials	21
	STAPHYLOCOCCUS AUREUS	25
	Characteristics	25
	The Disease	26
	Outbreaks	26
	Incidence in Dairy Products	28
	Growth and Survival in Dairy Products	31
	Effect of Antimicrobials	33
	SALMONELLA SPP.	35
	Characteristics	35
	The Disease	36
	Outbreaks	36
	Incidence in Dairy Products	37
	Growth and Survival in Cheese	38
	Effect of Antimicrobials	40
	REFERENCES	44
III	EFFECT OF STARTER CULTURE AND ANTIMICROBIALS ON THE SURVIVAL OF SELECTED MICROORGANISMS IN WHITE PICKLED CHEESE	58
	INTRODUCTION	58
	MATERIALS AND METHODS	59
	Starter Culture	59
	Bacterial Cultures	60
	Treatments	60
	Manufacture of Cheese	62
	Enumeration of *Listeria monocytogenes*	63
	Enumeration of *Staphylococcus aureus*	63
	Enumeration of *Salmonella typhimurium*	63
	Statistical Analyses	64
	RESULTS AND DISCUSSION	65
	Survival of *L. monocytogenes* in curd and brine solution	65

(Cont'd)

CHAPTER		Page
	Survival of *S. aureus* in curd and brine solution	72
	Survival of *S. typhimurium* in curd and brine solution	79
	REFERENCES	85
	APPENDIX	90
IV	DEVELOPMENT OF SALT-RESISTANT MUTANTS OF LACTIC STREPTOCOCCI	98
	INTRODUCTION	98
	MATERIALS AND METHODS	99
	Media	99
	Isolation and Identification of Lactic Streptococci	100
	Chemicals	100
	Mutant Isolation	101
	RESULTS AND DISCUSSION	102
	Isolation and Identification	102
	Mutant Isolation	104
	REFERENCES	105
V	EFFECT OF SALT ON CHEMICAL COMPOSITION AND SURVIVAL OF *L. MONOCYTOGENES* IN WHITE PICKLED CHEESE	107
	INTRODUCTION	107
	MATERIALS AND METHODS	108
	Cheese Manufacture	108
	Enumeration of *L. monocytogenes*	108
	Evaluation of Chemical Composition of Cheese	109
	Statistical Analyses	110
	RESULTS AND DISCUSSION	110
	Chemical Composition	110
	Fat Content	110
	Protein Content	113
	Salt Content	115
	Total Solids	115
	Ash Content	118
	Protein Degradation	118
	Acidity	120
	Survival of *L. monocytogenes* in Cheese Curd and Whey	125
	REFERENCES	128
	APPENDIX	131
VI	CONCLUSIONS	149
	VITA	152

LIST OF TABLES

Table		Page
1.	Selected differential characteristics of dairy starter cultures	6
2.	Incidence of *L. monocytogenes* in dairy products	14
3.	Survival of *L. monocytogenes* in various dairy products	18
4.	General outline of the additives used during the manufacture of cheese	61
5.	Enumeration of *L. monocytogenes* strain Scott A in curd and brine solution of white pickled cheese made from pasteurized milk and stored at 4°C for 60 d. Values represent the mean of all storage days sampled	67
6.	Mean $\log_{10}$ of *S. aureus* NRRL B-120 and pH values of curd and brine solution for white pickled cheese made from pasteurized milk and stored at 4°C for 60 d	76
7.	Isolation and identification of *S. typhimurium* NRRL B-4420 in curd of white pickled cheese made from pasteurized milk and stored at 4°C for 60 d	80
8.	Isolation and identification of *S. typhimurium* NRRL B-4420 and pH values in brine solution used to preserve cheese curd and stored at 4°C for 60 d during the study of the behavior of the pathogen in white pickled cheese	81
9.	Distinguishing features of the parent cell	103
10.	Average chemical composition of milk, curd and salted whey of white pickled cheese over all sampling times.	111
11.	Mean $\log_{10}$ CFU/g (ml) of *L. monocytogenes* Scott A and pH for curd and whey of white pickled cheese made from pasteurized milk with 8% salt.	124

LIST OF FIGURES

Figure		Page
1.	Behavior of *L. monocytogenes* Scott A in curd during storage of white pickled cheese at 4°C for 60 d. Cheese was made from pasteurized milk with 4% salt and preserved in 4% brine solution	66
2.	Behavior of *L. monocytogenes* Scott A in brine solution during storage of white pickled cheese at 4°C for 60 d	68
3.	Change in pH of brine solution of white pickled cheese challenged with *L. monocytogenes* and stored at 4°C for 60 d	69
4.	Behavior of *S. aureus* NRRL B-120 in curd of white pickled cheese made from pasteurized milk with 4% salt and stored in 4% brine solution at 4°C for 60 d	73
5.	Survival of *S. aureus* NRRL B-120 in brine solution of white pickled cheese made from pasteurized milk and stored at 4°C for 60 d	74
6.	Change in the pH of brine solution of white pickled cheese made from pasteurized milk inoculated with *S. aureus* cells and stored in 4% brine solution at 4°C for 60 d	75
7.	Change in pH of brine solution during storage of white pickled cheese at 4°C for 60 d. Cheese was made from pasteurized milk with 4% salt and preserved in 4% brine solution. Cheese was analyzed for the survival of *S. typhimurium* NRRL B-4420	82
8.	Change in fat, protein and salt in cheese curd during storage at 4°C for 60 d. Cheese was made from pasteurized milk with 8% salt. Starter culture (1%; *Lactococcus lactis ssp. cremoris*) was added to the starter treatment . . .	112
9.	Change in fat, protein and salt contents of whey during storage at 4°C for 60 d of cheese made from pasteurized milk with 8% salt	114
10.	Change in total solids and ash contents of white pickled cheese curd during storage at 4°C for 60 d	116

(Cont'd)

Figure Page

11. Change in total solids and ash contents of cheese whey from cheese made from pasteurized milk with 8% salt and stored at 4°C for 60 d . . . 117

12. Proteolysis (A_{420}) in cheese curd during storage at 4°C for 60 d. ABS=absorbance at wavelength 420 nm 119

13. Change in titratable acidity of curd and whey of white pickled cheese during storage at 4°C for 60 d. Cheese was made from pasteurized milk with 8% salt 121

14. Survival of *L. monocytogenes* Scott A and pH in white pickled cheese curd made from pasteurized milk with 8% salt and stored at 4°C for 65 d. Starter culture (*L. lactis ssp. cremoris*) at 1% was added to the starter treatment 122

15. Behavior of *L. monocytogenes* Scott A and pH in cheese whey made from milk with 8% salt and stored at 4°C for 65 d 123

CHAPTER I

INTRODUCTION

The white pickled cheese group is comprised of soft and semi-soft cheeses that are preserved in salted whey or brine (Abd El-Salam, 1987). These cheeses may be consumed fresh but are frequently stored after maturation in salt brine or salted whey in sealed containers. The brine or salted whey improves the flavor of cheese and acts as a preservative (Fahmi and Sharara, 1950). The pickling practice constitutes the primary difference between this group and other typical soft cheeses of temperate zones (Fahmi and Sharara, 1950).

Pickled cheeses are manufactured in many East European countries, the East Mediterranean region and North Africa (Khalid and Harrigan, 1984). The most important varieties include; Feta (Greece), Telema (Greece, Romania), Brinza (Bulgaria, USSR), Salamora (Turkey), Akaawi (Syria), Baladi (Lebanon) and Domiati (Egypt)(Abd El-Salam, 1987). Gebna Beyda is the most popular cheese of the Sudan that is consumed by all socioeconomic classes and age groups (Khalid and Harrigan, 1984). Gebna Beyda cheese is salted at the first step of manufacture. Salt is added directly to the milk prior to addition of rennet.

In Sudan, commercial cheesemaking is considered to be semi-traditional (Alla Gabo, 1986), with product manufactured

locally in milk producing areas. Alla Gabo (1986) and Khalid and Harrigan (1984) described the manufacturing method for Gebna Beyda. While Alla Gabo (1986) reported a salt content of 8-9%, Khalid and Harrigan (1984) reported 6-20% depending on the manufacturer. Milk is brought to the factory and either warmed (winter time) or kept at ambient temperature. Salt is added and rennet (one tablet per 40 kg milk) is added (the manufacturer specifies that "one tablet will coagulate 20 gallons of pure fresh milk at 94-96°F in 45 minutes"). After the curd is formed (6-8 h), it is poured into wooden molds lined with cheesecloth. The curd is pressed overnight and whey is collected for use as brine solution. The curd is cut into cubes and placed in the collected salted whey in metal tins and shipped to consumption areas. Commercially produced Gebna Beyda is shaped as small rectangular cubes. Fresh Gebna Beyda has a soft body, but, as it ripens in pickling solution (salted whey), the body usually becomes firmer. Since cheese is stored under warm atmospheric conditions, the keeping quality is short and it soon deteriorates, even before it ripens. Salt at high levels is added to act as a preservative and to prevent the formation of gas holes by undesirable microorganisms. Although this high salt level is meant to control the indigenous microflora, and particularly pathogens, it has a major influence on acid development and rennet coagulation. High salt level limits the development of naturally occurring lactic acid bacteria (Khalid and Harrigan,

1984). In addition, high lactic acid causes the curd to be fragile due to a decreased rate of casein particle aggregation during rennet coagulation and causes a decrease in the stability of the structural elements of the coagulum thereby increasing the coagulation time of milk (Abd El-Salam, 1987).

Since the indigenous flora relied upon for acid production is a mixed flora, the rate of acid production and growth of desirable acid producing bacteria is paralleled by the growth of undesirable bacteria leading to the production of gas and off-flavors (Fox, 1987). Pasteurization has the advantage of eliminating pathogenic bacteria (Abdalla and Davidson, 1990; Alla Gabo, 1986; Dariani *et al.*, 1980), while acidification has the advantages of affecting coagulant activity during coagulation, curd strength and bacterial growth (Fox, 1987).

The objectives of this study were: (1) to determine the effect of processing conditions on the microbial quality of white pickled cheese, and (2) to develop salt resistant mutants of lactic streptococci for use as starter cultures in high salt cheese.

CHAPTER II

REVIEW OF LITERATURE

LACTIC ACID BACTERIA

Taxonomy and Classification

Lactic acid bacteria important to the dairy industry belong to the genera *Streptococcus, Leuconostoc* and *Lactobacillus* (Cogan and Daly, 1987). The genus *Streptococcus* is widely used in the manufacture of fermented milk products and includes mesophilic cultures, *Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. diacetilactis*. The official designation of these three subspecies has been changed from *Streptococcus* to *Lactococcus* (Tamime, 1990). Lactococci are homofermentative; producing only lactic acid from glucose (Tamime, 1990). Although it is homofermentative, *Streptococcus thermophilus* has several properties distinguishing it from other streptococci. These differences include different enzymes at the ends of the glycolytic pathway and the use of glucose and the rejection of galactose (Garvie, 1984). The genus *Leuconostoc* includes heterofermentative organisms capable of producing lactic acid, acetic acid, diacetyl, CO_2 and ethanol (Sharpe, 1979; Tamime, 1990). The species which are associated with dairy starter cultures are *Leuconostoc mesenteroides ssp. cremoris, Leuc. mesenteroides ssp.*

mesenteroides, *Leuc. mesenteroides ssp. dextranicum* and *Leuc. lactis* (Cogan and Daly, 1987; Garvie, 1984; Tamime, 1990). The genus *Lactobacillus* is divided into three main groups, corresponding to Orla-Jensen's genera (I, II and III) but without designating them as subgeneric taxa (Kandler and Weiss, 1986; Tamime, 1990). Two groups are homofermentative, *Thermobacterium* and *Streptobacterium*, and one heterofermentative, *Betabacterium* (Sharpe, 1979). The species in the group *Thermobacterium* of interest to the dairy industry are *Lactobacillus delbrueckii ssp. lactis, Lac. delbrueckii ssp. bulgaricus, Lac. helveticus* and *Lac. acidophilus* (Sharpe, 1979; Tamime, 1990). *Streptobacterium* includes *Lac. casie ssp. casie* and *Lac. plantarum*. *Betabacterium* is heterofermentative but its species are not important in the dairy industry (Tamime, 1990). There are three other genera of importance to the dairy industry, namely *Bifidobacterium, Propionibacterium* and *Brevibacterium* (Tamime, 1990). Some differential characteristics of dairy starter cultures are presented in Table 1.

Media for Growth and Differentiation

The lactic acid bacteria found in starter cultures are fastidious, requiring several amino acids and vitamins (Anderson and Elliker, 1953). The general purpose medium for growing lactic streptococci and lactobacilli was the lactic agar medium of Elliker *et al.* (1956). However, this medium has been criticized for having an adverse effect on lactic

Table 1. Selected differential characteristics of dairy starter cultures.

Microorganisms	Growth at °C			Fermentation of			Citrate metabolism	NH_3 from arginine	% G+C[b]
	10	40	45	glu[a]	gal[a]	lac[a]			
L. lactis ssp. lactis	+	+	-	+	+	+	-	+	38.5
L. lactis ssp. cremoris	+	-	-	+	+	+	-	-	38-40
L. lactis ssp diacetilactis	+	+	-	+	+	+	+	±	38.5
S. thermophilus	-	+	+	+	-	+	-	-	40.0
Leuc. lactis	+	-	-	+	+	+	d	-	43-45
Leuc. mesen ssp. mesen.	+	-	-	+	+	(d)	d	-	37-41
Leuc. mesen. ssp. dextra.	+	-	-	+	d	+	d	-	37-40
Leuc. mesen. ssp. cremoris	+	-	-	+	d	+	+	-	38-40
Lac. bulgaricus	-	+	+	+	-	+	-	-	49-51
Lac. lactis	-	+	+	+	-	+	-	d	49-51
Lac. helveticus	-	+	+	+	+	+	-	-	37-40
Lac. casie ssp. casie	+	+	+	+	+	d		-	45-47
Lac. plantarum	+	+	+	+	+	+		-	44-46
Lac. acidophilus	-	+	+	+	+	+		-	32-37

Source: Tamime (1990); Cogan and Daly (1987).

Symbols: +, 90% or more of strains are positive, -, 90% or more of strains are negative ±, most strains positive but some negative, d, 11-89% of strains are positive; (d), delayed reaction.

[a] glu, glucose; gal, galactose; lac, lactose

[b] G+C, guanine plus cytosine in DNA.

acid bacteria since it has no buffering agent thus allowing the pH to drop such that it adversely affects growth of bacteria (Terzaghi and Sandine, 1975). Hunter (1946) incorporated sodium phosphate as a buffering agent so that the pH of the medium changed from 7.0 to 6.0. The unbuffered medium had a pH of 5.0. However, the buffered medium also had drawbacks since phosphate precipitated bacteriological media by sequestering alkaline earth metals (Terzaghi and Sandine, 1975).

The current medium of choice is called M17 developed by Terzaghi and Sandine (1975). In this medium, β-disodium glycerophosphate was incorporated as a buffering agent instead of inorganic phosphate. M17 medium is suitable for group N streptococci, *S. thermophilus* and their phages and lactobacilli especially *Lac. bulgaricus*. However, later it was found that this medium was inhibitory to many strains of *Lac. bulgaricus*, and in fact it was used for selective isolation of *S. thermophilus* from yogurt (Shankar and Davies, 1977). The most useful general purpose medium for lactobacilli is MRS broth (DeMan *et al.*, 1960).

Several differential media have been developed for distinguishing mesophilic starter cultures. Calcium citrate agar (KCA) medium of Nickles and Leesment (1964) was useful to differentiate flavor producing from non-flavor producing bacteria. Colonies were differentiated by the size of the surrounding clear zones. Other media to differentiate *L.*

lactis ssp. lactis and *L. lactis ssp. cremoris* (Mikolajcik, 1964; Reddy *et al.*, 1969; Turner *et al.*, 1963) were developed. These media were based on the ability of *L. lactis ssp. lactis* and the inability of *L. lactis ssp. cremoris* to hydrolyze arginine and produce ammonia which is detected by pH change. The pH indicators ranged from bromcresol purple (Mikolajcik, 1964; Reddy *et al.*, 1969) to 2, 3, 5—triphenyl tetrazolium chloride (TTC) (Turner *et al.*, 1963). The media used for differentiation of citrate-fermenting and non-citrate fermenting mesophilic lactic acid bacteria were based on the ability of *L. lactis ssp. diacetilactis* and the inability of *L. lactis ssp. lactis* and *L. lactis ssp. cremoris* to ferment citrate (Kempler and McKay, 1980; Reddy *et al.*, 1970; Reddy *et al.*, 1972). Calcium or sodium citrate was incorporated as specific substrates along with a pH indicator.

Production of Mutant Strains

Considerable research has taken place to produce phage-resistant mutants or other especially desirable characteristics. Physical as well as chemical mutation have been used in the dairy industry. Dilanian *et al.* (1976) produced proteolytically active mutant strains of lactic acid bacteria by x-ray and gamma irradiation and used these mutants in the production of cheese. The cheese had shortened time of ripening and increased quality over control cheese. Ethyl methanesulfonate (60 µl/ml) was used to induce mutation of *L. lactis ssp. lactis* C2 (Feirtag and McKay, 1987). Three

classes of mutants were isolated; mutants unable to grow and produce acid at 38 to 40°C, mutants unable to grow at 38 to 40°C but which continued to produce acid at a rate similar to temperature-sensitive strains of *L. lactis ssp. cremoris* and mutants that underwent lysis when cells were shifted to 38 to 40°C. The third class of these mutants, those with the ability to lyse at cooking temperatures of 38 to 40°C, were used in Cheddar cheese manufacture.

Mutants of *L. lactis ssp. diacetilactis* were produced by exposure of parent cells to N-methyl-N'-nitro-N-nitrosoguanidine (NTG) and ultraviolet (UV) irradiation (Kuila *et al.*, 1971). Mutants produced higher amounts of diacetyl and lower acid quantities compared to parent cells. Also, NTG was used to produce mutants of *L. lactis ssp. diacetilactis* with enhanced ability to produce acetoin and diacetyl (Chuang and Collins, 1971). Mutant cells grew and produced acid in lactose-citrate broth or litmus milk much more slowly than parent cells. In lactose-citrate broth, mutant cells produced 4.2 μg/ml acetoin plus diacetyl in 33 h, and 15.2 μg/ml in 10 days, but parent cells produced only 2.4 μg/ml of acetoin plus diacetyl in 33 h. Grieve *et al.* (1983) isolated mutants of *L. lactis ssp. lactis* that lost the ability to ferment lactose (lac^-) spontaneously. This was accomplished by growing cells on TYLB (modified M17 agar containing 0.5% (w/v) glucose instead of lactose, and 0.004% (w/v) bromcresol purple indicator) and picking white, non-acid producing colonies.

These lac^- mutants were also prt^-, having lost their surface-bound proteinase activity. These mutants were used successfully to increase starter populations in Cheddar cheese (Grieve and Dulley, 1983). Proteolysis in cheese, hence levels of free amino acids, was increased and no bitter flavors were developed.

Khosravi and Sandine (1987) used this mutagen to develop penicillin-resistant mutants of *L. lactis ssp. cremoris*. Penicillin resistance was found to be due to the production of β-lactamase. Cells of lactic acid bacteria resistant to 6-7% salt were produced by Karlikanova *et al.* (1978). It was, however an adaptation lasting over 200 days rather than a mutation. Strains of *Lactococcus lactis ssp. lactis, L. lactis ssp. cremoris, L. lactis ssp. diacetilactis* and *Leuconostoc dextranicum* were isolated and incorporated into starter for pickled cheese. The use of these adapted cells (6-7%) ensured normal development of the microbiological processes during ripening and improved the flavor of the cheese. However, because the adaptation was not permanent further research is necessary to produce mutant cells with similar capability.

LISTERIA MONOCYTOGENES

Characteristics

The genus *Listeria* includes seven species: *L. monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L.*

ivanovii, *L. grayi* and *L. murrayi* (Donlon, 1988; Farber and Peterkin, 1991). *L. monocytogenes* is a gram-positive, nonsporeforming, facultative anaerobic rod which grows between 1 and 50°C, is catalase positive and β-hemolytic on blood agar (Farber and Peterkin, 1991; Seeliger and Jones, 1986). The organism possesses peritrichous flagella which give it a characteristic tumbling motility at 20-25°C (Farber and Peterkin, 1991; Wehr, 1987). The pH range for growth of the organism is between 5.0 and 9.6, but it can survive down to pH 4.3 (Farber *et al.*, 1989b; Wehr, 1987). *L. monocytogenes* has been isolated from sewage, soil, silage, feces of healthy animals and man, birds, fish, insects, green plants, water, and food products including milk, cheeses, meats, fruits and vegetables (Seeliger and Jones, 1986; Wehr, 1987).

The Disease

The human intestine is thought to be the primary route of entry of *L. monocytogenes* (Lovett, 1989). Due to association of *L. monocytogenes* with several foodborne outbreaks, the source of infection is thought to be the contaminated food (Azadian *et al.*, 1989; Farber and Peterkin, 1991; Schlech *et al.*, 1983). The infectious dose is not known (Lovett, 1989). However, Azadian *et al.* (1989) estimated an oral dose of 2.5-4.3 $\times$ 10^9 cells to cause meningitis in a fit, immunocompetent individual. The most affected groups are very young or very old individuals (Ciesielski *et al.*, 1988; Farber and Peterkin, 1991), pregnant (McLauchlin, 1990) and immunocompromised

individuals (Dee and Lorber, 1986; McLauchlin, 1990; Schlech *et al.*, 1983). However, it was reported that immunocompetent individuals developed listeriosis also (Azadian *et al.*, 1989). In pregnant women, the stage of pregnancy determined the outcome of the disease, whether abortion, stillbirth or neonatal sepsis (Gellin *et al.*, 1991; Lovett, 1989). Symptoms include septicemia, meningitis, central nervous system infection, brain abscess, sepsis and abortion in pregnant women (Azadian *et al.*, 1989; Ciesielski *et al.*, 1988; Dee and Lorber, 1986; Farber and Peterkin, 1991; Lovett, 1989; McLauchlin, 1990; Schlech *et al.*, 1983).

Food-borne Outbreaks

Five major foodborne outbreaks were reported in the last decade. In 1979, listeriosis associated with serotype 4b occurred in Boston, MA. The outbreak was reported several years later (Ho *et al.*, 1986). Case control studies showed that three types of foods were preferred by patients: tuna fish, chicken salad and cheese. The common feature appeared to be serving of these foods with raw celery, tomatoes and lettuce. The raw vegetables may have been contaminated with *L. monocytogenes*, which survived ingestion. Fifty percent of patients were immunocompromised and mortality rate was 15%.

The second outbreak occurred in 1983 in Massachusetts, (Fleming *et al.*, 1985). The outbreak was associated with pasteurized whole or 2% milk which came from a group of farms in which listeriosis in dairy cows occurred during the

outbreak. Inspection of the plant where the milk was processed rèvealed no evidence of faulty pasteurization. These findings raised the question of whether or not *L. monocytogenes* in contaminated cows' milk was destroyed by pasteurization. Mortality rate was 29%. The third outbreak occurred in Canada in 1981 (Schlech *et al.*, 1983). This outbreak was associated with the consumption of cole slaw prepared from cabbage grown on soil containing the manure of infected sheep and stored at low temperature before the cole slaw was made. The mortality rate among infants was 27% and among adults was 28.6%.

The fourth outbreak occurred in California, in 1985 (Linnan *et al.*, 1988). It was associated with the consumption of Mexican-style soft cheese. A case control study showed an association with one brand of Mexican-style soft cheese. An investigation of the cheese plant suggested that the cheese was commonly contaminated with unpasteurized milk. The mortality rate was 34%. The fifth outbreak occurred in Switzerland during 1983-1987 (Farber and Peterkin, 1991). A soft cheese was implicated and the mortality rate was 25.4%.

Incidence in Dairy Products

L. monocytogenes has been found in a wide range of dairy products (Table 2). Because three of the outbreaks involved milk and milk products, such foods received major emphasis in surveillance programs. Lovett (1989) reviewed the surveys of milk and milk products for *Listeria spp*. *Listeria spp*. were

Table 2. Incidence of *L. monocytogenes* in dairy products.

Product type	No. of samples	No. (%) *L. monocytogenes* positive	No. major serotypes isolated
Hard cheese	88	0(0)	NK[a]
Semihard cheese	205	2(2.0)	
White mold-cured cheese	261	7(2.7)	NK
Red smear cheese	343	33(9.6)	
Other cheeses	107	6(5.6)	
Soft cheese[b]	222	23(10.0)	-[c]
Various cheeses	100	2(2.0)	1/2a
Soft cheeses	338	6(1.8)	NK
Soft cheeses	121	2(1.6)	1
Butter	20	0(0)	
Soft and semisoft cheese	374	2(0.5)	NK
Cheese	509	29(5.7)	1(76)
Cheese	140	1[d](0.7)	
Cheese	89	8(9.0)	1(100)
Cheese[e]	23	20(87.0)	1/2a
Cheese	350	31(8.9)	NK
Soft ripened cheese[f]	769	63(8.2)	1/2(71)
Soft unripened cheese	366	4(1.1)	
Hard cheese	66	1(1.5)	
Goat milk cheese[f]	476	22(4.6)	4b(55)
Ewe milk cheese	141	1(0.7)	
Yogurt	180	4(2.2)	
Ice cream	394	1(0.3)	NK
Ice cream mix	85	0(0)	
Ice cream novelties	51	1(1.9)	
Ice cream	150	3(2.0)	4

Source: Farber and Peterkin (1991).
[a] NK, not known. [b] Includes cheeses from seven countries.
[c] 13 serotype 1/2,; 6 serotype 4b; 3 both serotypes 1/2 and 4b.
[d] Homemade goat cheese. [e] All samples from one cheese plant.
[f] Most samples contained <500 CFU/g.

detected in 58% of raw milk samples, with *L. monocytogenes* detected in 20% of samples. Two and three percent of soft cheese from pasteurized and raw milk, respectively, were positive for *Listeria spp.* However, *L. monocytogenes* was not detected in 126 Blue cheese samples. One percent of Cottage cheese and 4% of semi-hard cheeses yielded *Listeria spp.*, being mostly *L. monocytogenes*. Lovett (1989) also compiled reports from Europe where 706 cheeses were examined for *Listeria spp.*, 8% of samples were positive for *Listeria spp.* and 2.5% were positive for *L. monocytogenes*.

Massa *et al.* (1990) examined samples of soft cheese, butter and raw milk for *Listeria spp.* *L. monocytogenes* and *L. innocua* were each found in 1.6% of soft cheese samples, while 5% of butter samples contained *L. innocua*. No *Listeria spp.* were detected in 40 raw milk samples.

Doyle and Schoeni (1987) isolated *L. monocytogenes* from soft, surface-ripened cheeses by different procedures. Forty-one of 90 samples tested (45.6%) were positive. Farber *et al.* (1989a) analyzed various foods for the presence of *Listeria spp.* Fourteen samples of pasteurized milk were free of *L. monocytogenes*, and only two of 530 samples of ice cream products were positive for *L. monocytogenes*.

Raw milk has been studied extensively for presence of *Listeria spp.* (Farber *et al.*, 1988; Fenlon and Wilson, 1989; Fernandez *et al.*, 1987; Hayes *et al.*, 1986; Lovett *et al.*, 1987; Massa *et al.*, 1990; Rohrbach *et al.*, 1992; Slade and

Collins-Thompson, 1988). Lovett *et al.* (1987) examined 650 raw milk samples from three areas: California, Massachusetts and the tri-state area around Cincinnati, OH for the presence of *Listeria spp.* They found that 12.6% samples contained *Listeria spp.*, with *L. monocytogenes* found in 4.2% of samples. Of the 26 *L. monocytogenes* isolates, 25 were pathogenic in adult mice.

Hayes *et al.* (1986) examined 121 raw milk samples from individual farms, the milk cooperative and the pasteurizing plant which was implicated in the 1983 outbreak in Massachusetts. They recovered *L. monocytogenes* from 12% of samples. Farber *et al.* (1988) isolated *Listeria spp.* from 12.4% of raw milk samples from bulk tanks in Ontario. *L. innocua* was isolated most frequently and accounted for 9.7%, while *L. monocytogenes* and *L. welshimeri* were each found in 1.3% of samples.

Fenlon and Wilson (1989) tested over 180 farm bulk milk tanks in Northeast Scotland for the presence of *L. monocytogenes* in periods when cows were grazing or confined inside on a silage diet. *L. monocytogenes* incidence ranged from 3.8% in summer to 1.0% in October.

Slade and Collins-Thompson (1988) found 36 (11.4%) of 315 raw milk samples in Ontario contained *Listeria spp.* *L. monocytogenes* was found in 5.4% of samples, *L. innocua* in 8.2% and *L. welshimeri* in 0.3% of samples. Fernandez *et al.* (1987) tested milk samples for presence of *L. monocytogenes* for one

year on a monthly basis. They found 30 samples (44.8%) to contain *L. monocytogenes*. The highest incidence was in February and December (75%), while the lowest incidence was in August (14.3%). Rohrbach *et al.* (1992) tested raw milk samples collected from farm bulk tanks in East Tennessee and Southwest Virginia for the presence of *L. monocytogenes* and other foodborne pathogens, and isolated *L. monocytogenes* from 12 (4.1%) of 292 samples tested.

Growth and Survival in Dairy Products

The association of dairy products (milk and cheese) in listeriosis outbreaks has prompted the study of the behavior of *L. monocytogenes* during the manufacture and ripening of different dairy products. Cheese has been studied more extensively than other dairy products (Table 3). *L. monocytogenes* was found to concentrate in the curd with only small numbers of the organisms found in the whey (Papageorgiou and Marth, 1989a; Ryser and Marth, 1989; Yousef and Marth, 1988). It appears that lactic acid bacteria slowed but did not inhibit growth of *L. monocytogenes* (Farber and Peterkin, 1991).

The organism did not grow to any appreciable extent during the manufacture of Colby cheese (Yousef and Marth, 1988). Early in storage, numbers of *L. monocytogenes* in cheese remained relatively constant, then decreased steadily thereafter. *L. monocytogenes* was detected in the cheese for up to 140 d. After 140 d, numbers detected in cheese were

Table 3. Survival of *L. monocytogenes* in various dairy products.

Product type	Manufacture		Ripening or storage		Reference
	survival[a]	growth[b]	survival[a]	growth[b]	
Blue cheese	+	+	+	-	Papag & Marth (1989b)
Cold pack cheese	+	-	±	-	Ryser & Marth (1988)
Cheddar cheese	+	+	±	-	Ryser & Marth (1987b)
Brick cheese	+	++	+	+	Ryser & Marth (1989)
Colby cheese	+	-	±	-	Yousef & Marth (1988)
Feta cheese	+	++	±	+	Papag & Marth (1989a)
Camembert cheese	+	++	+	++	Ryser & Marth (1987b)
Cottage cheese	+	-	±	-	Ryser *et al.* (1985)
Parmesan cheese	+	+	±	-	Yousef & Marth (1990)
Swiss cheese	+	±	±	-	Buazzi *et al.* (1992)
Yogurt	+	+	±	-	Schaack & Marth(1988a)
Skim milk	+	+	±[c]	±[c]	Schaack & Marth (1988b)
Skim milk	+	+	±	±[d]	Schaack & Marth (1988c)
Nonfat dry milk	+	±	±	-	Doyle *et al.* (1985)
Cultured milk	+	+	±	-[e]	Schaack & Marth (1988a)
Chocolate milk[f]			+	++	Rosenow & Marth (1987)
Whole milk[f]			+	++	Rosenow & Marth (1987)
Skim milk[f]			+	++	Rosenow & Marth (1987)
Cream[f]			+	++	Rosenow & Marth (1987)

[a] ±, numbers decreased during ripening
[b] -, no growth; +, limited growth; ++, good growth
[c] Mesophilic starter culture had an inhibitory effect on *L. monocytogenes*
[d] Some strains showed slight increase during 15 h fermentation.
[e] *L. monocytogenes* was not detected in some samples. Combination of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* had detrimental effect.
[f] Autoclaved samples were inoculated with *L. monocytogenes* and incubated at 4, 8, 13, 21 or 35°C (no manufacturing step).

related to initial concentration of cells in milk, moisture content of cheese and strain of *L. monocytogenes*. During the manufacture of Cheddar cheese (Ryser and Marth, 1987a), *Listeria* counts remained relatively constant at about 5 x 10^2 CFU/ml but increased to about 1 x 10^3 CFU/g after overnight pressing of the curd. After 14 days of ripening, numbers increased to about 5 x 10^3 CFU/g. Further ripening resulted in a decrease in numbers. Depending on strain, *L. monocytogenes* survived for a maximum of 434 d of storage (after 70 d, analyses continued at 28 d intervals until *L. monocytogenes* could no longer be quantified by direct plating and until the organism was not detected in two successive samples after enrichment at 3°C for 8 wk).

Small numbers of the organism survived the Cottage cheesemaking process (Ryser *et al.*, 1985). *Listeria* remained steadily constant until after cooking of curd was completed. For Cottage cheese stored at 3°C for 28 d, *L. monocytogenes* was recovered from 38.4% and 52.7% of samples by direct plating and cold enrichment, respectively. In Camembert cheese, *Listeria* increased during its manufacture (Ryser and Marth, 1987b). Depending on the strain, numbers either decreased or remained unchanged during the first 18 d of ripening. After 65 d, numbers increased from 1 x 10^6 to 5 x 10^7 CFU/g, and growth paralleled the increase in pH of cheese during ripening.

During the manufacture of Parmesan cheese (Yousef and Marth, 1990), numbers of *L. monocytogenes* increased to a maximum just before cooking the curd, and decreased during cooking. During cheese ripening, numbers decreased. No cells were detected after 16 wk of ripening. Feta cheese curd entrapped *L. monocytogenes* (Papageorgiou and Marth, 1989a). Numbers increased during cheesemaking and after 2 d of ripening. During storage, cells survived for more than 90 d.

For Swiss cheese (Buazzi *et al.*, 1992), numbers remained unchanged during milk clotting, increased just before cooking and decreased during cooking. However, numbers increased after curd was pressed under whey, and reached their maxima at the end of this stage. During brining of cheese blocks, a sharp decrease was noticed and counts continued to decline during ripening. Although *L. monocytogenes* survived the manufacture and ripening of cold-pack cheese food, it did not grow (Ryser and Marth, 1988). After 182 d, numbers had decreased less than 10-fold.

Populations of *L. monocytogenes* increased during the first 24 h of Blue cheesemaking (Papageorgiou and Marth, 1989b) and during the 32 h of Brick cheesemaking (Ryser and Marth, 1989). Populations decreased during the first 50 d and 20-22 wk of ripening of Blue and Brick cheeses, respectively. However, between days 50-120, the environment of Blue cheese favored improved survival but not growth of the pathogen.

L. monocytogenes decreased during spray drying of nonfat dry milk at a temperature of 165°C and it progressively died during storage at 25°C with no cells detected after 14 wk of storage (Doyle *et al.*, 1985). Growth rates of *L. monocytogenes* were similar at a given temperature during the incubation of skim, whole and chocolate milk and whipping cream at 4, 8, 13, 21 and 35°C, and increased with an increase in temperature (Rosenow and Marth, 1987). The highest number of cells were produced in chocolate milk. *L. monocytogenes* survived during the manufacture and storage of yogurt (Schaack and Marth, 1988a) and yogurt mix (Schaack and Marth, 1988b).

Effect of Antimicrobials

Effects of acetic, lactic, citric or HCl acid on the ability of four strains of *L. monocytogenes* to initiate growth in Brain Heart Infusion (BHI) broth were studied by Farber *et al.* (1989b). In double strength BHI broth adjusted to various pH values (ranging from 4.0-6.0) with either 1 N HCl or 10% (w/v) lactic, acetic or citric acids, acetic acid was the most effective acidulant against *L. monocytogenes*. None of the strains grew at pH < 5.6 at 4°C or < 5.0 at 30°C. The minimum deleterious effect was caused by HCl. The minimum pH value required for initiation of growth ranged from 5.0 to 5.7 at 4°C and from 4.3 to 5.2 at 30°C, depending on the acidulant used.

Schaack and Marth (1988c) studied the ability of *L. monocytogenes* to grow and compete with mesophilic lactic acid

bacteria. Autoclaved skim milk was inoculated with 10^3 cells/ml of *L. monocytogenes* and with 5.0, 1.0, 0.5, and 0.1% of milk culture of either *L. lactis ssp. lactis* or *L. lactis ssp. cremoris*, and samples were incubated for 15 h at 21 or 30°C. *L. monocytogenes* survived the 15-h fermentation at both temperatures regardless of level of inoculum of *L. lactis ssp. lactis* or *L. lactis ssp. cremoris*. *L. lactis ssp. lactis* reduced the pH of fermented milk more than did *L. lactis ssp. cremoris*. In most instances, growth appeared to be inhibited when pH dropped below 4.75.

Schaack and Marth (1988b) also studied survival of *L. monocytogenes* in skim milk inoculated with cultures of *S. thermophilus*, *Lac. bulgaricus* or a mixture of the two species. A decrease in pH below 4.0 caused by *L. bulgaricus* was accompanied by rapid death of the pathogen. The combination of the two species was more inhibitory than *S. thermophilus* but less than *L. bulgaricus* alone.

Potassium sorbate (a salt of sorbic acid) is used widely in food systems due to its high solubility in water compared to sorbic acid (at 25°C the solubilities of acid and salt in water are 0.16 and 50%, respectively) (Sofos and Busta, 1981). Elshenawy and Marth (1988) studied inhibition and inactivation of *L. monocytogenes* by potassium sorbate by supplementing tryptose broth with 0, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3% potassium sorbate, adjusting to pH 5.6 or 5.0 and incubating at 4, 13, 21 or 35°C. *L. monocytogenes* grew in sorbate free

medium under all conditions except at 4°C and pH 5.0. At 4°C and pH 5.6 inactivation by 0.25 or 0.3% potassium sorbate was achieved after 66 and 60 days, respectively. Other concentrations permitted slight growth followed by a decrease in population. However, at 4°C and pH 5.0, *L. monocytogenes* was not detected after 48 or 36 d at concentrations of 0.25 or 0.3% respectively, or after 60 d at concentrations of 0.15 or 0.2%. Other concentrations caused a gradual decrease in population. At 13°C and pH 5.6, growth occurred in all concentrations of potassium sorbate, and maximum population was directly related to concentration. At pH 5.0, concentrations of 0.2, 0.25 or 0.3% potassium sorbate caused complete inhibition, whereas 0.15% or lower allowed growth of the pathogen. At 21 and 35°C, appreciable growth occurred at pH 5.6 for all concentrations, while reducing the pH to 5.0 allowed limited growth of the pathogen at 0.05, 0.1 or 0.15%.

A study on milk treated with hydrogen peroxide showed that *L. monocytogenes* was less affected than the normal flora of raw milk stored at 4°C, and treatment could have acted as a selective enrichment of the pathogen in milk (Dominguez *et al.*, 1987). In sterilized milk, a concentration of 0.0495% hydrogen peroxide and 9 h were required to produce complete inhibition of a pure culture of *L. monocytogenes*.

When milks containing 0, 10 or 50 IU/ml nisin and varying fat percentages were challenged with approximately $\log_{10}$ 7.0-7.5 CFU/ml of *L. monocytogenes* (Jung *et al.*, 1992), after 2 h

the count decreased to $\log_{10}$ 0.3 CFU/ml in skim milk with 50 IU/ml, decreased to $\log_{10}$ 2.9 CFU/ml in skim milk with 10 IU/ml and increased slightly to $\log_{10}$ 7.8 CFU/ml in skim milk without nisin. In milk with 12.9% fat, populations decreased to $\log_{10}$ 6.6 CFU/ml for 10 IU/ml and $\log_{10}$ 5.9 CFU/ml for 50 IU/ml.

In another study, fourteen bacteriocin-producing strains from *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Lactococcus* were evaluated for their ability to inhibit growth of *L. monocytogenes* (Harris *et al.*, 1989). Seven strains were antagonistic to the pathogen by deferred antagonism testing on agar. Of these seven strains, three also inhibited growth in well diffusion assays. The strains were: *Lactobacillus sp.* UAL 11 which produced an unnamed bacteriocin, *L. lactis ssp. lactis* ATCC 11454 which produced nisin, *L. lactis ssp. lactis* SIK 83 which produced nisin, *Pediococcus sp.* UAL 14 which produced an unnamed bacteriocin, *P. acidilactici* PAC 1.0 which produced pediocin PA-1, *P. pentosaceus* FBB 61-1 which produced pediocin A, and *P. pentosaceus* L-7230 which produced pediocin A.

Payne *et al.* (1989) used a model milk system with 1000 µg/ml propyl paraben, 150 µg/ml tertiary butylhydroquinone (TBHQ) and 1000 µg/ml potassium sorbate to determine the effect of these compounds on survival of *L. monocytogenes* inoculated at 10 CFU/ml and 1000 CFU/ml. For the 10 CFU/ml inoculum, after 24 h the control (1% ethanol) and potassium sorbate samples reached $\log_{10}$ 7.0 and 6.8 CFU/ml, respectively,

while counts in propyl paraben were as low as $\log_{10}$ 3.0 CFU/ml, and for TBHQ counts ranged from $\log_{10}$ <1.0 to $\log_{10}$ 3.0 CFU/ml depending on the replicate. For 1000 CFU/ml inoculum, counts increased to $\log_{10}$ 7.5 and 6.9 CFU/ml in control and potassium sorbate, respectively. For TBHQ and propyl paraben at 24 h, counts reached $\log_{10}$ 4.8 and $\log_{10}$ 3.7 CFU/ml, respectively.

STAPHYLOCOCCUS AUREUS

Characteristics

The genus *Staphylococcus* is composed of species that are gram positive cocci, nonmotile, catalase positive, and facultative anaerobes. Natural populations are associated mainly with skin, skin glands and mucous membranes of warm blooded animals. This genus consists of 19 recognized species; several others are currently under investigation (Kloos and Schleifer, 1986). *Staphylococcus aureus* produces acid from mannitol, glucose, lactose and maltose in aerobic and anaerobic conditions. Typical *S. aureus* strains produce α-toxin, coagulase and a heat-stable nuclease (Benwart, 1989). *S. aureus* is wide spread in nature. However, the main reservoirs are the nasal cavities, from these it finds its way to the skin. The most common skin sources are the arms, hands and face, it is also found in the eyes, throat and in the intestinal tract (Benwart, 1989; Jay, 1986). Most domesticated animals harbor *S. aureus*, and staphylococcal mastitis is known among dairy herds (Jay, 1986).

The Disease

It is believed that 10^5 to 10^6 cells per gram of *S. aureus* must be present before enterotoxin is produced to a level that causes intoxication (Benwart, 1989; Holmberg and Blake, 1984). Symptoms occur within 1-6 h after ingestion of food containing enterotoxin (Bergdoll, 1989). Major symptoms include: nausea, vomiting, abdominal pain, diarrhea and mild headache (Bergdoll, 1989; Holmberg and Blake, 1984; Minor and Marth, 1972a). Death from staphylococcal food poisoning is uncommon but it has occurred. Weed *et al.* (1943) reported the death of two children within 24 h after drinking milk from a goat infected with staphylococcal mastitis. The autopsy indicated that children received enterotoxin greater than the amount usually present in food.

Outbreaks

Staphylococcus aureus is one of three important agents of foodborne outbreaks world wide (Benwart, 1989). Various foods have been involved in staphylococcal outbreaks including meat products, poultry products, bakery products and dairy products (Benwart, 1989; Bergdoll, 1989; Holmberg and Blake, 1984; Minor and Marth, 1972b). Holmberg and Blake (1984) investigated 131 outbreaks reported to the Centers for Disease Control (CDC) in Atlanta, from 1977 to 1981. They found that all outbreaks were caused by previously cooked proteinaceous foods. Ham was implicated the most times (24% of outbreaks), followed by salad mixtures (15%), and baked foods involving

cream or custard-filled pastries allowed to sit at room temperature (10%). The largest single outbreak involved consumption of chocolate milk to which chocolate, milk solids and sugar were added after cream had been removed.

Minor and Marth (1972b) reviewed outbreaks related to milk, cheese, nonfat dry milk and other dairy products. Seven outbreaks caused by consumption of raw milk occurred between 1907 and 1939 and involved 506 persons. Other outbreaks associated with raw milk included one outbreak involving 75 persons in Africa, a 57-case outbreak in a World War II defense plant, and a family outbreak due to mastitic milk. There were also two outbreaks involving post-pasteurization contaminated milk, one affecting 32 persons and the other affecting 29 persons. Nonfat dry milk was the cause of eight outbreaks in England in 1953 and a total of 19 separate outbreaks in a single month in Puerto Rico. Cheese was implicated in an outbreak in 1965 over a wide spread area of the United States. Cheddar, Kuminost and Monterey cheeses produced in a dairy plant in Missouri were incriminated. In 1958, an outbreak of 200 cases was associated with consumption of Cheddar cheese made from raw milk. Also an outbreak associated with the consumption of butter was reported in 1970. Although staphylococci were not recovered from the milk, they were recovered from the butter, suggesting that contamination was caused by ingredients used to modify the milk.

Allen and Stovall (1960) reported on a series of outbreaks involving Colby-type cheese in Wisconsin. Investigation revealed that 78 of 87 samples contained staphylococci and 72 of the 78 isolates were coagulase-positive (47 from cheese, 24 from milk used in making cheese and one from a factory swab). Bergdoll (1989) reported on an outbreak that occurred among school children in Kentucky in 1985. It was associated with consumption of 2% chocolate milk. Staphylococci were not isolated from the milk but enterotoxin A was detected. It was believed that milk had been held at warm temperatures for 4-5 h before pasteurization, during which staphylococci multiplied and produced toxin. Khalid and Harrigan (1981) reported staphylococcal food poisoning cases in Sudan between 1977 and 1979 involving milk and white cheese. Data indicated that *S. aureus* food poisoning accounted for 25% of all incidents. Upon investigation of 25 cases, six were staphylococcal food poisoning. Of these six, three involved children and milk was implicated, while in the other three, cheese was the vehicle.

Incidence in Dairy Products

Clark and Nelson (1961) tested Grade A raw milk samples and reported 25-330 coagulase-positive staphylococci/ml. Holding milk at 4°C in a bulk tank and at 10°C in cans caused significant growth of staphylococci at 10°C for seven days but not at 4°C. Olson *et al.* (1970) found 23 of 157 strains isolated from mastitic milk to produce enterotoxins C and D.

Sharpe *et al.* (1965) tested 667 raw milk and 287 heat-treated market milk samples in England and Wales for the occurrence of coagulase-positive staphylococci. About 30% of samples contained $<10^3$ staphylococci/ml, while the rest contained between 10^3 and 10^6. Approximately 90% of heat-treated milk samples contained $<10^3$ staphylococci/ml.

Tham *et al.* (1990) tested 198 ripened soft or semi-soft cheeses made from goat milk in Sweden and found 43 samples having $\geq 10^2$ coagulase-positive staphylococci/g. Enterotoxins A, C and both A and C were produced by 22, 32 and 5% of strains, respectively, by the 63 strains isolated (Tham *et al.*, 1990). Mickelsen *et al.* (1961) tested 20 different varieties of cheeses and found 76% contained staphylococci. Among these, 70% were *S. aureus* and 26% were *S. epidermidis*. Coagulase-positive staphylococci were isolated from Cheddar, Bondost, Blue and Brick cheeses. Cheddar cheese samples incriminated in staphylococcal food poisoning outbreaks (11 of 13 samples) contained coagulase-positive staphylococci in numbers ranging from 50 to several million/g, and 20% of 343 samples contained coagulase-positive staphylococci in numbers ranging from 50 to $>2 \times 10^5$/g (Donnelly *et al.*, 1964). Mickelsen *et al.* (1963) tested 24 samples of Cottage cheese during winter and 42 samples during summer seasons and found that 11 samples (45%) tested during winter contained staphylococci, and only one out of these 11 samples contained *S. aureus*. For the samples tested during summer, 24 samples

(57%) contained staphylococci, of which eight contained *S. aureus*.

Khayat *et al.* (1988) collected cheese samples from California and Utah and analyzed them for *S. aureus*. Of the 200 samples, five samples (2%) contained over 10^3 CFU *S. aureus*/g. Zehren and Zehren (1968) examined 2,112 vats of cheese for the presence of staphylococcal enterotoxin A. They found that 56 vats of Cheddar, two vats of Monterey and one vat of Kuminost cheese contained enterotoxin A at concentration as high as 12 μg/100 g cheese. In a study of 237 samples of cheese in Ontario for the presence of *S. aureus*, Brodsky (1984) did not detect *S. aureus* above the screening level of 1000/g cheese in any of the samples. All the 103 strains of *S. aureus* isolated from 72 samples of cheese made from unpasteurized milk in Chile (Castro *et al.*, 1986) were coagulase and thermonuclease-positive, but were negative for the production of the identified enterotoxins (A, B, C, D, E).

Abbar and Mohammed (1986) collected 23 samples of cheese from Iraq and tested them for staphylococci. They found that of the 80 organisms isolated, 72.5% were staphylococci. *S. aureus* constituted 31% and six strains produced enterotoxin A. Chavarri *et al.* (1985) analyzed 94 samples of Burgos and 50 samples of Villalon cheeses in Spain for staphylococci. They found the staphylococci at levels of $\log_{10}$ 5.8 and $\log_{10}$ 6.2/g

for Burgos and Villalon cheeses, respectively. Coagulase-positive staphylococci represented 24.8% of the isolates.

Growth and Survival in Dairy Products

In whole and skim milk inoculated with *S. aureus* and incubated at 5, 10, 22, 30 and 37°C (Walker and Harmon, 1965), a continuous decline in numbers at 5°C was observed in both media. The inoculum had little effect on the duration of the logarithmic phase which varied from 12 h at 37°C to 20 d at 10°C. Sterile reconstituted nonfat dry milk was inoculated with *S. aureus* and incubated at 4°C and -10°C for five days (Marshall *et al.*, 1966). There was a 52% decrease in population at 4°C, compared to 3% decrease at -10°C. *S. aureus* numbers increased during the preparation of Domiati cheese and decreased rapidly during storage in cheese made with unsalted milk and cheese made with 5% salt, while in cheese made with 10% salt, *S. aureus* survived until the end of the fourth week with a population of $\log_{10}$ 4.7 cells/g (Ahmed *et al.*, 1983).

Tuckey *et al.* (1964) inoculated approximately 1 x 10^5 CFU/ml milk and made Cheddar, Colby, Swiss-type, Limburger and Cottage cheeses. Conditions of manufacture were favorable for growth of *S. aureus* in all cheeses except Cottage cheese, and *S. aureus* increased in numbers until cheese was salted. After pressing the curd, another increase occurred during the first 21 days of aging, after which a gradual decrease occurred throughout aging. However, *S. aureus* count never became zero

at any time. The manufacturing procedure for Cottage cheese was the only effective one in destroying *S. aureus*. No staphylococci were recovered from fresh Cottage cheese after cooking at 130°F for 40 min at pH 4.5, but when curd was stored dry or creamed for 10 d at either 40 or 50°F, viable *S. aureus* cells were recovered. In Manchego-type cheese (Gomez-Lucia *et al.*, 1992), *S. aureus* count was one $\log_{10}$ higher in 0.1% than in 1% starter culture. *S. aureus* numbers declined markedly after day 35 to 42, and were never detected by the end of ripening period of 60 d.

Nunez *et al.* (1988) studied the growth and survival of *S. aureus* in Manchego cheese made with raw ewes' milk. The highest *S. aureus* counts were detected after 24 h in cheese with 1% starter culture and cheese without starter. Populations declined during storage at 10°C. The death rate of *S. aureus* (mean decrease in $\log_{10}$ CFU/g/wk of ripening) from day 1 to day 60 was 0.421 and 0.404 in cheese made with starter culture and without starter culture, respectively. Burgos cheese was made from ewes' milk inoculated with *S. aureus* and stored at 4, 10, 15°C and room temperature (Otero *et al.*, 1988). Growth was observed during the storage period, with larger levels of *S. aureus* corresponding to higher temperatures. At 4°C, strain FRI 361 grew slightly while strain FRI 137 increased two generations by the end of the storage period. At 10°C, both strains increased two or more generations after 6 d. At room temperature and 15°C, higher

populations of 4 to 12 generations were attained at 7 d. *S. aureus* in Manchego-type cheese made from cow, goat and sheep milk with 1% and 0.1% starter culture (Gomez-Lucia *et al.*, 1986), decreased during the first three days and the first three weeks in 0.1% and 1% starter cheese, respectively. After 3 wk of ripening, growth decreased in both starter concentrations, and by the end of the ripening period of 58 d, no viable staphylococci were detected in the cheese.

Effect of Antimicrobials

Effect of starter culture on the survival of *S. aureus* was investigated in Brazilian Minas cheese by Clemente dos Santos and Genigeorgis (1981). By decreasing the pH to about 5.9 within the first 18 h of processing, and to 5.1-5.2 during ripening, starter culture bacteria had an inhibitory effect on *S. aureus* compared to cheese made without starter culture. Gilliland and Speck (1975) indicated that the antagonistic action of lactic streptococci toward *S. aureus* was not only due to low pH obtained by acid production, but due in part to other inhibitors produced by lactic streptococci in milk, one of which was a neutral material of low molecular weight.

Ibrahim *et al.* (1981) tested the effect of starter culture activity on *S. aureus* in Cheddar cheese and found that, at the end of cheddaring, *S. aureus* grew and produced toxin in detectable amounts in batches with a large inoculum of *S. aureus* and/or low starter activities. At the end of pressing, counts of *S. aureus* and pH of the unsalted cheese

were significantly lower than in salted cheese (salting suppressed the growth of salt-sensitive bacteria, including starter streptococci). Increasing concentration of NaCl from 0-12% had pronounced inhibition on the growth of *S. aureus* and enterotoxin B production (Hojvat and Jackson, 1969).

In Jibna Baida cheese (white pickled cheese) of Sudan, the pH is not considered a limiting factor since cheese is made without starter culture (Khalid and Harrigan, 1984). An investigation on the effect of salt level on growth of *S. aureus* in Sudanese semi-soft white brined cheese with salt content in milk of 8%, and preserved in brine solution with different salt concentrations, revealed that salt concentration had an inhibitory effect on *S. aureus* reduction (Khalid and Harrigan, 1984). Khalid and Harrigan (1986) submerged in tap water for 7 days Sudanese white cheese inoculated with *S. aureus* and found that numbers increased from 4.0×10^3 CFU/g after one day of storage to 8.3×10^7 CFU/g after seven days.

Milk was treated with 0.05% H_2O_2 and inoculated with *S. aureus* to determine the effect of treatment conditions and the characteristic of culture on effectiveness of H_2O_2 (Amin and Olson, 1967). Destruction of *S. aureus* (90-99.99%) required less time as temperature was increased from 37.8 to 57.2°C. At 54.4°C, *S. aureus* was significantly more resistant to heat treatment without added H_2O_2 than with 0.05% H_2O_2. Naguib (1972) made Domiati cheese with 0.15% and 0.1% H_2O_2 and found

that treatment with H_2O_2 caused reduction in coagulase-positive staphylococci.

The effect of potassium sorbate (0.25%), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on growth of *S. aureus* in synthetic media was studied (Parada *et al.*, 1982). At the end of the incubation period, the population of *S. aureus* was not affected by addition of potassium sorbate at pH 6.0 at a_w of 0.97 or 0.95. However when pH was lowered to 5.5, and in the presence of potassium sorbate, cells were reduced by one log as compared to that of pH 6.0. BHA and BHT at 100 and 200 ppm showed some growth inhibition. Neither antioxidant was effective in preventing growth of *S. aureus* in inoculated cheese spread at a_w of 0.97, pH 6.0 and 30°C.

SALMONELLA SPP.

Characteristics

The genus *Salmonella* belongs to the family *Enterobacteriaceae*, and, while only five species are recognized, over 2,000 serotypes are known with new ones added each year (Jay, 1986). *Salmonella spp.* are gram-negative, facultatively anaerobic, short rods (Minor, 1986), and are pathogenic to humans causing enteric fevers, gastroenteritis and septicemia (Minor, 1986). The primary habitat of *Salmonella spp.* is the intestinal tract of animals, although the organism may be found in other parts of the body from time

to time. Cells are excreted in the feces and transmitted by insects and other living creatures to a large number of places.

The Disease

Gastroenteritis is caused by foods that contain significant numbers (10^7- 10^9/g), and symptoms develop in 8-72 h from ingestion (Jay, 1986). Symptoms include diarrhea, nausea, vomiting, abdominal pain, chills, headache and fever. In normal healthy adults, gastroenteritis symptoms usually persist for two to three days, but if further infection occurs, the illness may persist for months or years (Benwart, 1989; Jay, 1986). Average mortality rate is 4.1% varying from 5.8% during the first year of life to 2% between the first and fiftieth year and 15% in persons over 50 years of age (Jay, 1986).

Outbreaks

Bean *et al.* (1990) summarized outbreaks reported to the CDC, Atlanta, between 1983 and 1987 and found that bacterial outbreaks accounted for 66% of foodborne illnesses. *Salmonella* accounted for 57% of total bacterial outbreaks, and it was the most frequently reported bacterial pathogen for each year. Tauxe (1991) reported a summary of food vehicles from outbreaks reported to the CDC between 1983 and 1987. Beef accounted for 11.2% of outbreaks, chicken 8.8%, eggs 5.3%, turkey 4.1%, dairy products 3.5%, vegetables 2.9%,

seafood 1.8% and other foods including multiple vehicles accounted for 59.4%.

Incidence in Dairy Products

Salmonella spp. were isolated from people who had consumed raw milk in 21 outbreaks of milkborne salmonellosis affecting 1090 persons (Reilly *et al.*, 1983). Four serotypes were involved including 12 phage types belonging to *S. typhimurium*. In another outbreak occurring in Wisconsin, Michigan and Illinois, *Salmonella spp.* were isolated from 32 of 678 (4.7%) raw milk samples collected from bulk tanks of milk suppliers (McManus and Lanier, 1987). *S. typhimurium* was found in eight of 21 lots of Cheddar cheese samples implicated in a foodborne outbreak in Canada at levels ranging from 0.36 to 9.3 CFU/100 g cheese (D'Oust *et al.*, 1985). Information on phosphatase test was lacking. However, three of six household cheese samples incriminated in the outbreak were phosphatase positive, and further analysis showed the presence of the enzyme in some lots. This study showed that the pathogen was able to survive in Cheddar cheese for up to eight months of refrigeration storage at 5°C.

Wood *et al.* (1984) found *S. muenster* in raw milk Cheddar cheese. The source was traced to a particular milk supplier shipping milk to the cheese factory. Analysis of milk samples from the herd of 35 cattle revealed only one cow shedding the pathogen into the milk at a rate of about 200 CFU/ml. Eleven of the 181 vats of cheese produced at the factory were

positive for *S. muenster* at the curd stage, but only two vats of the finished cheese were positive. *S. typhimurium* was isolated from Cheddar cheese implicated in a major outbreak of gastroenteritis in numbers ranging from <3 CFU/100 g to 9 CFU/100 g cheese from factory sealed blocks and samples from homes of known cases of salmonellosis (Ratnam and March, 1986). It was suggested that the infective dose in contaminated cheese was no greater than 10^4 organisms, and that a rapid decline in numbers must have occurred after the outbreak (Ratnam and March, 1986).

Growth and Survival in Cheese

Growth of *S. typhimurium* during the manufacture and ripening of Cheddar cheese was studied by Geopfert *et al.* (1968). Rapid growth occurred during manufacture before adding salt. Salting induced a reduction in multiplication rate, which was followed by a decline in numbers that continued during ripening. A 90% reduction was achieved by storing cheese for 2.5-3.0 wk at 13°C or 3.5-4.0 wk at 7°C. Regardless of the serotype, *Salmonella* survived longer when Cheddar cheese was stored at 4.5°C rather than 10°C (White and Custer, 1976). After nine months of storage, *S. new-brunswick* was present in nine of 16 lots of cheese at 4.5°C, and in six lots at 10°C. For *S. infantis*, one sample was positive at 4.5°C and 10°C. Six samples were positive for *S. newport* at 4.5°C but none were at 10°C.

Cheddar cheese was made from pasteurized milk with a low-acid producing strain of *L. lactis ssp. lactis* (Park *et al.*, 1970a). Salmonellae grew rapidly during manufacture, grew slightly during the first week of ripening at 13°C, and declined slightly in numbers thereafter. Growth of salmonellae during the early stages of ripening and subsequent extended survival was attributed to high moisture (43.2%) and high pH (5.75) of cheese which resulted from use of a low-acid producing starter.

Numbers of *S. javiana* increased during manufacture of Mozzarella-type cheese, reaching a maximum at the end of draining (Eckner *et al.*, 1990). Stretching and molding at 60°C completely eliminated any cells from all further processing steps. *S. typhimurium* rapidly declined during the first week of storage of cold-pack cheese food at 4.4 and 12.8°C regardless of temperature (Park *et al.*, 1970b). However, survival beyond this time was dependent on temperature and composition of cheese. No viable salmonellae were recovered after 3 wk at 12.8°C or 5 wk at 4.4°C from cheese food adjusted to pH 5.0 with lactic acid and fortified with 0.24% potassium sorbate.

During the manufacture of high moisture Monterey Jack cheese from ultrafiltered milk, *S. typhimurium* increased in number until the starter was added, and then grew steadily until day 7, after which it declined through day 18 (Eckner and Zottola, 1991). This pattern was similar in all

treatments. *S. typhimurium* was sensitive to acid development and salt during ripening since numbers declined during the six month ripening period. Medina *et al.* (1982) studied the behavior of salmonellae during the manufacture and ripening of Manchego cheese. Maximum growth of *S. typhimurium* occurred after 6 h, with a mean increase in $\log_{10}$ counts of 1.7, while *S. enteritidis* and *S. typhi* continued to grow for 9 h, with mean increase in $\log_{10}$ counts of 1.7 and 1.5, respectively. A considerable reduction occurred during the first week. During brine salting (9-48 h), a mean decline of 2.1, 0.2 and 1.2 $\log_{10}$ for *S. enteritidis, S. typhi* and *S. typhimurium*, respectively, was achieved. After 7 d, the average decrease in $\log_{10}$ counts of the three serotypes was 4.4, 1.2 and 4.0, respectively. No *Salmonella* was detected in the 8-wk-old cheese stored at 10°C (Medina *et al.*, 1982).

Effect of Antimicrobials

Effects of milk pasteurization, size of starter inoculum, titratable acidity and pH, type of lactic culture, chemical additives, salt and moisture content and curing temperature on *Salmonella* in the manufacture and ripening of Cheddar and Colby cheeses were studied (Hargrove *et al.*, 1969). Rate and amount of acid produced during cheesemaking, pH of the cheese and type and size of the starter inoculum had significant effects on growth and survival of salmonellae. However, salt, moisture, chemical additives and pasteurization of milk had little or no effect on salmonellae. Growth of salmonellae in

acidic media was more dependent on the acid molecule than on the pH (Chung and Geopfert, 1970). The organisms grew at pH 4.05 when the acid was HCl or citric. However, when the acid was acetic or propionic, the limiting pH was 5.4 or 5.5, respectively. Subramanian and Marth (1968) inoculated sterile skim milk acidified with HCl, lactic or citric acids with *S. typhimurium* and incubated it at 22 and 37°C. Acids were added at 2 h intervals so that the titratable acidity was raised from 0.2% at 2 h to 0.9% at 16 h of inoculation. At 37°C, in control milk, maximum numbers of 10^9 cells/ml were attained after 12 h, whereas in acidified milk maximum numbers of more than 10^8 were reached after 16 h incubation. At 22°C, highest numbers appeared in all milks after 16 h of incubation. HCl was the least inhibitory followed by lactic and acetic acids.

Sorrells and Speck (1970) studied the effect of acetic and lactic acids produced from *Leuc. citrovorum* on *S. gallinarum* in filter sterilized yeast extract medium after adjusting the pH to 7.0 and 4.4 and incubating at 37°C. Inhibition was caused primarily by acetic acid, although lactic acid was slightly inhibitory at lower pH. At pH 7.0, the pathogen grew in the presence of acetic and lactic acids, but growth was slower than in the control.

The behavior of *S. typhimurium* in skim milk inoculated with different concentrations of mesophilic and thermophilic lactic acid bacteria and incubated at different temperatures was studied (Park and Marth, 1972a). An inoculum of 0.25% of

L. lactis ssp. cremoris, *L. lactis ssp. lactis* and mixtures of the two repressed growth but did not inactivate the pathogen during incubation at 21 or 30°C for 18 h. Both *L. lactis ssp. diacetilactis* and *Leuc. citrovorum* were less inhibitory to *S. typhimurium* than *L. lactis ssp. cremoris* or *L. lactis ssp. lactis*. At 1% inoculum, *S. thermophilus* was more detrimental to the pathogen than *Lac. bulgaricus* at 42°C. With mixtures of *S. thermophilus* and *Lac. bulgaricus* at 1% and 5% concentration, complete inhibition was achieved during incubation at 42°C between 8 and 18 h. Another study was conducted on the effect of mesophilic lactic streptococci on *S. gallinarum* in nonfat dry milk (Speck, 1972). A marked decrease in the growth of *S. gallinarum* was observed when it was grown together with the mixed starter culture compared to the pathogen alone.

Three serotypes of *Salmonella*, namely *S. heidelberg, S. typhi* and *S. derby* showed resistance to 0-4%, 5-8% and 7-8% NaCl at 12, 22 and 37°C, respectively. However, at 8°C, *S. heidelberg* grew in 1—2% added NaCl, *S. typhi* grew in 1% added NaCl while *S. derby* failed to grow (Matches and Liston, 1972).

Park and Marth (1972b) studied the effect of sorbic acid on *S. typhimurium*. Sorbic acid at 2,000 and 3,000 ppm, was added to nutrient broth, skim milk and evaporated milk and adjusted to pH 5.0 and 5.5 with acetic or lactic acids and incubated at 7, 13 and 37°C. The combination of 3,000 ppm sorbic acid and acetic acid at pH 5.0 was most inhibitory in

all media and all temperatures, and complete inactivation required from 12 h or less in nutrient broth at 37°C, to 55 d in evaporated milk at 7°C. Reduction in concentration to 2,000 ppm or raising the pH to 5.5 increased the time of inactivation of the pathogen.

In conclusion, foodborne pathogens cause diseases to human if found in food products in significant numbers. The diseases affect health and decrease productivity. Additionally, some are fatal, particularly to individuals with health problems. Controlling these pathogens in food is an important factor in reducing the incidence of foodborne diseases. Control measures could be achieved through proper manufacture and handling of foods.

It is also possible that foodborne pathogens can be controlled by use of antimicrobials. Lactic acid bacteria are capable of inhibiting growth of bacterial pathogens in food products. However, in food products with high salt levels, lactic acid bacteria can not grow and inhibit the pathogen. Therefore, it is necessary to produce strains capable of growing under adverse conditions such as high salt levels.

This investigation was undertaken to evaluate and study the processing conditions suitable for the production of microbiologically safe white pickled cheese, and to develop salt-resistant mutants of lactic streptococci for use in high salt cheese.

REFERENCES

1. Abbar, F. M. and M. T. Mohammed. 1986. Identification of some enterotoxigenic strains of staphylococci from locally processed cheese. Food Microbiol. 3:33-36.

2. Abdalla, O. M. and P. M. Davidson. 1990. Effect of starter culture and antimicrobials on the growth of *Staphylococcus aureus* in Gebna Beyda cheese. Institute of Food Technologists' Annual Meeting, June, 16-20, 1990. Anaheim, California.

3. Abd El-Salam, M. H. 1987. Domiati and Feta type cheeses. pp. 277-309. In Fox, P.F. (ed.), *Cheese: chemistry, physics and microbiology*. Vol. 2. Elsevier Appl. Sci., N.Y.

4. Ahmed, A. A. H., M. K. Moustafa, and E. H. Marth. 1983. Growth and survival of *Staphylococcus aureus* in Egyptian Domiati cheese. J. Food Prot. 46:412-415.

5. Alla Gabo, I. H. 1986. *Studies on the composition and quality of Gibna Beyda*. M.Sc. Thesis, University of Khartoum, Khartoum, Sudan.

6. Allen, V. D. and W. D. Stovall. 1960. Laboratory aspects of staphylococcal poisoning from Colby cheese. J. Milk Food Technol. 23:271-274.

7. Amin, V. M. and N. F. Olson. 1967. Factors affecting the resistance of *Staphylococcus aureus* to hydrogen peroxide treatments in milk. Appl. Microbiol. 15:97-101.

8. Anderson, A. W. and P. R. Elliker. 1953. The nutritional requirements of lactic streptococci isolated from starter cultures. I. Growth in a synthetic medium. J. Dairy Sci. 36:161-167.

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

10. Bean, N. H., P. M. Griffin, J. S. Goulding, and C. B. Ivey. 1990. Foodborne disease outbreaks, 5-year summary, 1983-1987. J. Food Prot. 53:711-728.

11. Benwart, G. J. 1989. *Basic food microbiology*. 2nd Ed. Van Nostrand Reinhold Co., N.Y.

12. Bergdoll, M. S. 1989. *Staphylococcus aureus*. pp. 463-523. In Doyle, M. P. (ed), *Foodborne bacterial pathogens*. Marcell Dekker, Inc., N.Y.

13. Brodsky, M. H. 1984. Bacteriological survey of freshly formed Cheddar cheese. J. Food Prot. 47:546-548.

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

15. Castro, R., R. Schoebitz, L. Montes, and M. S. Bergdoll. 1986. Enterotoxigenicity of *Staphylococcus aureus* strains isolated from cheese made from unpasteurized milk. Lebensm. Wiss. Technol. 19:401-402.

16. Chavarri, F. J., Nunez, J. A., L. Bautista, and Nunez M. 1985. Factors affecting the microbiological quality of Burgos and Villalon cheeses at the retail level. J. Food Prot. 48:865-869.

17. Chuang, L. F. and E. B. Collins. 1971. Isolation of nitrosoguanidine-induced mutants of *Streptococcus diacetilactis* with enhanced ability to produce acetoin and diacetyl. J. Dairy Sci. 54:282-283.

18. Chung, K. C. and J. M. Geopfert. 1970. Growth of *Salmonella* at low pH. J. Food Sci. 35:326-328.

19. Ciesielski, C. A., A. W. Hightower, S. K. Parsons, and C. V. Broome. 1988. Listeriosis in the United States: 1980-1982. Arch. Intern. Med. 148:1416-1419.

20. Clark, W. S., Jr. and F. E. Nelson. 1961. Multiplication of coagulase-positive staphylococci in Grade A raw milk samples. J. Dairy Sci. 44:232-236.

21. Clemente dos Santos, E. and C. Genigeorgis. 1981. Survival and growth of *Staphylococcus aureus* in commercially manufactured Brazilian Minas cheese. J. Food Prot. 44:177-184.

22. Cogan, T. M. and C. Daly. 1987. Cheese starter cultures. pp. 179-249. In Fox, P.F. (ed.), *Cheese: chemistry, physics and microbiology*. Vol. 1. Elsevier Appl. Sci., N.Y.

23. Dariani, N. D., K. M. Galal, S. J. Speck, and M. Loewenstein. 1980. I. Manufacturing soft pickled cheese using cow and goat milk. Dairy Field 163(6):81-84.

24. Dee, R. R. and B. Lorber. 1986. Brain abscess due to *Listeria monocytogenes*: case report and literature review. Rev. Infect. Dis. 8:968-977.

25. DeMan, J. C., M. Rogosa, and M. E. Sharpe. 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23:130-135.

26. Dilanian, Z., K. Makarian, and D. Chuprina. 1976. Use of x-ray and gamma-induced mutants of lactic acid bacteria in the manufacture of dairy products. Milchwissenschaft 41:217-221.

27. Dominguez, L., J. F. F. Garayazabal, E. R. Ferri, J. A. Vazquez, E. Gomezlucia, C. Ambrosio, and G. Suarez. 1987. Viability of *Listeria monocytogenes* in milk treated with hydrogen peroxide. J. Food Prot. 50:636-639.

28. Donnelly, C. B., L. A. Black, and K. H. Lewis. 1964. Occurrence of coagulase-positive staphylococci in Cheddar cheese. Appl. Microbiol. 12:311-315.

29. Donlon, T. P. 1988. *Listeria monocytogenes*: a review of identification and prevention. Cultured Dairy Products J. 23:24-26.

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

31. Doyle, M. P. and J. L. Schoeni. 1987. Comparison of procedures for isolating *Listeria monocytogenes* in soft, surface-ripened cheese. J. Food Prot. 50:4-6.

32. Doyle, M. P., L. M. Meske, and E. H. Marth. 1985. Survival of *Listeria monocytogenes* during the manufacture and storage of nonfat dry milk. J. Food Prot. 48:740-742.

33. Eckner, K. F. and E. A. Zottola. 1991. The behavior of selected microorganisms during the manufacture of high moisture Jack cheeses from ultrafiltered milk. J. Dairy Sci. 74:2820-2830.

34. Eckner, K. F., R. F. Roberts, A. A. Strantz, and E. A. Zottola. 1990. Characterization and behavior of *Salmonella javiana* during manufacture of Mozzarella-type cheese. J. Food Prot. 53:461-464.

35. Elliker, P. R., A. W. Anderson, and G. Hannesson. 1956. An agar culture medium for lactic acid bacteria. J. Dairy Sci. 39:1611-1612.

36. El-Shenawy, M. A. and E. H. Marth. 1988. Inhibition and inactivation of *Listeria monocytogenes* by sorbic acid. J. Food Prot. 51:842-847.

37. Fahmi, A. H. and H. A. Sharara. 1950. Studies on Egyptian Domiati cheese. J. Dairy Res. 17:312-328.

38. Farber M. J. and P. I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. 55:476-511.

39. 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.

40. Farber, J. M., G. W. Sanders, and M. A. Johnson. 1989a. A survey of various foods for the presence of *Listeria* species. J. Food Prot. 52:456-458.

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

42. Feirtag, J. M. and L. L. McKay. 1987. Isolation of *Streptococcus lactis* C2 mutants selected for temperature sensitivity and potential use in cheese manufacture. J. Dairy Sci. 70:1773-1778.

43. Fenlon, D. R. and J. Wilson. 1989. The incidence of *Listeria monocytogenes* in raw milk from farm bulk tanks in North-East Scotland. J. Appl. Bacteriol. 66:191-196.

44. Fernandez Garayzabal, J. F., L. Dominguez, J. A. Vazquez, E. Gomez-Lucia, E. R. Rodriguez Ferri, and G. Suarez. 1987. Occurrence of *Listeria monocytogenes* in raw milk. Vet. Rec. 120:258-259.

45. Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. 312:404-407.

46. Fox, P. F. 1987. Cheese: An overview. pp. 1-32. In Fox, P. F. (ed.), *Cheese: chemistry, physics and microbiology*. Vol. 1. Elsevier App. Sci., N.Y.

47. Garvie, E. I. 1984. Taxonomy and identification of bacteria important in cheese and fermented dairy products. pp. 35-66. In Davies, F. L. and B. A. Law (ed.), *Advances in the microbiology and biochemistry of cheese and fermented milk*. Elsevier App. Sci., N.Y.

48. Gellin, B. G., C. V. Broome, W. F. Bibb, R. E. Weaver, S. Gaventa, L. Mascola, and the listeriosis study group. 1991. The epidemiology of listeriosis in the United States—1986. Am. J. Epidemiol. 133:392-401.

49. Geopfert, J. M., N. F. Olson, and E. H. Marth. 1968. *Salmonella* in Cheddar cheese. J. Dairy Sci. 51:934.

50. Gilliland, S. E. and M. L. Speck. 1975. Antagonism of lactic streptococci toward *Staphylococcus aureus* in associative milk cultures. Appl. Microbiol. 28:1090-1093.

51. Gomez-Lucia, E., J. L. Blanco, J. Goyache, R. DeLaFuente, J. A. Vazquez, E. F. R. Ferri, and G. Suarez. 1986. Growth and enterotoxin A production by *Staphylococcus aureus* S6 in Manchego type cheese. J. Appl. Bacteriol. 61:499-503.

52. Gomez-Lucia, E., J. Goyache, J. A. Orden, A. Domenech, F. J. Hernandez, J. A. R. Quiteria, B. Lopez, J. L. Blanco, and G. Suarez. 1992. Growth of *Staphylococcus aureus* and synthesis of enterotoxin during ripening of experimental Manchego-type cheese. J. Dairy Sci. 75:19-26.

53. Grieve, P. A. and J. R. Dulley. 1983. Use of *Streptococcus lactis* lac$^-$ mutants for accelerating Cheddar cheese ripening. 2. Their effect on the rate of proteolysis and flavor development. Aust. J. Dairy Technol. 38:49-54.

54. Grieve, P. A., B. A. Lockie, and J. R. Dulley. 1983. Use of *Streptococcus lactis* lac$^-$ mutants for accelerating Cheddar cheese ripening. 1. Isolation, growth and properties of a C2 lac$^-$ variant. Aust. J. Dairy Technol. 38:10-13.

55. 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.

56. Harris, L. J., M. A. Daeschel, M. E. Stiles, and T. R. Klaenhammer. 1989. Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes*. J. Food Prot. 52:384-387.

57. 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.

58. Ho, J. L., K. N. Shands, G. Friedland, P. Eckind, and D. W. Fraser. 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Arch. Intern. Med. 146:520-524.

59. Hojvat, S. A. and H. Jackson. 1969. Effects of sodium chloride and temperature on the growth and production of enterotoxin B by *Staphylococcus aureus*. Can. Inst. Food Technol. J. 2:56-59.

60. Holmberg, S. D. and P. A. Blake. 1984. Staphylococcal food poisoning in the United States. J. Amer. Med. Assoc. 251:487-489.

61. Hunter, G. J. E. 1946. A simple agar medium for the growth of lactic streptococci: the role of phosphate in the medium. J. Dairy Res. 14:283-290.

62. Ibrahim, G. F., A. K. Baldock, D. R. Bradford, and L. B. Ireland. 1981. Inhibition of *Staphylococcus aureus* growth and enterotoxin A production in Cheddar cheese produced with variable starter activity. J. Food Prot. 44:263-267.

63. Jay, J. M. 1986. *Modern food microbiology*. 3rd. Ed. Van Nostrand Reinhold Co., N.Y.

64. Jung, D., F. W. Bodyfelt, and M. A. Daeschel. 1992. Influence of fat and emulsions on the efficacy of nisin in inhibiting *Listeria monocytogenes* in fluid milk. J. Dairy Sci. 75:387-393.

65. Kandler, O. and N. Weiss. 1986. Genus *Lactobacillus* Beijerinck 1901, 212[AL]. pp. 1209–1235. In Sneath, A. H. P. S. N. Mair, M. E. Sharpe, and G. J. Holt (ed.), *Bergey's manual of systematic bacteriology*. Vol. 2. Williams and Wilkins Co., Baltimore, MD.

66. Karlikanova, S. N., I. U. Ramazanov, and O. P. Ramazanova. 1978. Production of salt-resistant mutants of lactic acid streptococci and their use in starters for pickled cheese. XX Intern. Dairy Cong. E. 525-526.

67. Kempler, G. M. and L. L. McKay. 1980. Improved medium for detection of citrate-fermenting *Streptococcus lactis ssp. diacetylactis*. Appl. Environ. Microbiol. 39:926-927.

68. Khalid, S. A. and W. F. Harrigan. 1981. Incidence of food poisoning in the Sudan with special reference to *Staphylococcus aureus* in milk and white cheese (Jibna Baida). J. Appl. Bacteriol. 51:23.

69. Khalid, S. A. and F. W. Harrigan. 1984. Investigation into the effect of salt level on growth of, and toxin production by *Staphylococcus aureus* in Sudanese cheese. Lebensm-Wiss Technol. 17:99-103.

70. Khalid, S. A. and W. F. Harrigan. 1986. The effect of desalting Sudanese cheese on growth of and toxin production by *Staphylococcus aureus* 196E. J. Food Sci. Technol. 23:243-245.

71. Khayat, F. A., J. C. Bruhn, and G. H. Richardson. 1988. A survey of coliforms and *Staphylococcus aureus* in cheese using impedimetric and plate count methods. J. Food Prot. 51:53-55.

72. Khosravi, L. and W. E. Sandine. 1987. β-lactamase-producing mutants of *Streptococcus cremoris*. J. Dairy Sci. 70:1529-1543.

73. Kloos, E. W. and K. H. Schleifer. 1986. Genus *Staphylococcus* Rosenbach 1884, 18[AL]. pp. 1013-1035. In Sneath, A. H. P., S. N. Mair, M. E. Sharpe, and G. J. Holt (ed.), *Bergey's manual of systematic bacteriology*. Vol. 2. Williams and Wilkins Co., Baltimore, MD.

74. Kuila, R. K., B. Ranganathan, S. M. Dutta, and H. Laxminarayana. 1971. Induction of mutation in *Streptococcus diacetilactis* by nitrosoguanidine and ultraviolet irradiation. J. Dairy Sci. 54:331-334.

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

76. Lovett, J. 1989. *Listeria monocytogenes*. pp. 283-310. In Doyle, P. M. (ed.), *Foodborne bacterial pathogens*. Marcel Dekker, Inc., N.Y.

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

78. Marshall, R. T., B. Singh, S. A. S. Shahidi, and J. E. Edmondson. 1966. *Staphylococcus aureus* counts as affected by time and temperature of storage of milk samples. J. Dairy Sci. 49:703-704.

79. Massa, S., D. Cesaroni, G. Poda, and L. D. Trovatelli. 1990. The incidence of *Listeria spp.* in soft cheese, butter and raw milk in the province of Bologna. J. Appl. Bacteriol. 68:153-156.

80. Matches, J. R. and J. Liston. 1972. Effects of incubation temperature on the salt tolerance of *Salmonella*. J. Milk Food Technol. 35:39-44.

81. McLauchlin, J. 1990. Human listeriosis in Britain, 1967-85, a summary of 722 cases. 2. Listeriosis in non-pregnant individuals, a changing pattern of infection and seasonal incidence. Epidemiol. Infect. 104:191-201.

82. McManus, C. and J. M. Lanier. 1987. *Salmonella, Campylobacter jejuni* and *Yersinia enterocolitica* in raw milk. J. Food Prot. 50:51-55.

83. Medina, M., P. Gaya, and M. Nunez. 1982. Behavior of salmonellae during manufacture and ripening of Manchego cheese. J. Food Prot. 45:1091-1095.

84. Mickelsen, R., V. D. Foltz, W. H. Martin, and C. A. Hunter. 1961. The incidence of potentially pathogenic staphylococci in dairy products at the consumer level. II. Cheese. J. Milk Food Technol. 24:342-345.

85. Mickelsen, R., V. D. Foltz, W. H. Martin, and C. A. Hunter. 1963. Staphylococci in Cottage cheese. J. Milk Food Technol. 26:74-77.

86. Mikolajcik, E. M. 1964. Single broth for the differentiation of *Streptococcus lactis* from *Streptococcus cremoris*. J. Dairy Sci. 47:437-438.

87. Minor, L. L. 1986. *Salmonella* lignieres 1900, 389[AL]. pp. 427-458. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*. Vol. 1. Williams and Wilkins Co., Baltimore, MD.

88. Minor, T. E. and E. H. Marth. 1972a. *Staphylococcus aureus* and staphylococcal food intoxications. A review. II. Enterotoxins and epidemiology. J. Milk Food Technol. 35:21-29.

89. Minor, T. E. and E. H. Marth. 1972b. *Staphylococcus aureus* and staphylococcal food intoxications. A review. III. Staphylococci in dairy products. J. Milk Food Technol. 35:77-82.

90. Naguib, K. 1972. Hydrogen peroxide treatment of milk for Domiati cheese. Aust. J. Dairy Tech. 27:56-60.

91. Nickles, C. and H. Leesment. 1964. Method for the differentiation and quantitative determination of starter bacteria. Milchwissenschaft 19:374-378.

92. Nunez, M., L. Bautista, M. Medina, and P. Gaya. 1988. *Staphylococcus aureus*, thermostable nuclease and staphylococcal enterotoxins in raw ewes' milk Manchego cheese. J. Appl. Bacteriol. 65:29-34.

93. Olson, J. C., Jr., E. P. Casman, E. F. Baer, and J. E. Stone. 1970. Enterotoxigenicity of *Staphylococcus aureus* cultures isolated from acute cases of bovine mastitis. Appl. Microbiol. 20:605-607.

94. Otero, A., M. C. Garcia, M. L. Garcia, M. Prieto, and B. Moreno. 1988. Behavior of *Staphylococcus aureus* strains, producers of enterotoxins C_1 or C_2, during the manufacture and storage of Burgos cheese. J. Appl. Bacteriol. 64:117-122.

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

96. 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.

97. Parada, J. L., J. Chirife, and R. C. Magrini. 1982. Effect of BHA, BHT and potassium sorbate on growth of *Staphylococcus aureus* in a model system and process cheese. J. Food Prot. 45:1108-1111.

98. Park, H. S. and E. H. Marth. 1972a. Behavior of *Salmonella typhimurium* in skim milk during fermentation by lactic acid bacteria. J. Milk Food Technol. 35:482-488.

99. Park, H. S. and E. H. Marth. 1972b. Inactivation of *Salmonella typhiurium* by sorbic acid. J. Milk Food Technol. 35:532-539.

100. Park, H. S., E. H. Marth, J. M. Geopfert, and N. F. Olson. 1970a. The fate of *Salmonella typhimurium* in the manufacture and ripening of low-acid Cheddar cheese. J. Milk Food Technol. 33:280-284.

101. Park, H. S., E. H. Marth, and N. F. Olson. 1970b. Survival of *Salmonella typhimurium* in cold-pack cheese food during refrigerated storage. J. Milk Food Technol. 33:383-388.

102. Payne, K. D., E. Rico-Munos, and P. M. Davidson. 1989. The antimicrobial activity of phenolic compounds against *Listeria monocytogenes* and their effectiveness in a model milk system. J. Food Prot. 52:151-153.

103. 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.

104. Reddy, M. S., E. R. Vedamuthu, C. J. Washam, and G. W. Reinbold. 1969. Differential agar medium for separating *Streptococcus lactis* from *Streptococcus cremoris*. Appl. Microbiol. 18:755-759.

105. Reddy, M. S., E. R. Vedamuthu, and G. W. Reinbold. 1970. A differential broth for separating the lactic streptococci. J. Milk Food Technol. 34:43-45.

106. Reddy, M. S., E. R. Vedamuthu, C. J. Washam, and G. W. Reinbold. 1972. Agar medium for differential enumeration of lactic streptococci. Appl. Microbiol. 24:947-952.

107. Reilly, W. J., J. C. M. Sharp, G. I. Forbes, and G. M. Paterson. 1983. Milkborne salmonellosis in Scotland 1980 to 1982. Vet. Rec. 112:578-580.

108. Rohrbach, W. B., F. A. Draughon, P. M. Davidson, and S. P. Oliver. 1992. Prevalence of *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Salmonella* in bulk tank milk: Risk factors and risk human exposure. J. Food Prot. 55:93-97.

109. Rosenow, E. M. and E. H. Marth. 1987. Growth of *Listeria monocytogenes* in skim, whole and chocolate milk, and in whipping cream during incubation at 4, 8, 13, 21 and 35°C. J. Food Prot. 50:452-459.

110. 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.

111. 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.

112. 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.

113. 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.

114. 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.

115. Schaack, M. M. and E. H. Marth. 1988a. Survival of *Listeria monocytogenes* in refrigerated cultured milks and yogurt. J. Food Prot. 51:848-852.

116. 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.

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

118. Schlech, W. F., III, P. M. Lavinge, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome. 1983. Epidemic listeriosis-evidence for transmission by food. N. Engl. J. Med. 308:203-206.

119. Seeliger R. P. H. and D. Jones. 1986. Genus *Listeria* Pirie, 1940, 383[AL]. pp. 1235-1245. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*. Vol. 2. The Williams and Wilkins Co., Baltimore.

120. Shankar, P. A. and F. L. Davies. 1977. A note on the suppression of *Lactobacillus bulgaricus* in media containing β-glycerophosphate and application od such media to selective isolation of *Streptococcus thermophilus* from yoghurt. J. Soc. Dairy Technol. 30:28-30.

121. Sharpe, M. E. 1979. Lactic acid bacteria in the dairy industry. J. Soc. Dairy Technol. 32:9-17.

122. Sharpe, M. E., B. G. Fewins, B. Reiter, and W. A. Cuthbert. 1965. A survey of the incidence of coagulase-positive staphylococci in market milk and cheese in England and Wales. J. Dairy Res. 32:187-192.

123. Slade, P. J., and D. L. Collins-Thompson. 1988. Incidence of *Listeria* species in Ontario raw milk. Can. Inst. Food Sci. Technol. J. 21:425-429.

124. Sofos, J. N. and F. F. Busta. 1981. Antimicrobial activity of sorbate. J. Food Prot. 44:614-622.

125. Soreels, K. M. and M. L. Speck. 1970. Inhibition of *Salmonella gallinarum* by culture filtrates of *Leuconostoc citrovorum*. J. Dairy Sci. 53:239-241.

126. Speck, M. L. 1972. Control of foodborne pathogens by starter cultures. J. Dairy Sci. 55:1019-1022.

127. Subramanian, C. S. and E. H. Marth. 1968. Growth of *Salmonella typhimurium* in acidified milks. J. Dairy Sci. 51:934.

128. Tamime, A. Y. 1990. Microbiology of starter cultures. pp. 131-201. In Robinson R. K. (ed.), *Dairy microbiology. The microbiology of milk products*. 2nd Ed., vol. 2. Elsevier Appl. Sci., N.Y.

129. Tauxe, R. V. 1991. *Salmonella*: A postmodern pathogen. J. Food Prot. 54:563-568.

130. Terzaghi, B. E. and W. E. Sandine. 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. 29:807-813.

131. Tham, W. A., L. J. Hajdu, and M. L. V. Danielsson-Tham. 1990. Bacteriological quality of on-farm manufactured goat cheese. Epidemiol. Infect. 104:87-100.

132. 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 cheeses. J. Dairy Sci. 47:604-611.

133. Turner, N., W. E. Sandine, P. R. Elliker, and E. A. Day. 1963. Use of tetrazolium dyes in an agar medium for differentiation of *Streptococcus lactis* and *Streptococcus cremoris*. J. Dairy Sci. 46:380-385.

134. Walker, G. C. and L. G. Harmon. 1965. The growth and persistence of *Staphylococcus aureus* in milk and broth substrates. J. Food Sci. 30:351-358.

135. Weed, L. A., A. C. Michael, and R. N. Harger. 1943. Fatal *Staphylococcus* intoxication from goat milk. Amer. J. Publ. Health 33:1314-1318.

136. Wehr, H. M. 1987. *Listeria monocytogenes* – A current dilemma. J. Assoc. Off. Anal. Chem. 70:769-772.

137. White, C. H. and E. W. Custer. 1976. Survival of *Salmonella* in Cheddar cheese. J. Milk Food Technol. 39:328-331.

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

139. 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.

140. Yousef, A. E. and E. H. Marth. 1990. Fate of *Listeria monocytogenes* during the manufacture and ripening of Parmesan cheese. J. Dairy Sci. 73:3351-3356.

141. Zehren, V. L. and V. F. Zehren. 1968. Examination of large quantities of cheese for staphylococcal enterotoxin A. J. Dairy Sci. 51:635-644.

CHAPTER III

EFFECT OF STARTER CULTURE AND ANTIMICROBIALS ON THE SURVIVAL OF SELECTED MICROORGANISMS IN WHITE PICKLED CHEESE

INTRODUCTION

White pickled cheese is usually made from raw milk containing 6%-20% NaCl (Alla Gabo, 1986; Khalid and Harrigan, 1984). The purpose of the high salt level is to control the indigenous microflora, particularly pathogens. However, the salt has a major influence on acid development and rennet coagulation. In order to avoid use of excessive salt, pasteurization is recommended for eliminating pathogenic bacteria (Alla Gabo, 1986; Dariani *et al.*, 1980). The behavior of several pathogenic microorganisms during manufacture and ripening of cheeses and milk products have been studied (Buazzi *et al.*, 1992; Papageorgiou and Marth, 1989a, 1989b; Rosenow and Marth, 1987; Ryser and Marth, 1987a, 1987b; Schaack and Marth, 1988a, 1988b, 1988c; Yousef and Marth, 1988). Mesophilic lactic streptococci were found to have variable effects on *Listeria monocytogenes* growth and survival depending on the level of inoculum (Schaack and Marth, 1988c). Antimicrobials such as potassium sorbate, H_2O_2, NaCl, nisin, and organic and inorganic acids have also been

evaluated for their effects on various pathogens (Dominguez *et al.*, 1987; El-Shenawy and Marth, 1988; Farber *et al.*, 1989; Jung *et al.*, 1992; Park and Marth, 1972). Various cheese varieties have been evaluated for the survival of a variety of pathogens. However, little or no information has been published on behavior of pathogenic microorganisms other than for *S. aureus* (Khalid and Harrigan, 1984) in white pickled cheese which is made in a way that could favor the growth of pathogens.

Outbreaks of listeriosis due to consumption of milk (Fleming *et al.*, 1985) and cheese (Linnan *et al.*, 1988), staphylococcal food poisoning from consumption of white pickled cheese (Khalid and Harrigan, 1981) and the fact that *Salmonella spp.* are frequently isolated from raw milk and cheese (D'Oust *et al.*, 1985; McManus and Lanier, 1987; Reilly *et al.*, 1983) encouraged the researchers to undertake this study to evaluate the effect of selected antimicrobials on *L. monocytogenes, Staphylococcus aureus* and *Salmonella typhimurium* during the manufacture and storage of white pickled cheese.

MATERIALS AND METHODS

Starter Culture

A commercial starter culture was obtained from Chr. Hansen's Laboratory Inc., Milwaukee, WI. The freeze-dried starter culture was added to heat-treated (87.8-90.6°C for 1

h) nonfat dry milk reconstituted to 12% solids, cooled to 21.1-22.2°C, and incubated at 21.1-22.2°C to a developed acidity of 0.75-0.85% titratable acidity (pH 4.95). The culture was stored at 7.2°C until used (generally within 2 d).

Bacterial Cultures

Staphylococcus aureus NRRL B-120 and *Salmonella typhimurium* NRRL B-4420 were kindly provided by Northern Regional Research Center, Agricultural Research Service, U.S.D.A., Peoria, IL. *Listeria monocytogenes* Scott A was obtained from the microbiology laboratory, Food Science and Technology Department, University of Tennessee, Knoxville. Cultures were stored frozen (-70°C in trypticase soy broth (TSB) containing 20% glycerol). When needed, the cultures were thawed and incubated in TSB at 35°C for 24 h. The initial number of cells used to inoculate milk was determined by plating in a suitable medium and incubating at 35°C for 48 h. Each culture was added to pasteurized milk at about $\log_{10}$ 4.0-5.0 colony forming units (CFU)/ml.

Treatments

Five treatments were used (Table 4). All treatments contained equivalent amounts of NaCl, $CaCl_2$ and rennet. The first treatment was the control, the second had starter culture (1% w/w), the third had H_2O_2 (0.1% v/v) (Fisher Scientific Company, Fair Lawn, N.J.), the fourth had potassium sorbate (0.1% w/w) (Sigma Chemical Company, St. Louis, MO) and the fifth had nisin (25 IU/ml) (Sigma) added.

Table 4. General outline of the additives used during the manufacture of cheese.

Additives	Treatment Number[a]				
	1	2	3	4	5
NaCl (4% w/w)	+	+	+	+	+
$CaCl_2$ (0.02% w/w)	+	+	+	+	+
Rennet (11 ml/45.5 kg milk)	+	+	+	+	+
Starter culture (1% w/w)	-	+	-	-	-
H_2O_2 (0.1% v/v)	-	-	+	-	-
Potassium sorbate (0.1% w/w)	-	-	-	+	-
Nisin (25 IU/ml)	-	-	-	-	+

[a] Treatments number 1, 2, 3, 4 and 5 are control, starter culture, hydrogen peroxide, potassium sorbate and nisin, respectively.

Manufacture of Cheese

Pasteurized milk (62.7°C for 30 min) was cooled to 37.8-40.0°C in a 5 L bucket. NaCl (4% w/w), $CaCl_2$ (0.02% w/w) and calf rennet (Chr. Hansen's Laboratory Inc., Milwaukee, WI) were added to all treatments. Rennet was diluted 1:40 in water and the whole volume was added to the milk to give a final concentration of 11 ml/45.5 kg of milk. Starter culture (1% w/w) was added to starter treatment before addition of pathogens. Potassium sorbate (0.1% w/w) and nisin (25 IU/ml) were added after pathogens were added. Hydrogen peroxide (0.1% v/v of 30% concentration) was added after addition of pathogens followed by incubation at 30°C for 30 minutes. Remaining H_2O_2 was decomposed by 0.0006% catalase. After addition of rennet (at 36°-38°C), milk was stirred and left to develop a curd. The curd was cut with a knife into small cubes. Whey was drained and curd ladled into small cylindrical metal molds (14.5 cm I.D. x 13 cm height) lined with cheesecloth and pressed lightly (627.3 kg weight) overnight. The following day, the curd was cut into small cubes (ca. 5.5 x 3.5 x 2.0 cm) and placed in sterile 4% (w/v) brine solution. Cheese was stored in brine solution at 4°C and pH of brine was determined (Fisher Accumet pH meter Model 600, Fisher Scientific, Pittsburgh, PA). Pathogens were enumerated in milk at day 0 (milk before addition of rennet), in curd just prior to addition of brine solution (day 1), and in curd and brine solution at 3, 10, 20, 30, 40, 50 and 60 d.

Enumeration of *Listeria monocytogenes*

Cheese curd (11 g) and whey (11 ml) were diluted 1:10 with sterile 0.1% peptone water, diluted serially and spread-plated on Listeria Selective Agar Base (Oxford Formulation, OXOID, Columbia, MD, supplemented with 0.1% colistin and 0.2% moxalactam, Sigma). Cheese curd was blended in a stomacher (Laboratory Blender Stomacher 400, Tekmar, Cincinnati, OH) for 2 min prior to plating. Plates were incubated at 35°C for 48 h. Typical colonies (creamy surrounded with black zone) were counted. Representative colonies (four colonies from a plate) were picked for confirmatory tests which included gram staining, tumbling motility, catalase, oxidase, umbrella motility, xylose, mannitol and rhamnose utilization (Lovett, 1988).

Enumeration of *Staphylococcus aureus*

Cheese curd (11 g) was blended in 99 ml of 0.1% sterile peptone water with a stomacher. Whey (11 ml) was mixed with 99 ml of peptone water. Serial dilutions were prepared and surface plated on Baird-Parker Complete Medium (Difco Laboratories, Detroit, MI). Plates were incubated at 35°C for 48 h. Colonies typical of *S. aureus* were counted and suspected colonies were confirmed by gram staining, catalase and coagulase tests (Tatini *et al.*, 1984).

Enumeration of *Salmonella typhimurium*

Cheese curd (11 g) or brine solution (11 ml) were inoculated into 99 ml Tetrathionate Broth Base (Difco) and

incubated at 35°C for 24 h for enrichment. Broth cultures were streaked on Brilliant Green Agar (BGA) (Difco) and incubated at 35°C for 48 h. Typical colonies (slightly pink-white opaque) were inoculated into Triple Sugar Iron Agar slants (TSI) (Difco) and incubated at 35°C for 24 h as a presumptive test. For confirmation, cultures from TSI with reactions typical for *Salmonella* were subjected to biochemical tests (catalase, oxidase, indole, urease, methyl red, Voges Proskauer and utilization of lactose, sucrose and dulcitol tests) (Poelma *et al.*, 1984).

Statistical Analyses

Data analyses were performed using Statistical Analysis System (SAS, 1990, Version 6.06, Cary, NC). Two replications were performed for each treatment. Separate models for each microorganism tested were used to determine effects of days of storage, treatment (control, starter culture, nisin, hydrogen peroxide and potassium sorbate), replication and the interaction between day and treatment. These variables were analyzed using general linear models statement to test the effect of pH of the brine solution and $\log_{10}$ (CFU)/g or ml of curd and brine solution and an LSD mean separation procedure was performed. Significance was established at $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Survival of *L. monocytogenes* in curd and brine solution

Three different antimicrobials (H_2O_2, nisin and potassium sorbate) and starter culture were added to milk prior to renneting. In general, cells were 1000 fold higher in curd at day 1 (Fig. 1) than the initial inoculum (Table 5). Thus, cells were entrapped by the curd during cheesemaking. Starter culture caused a decrease in viable *L. monocytogenes* cells in curd and brine (Figs. 1 and 2). Viable cells of the pathogen decreased with time, and after 50 d in curd and 30 d in brine solution, no viable cells were detected by direct plating. By day 3, cells were about $\log_{10}$ 4.0 CFU/ml in the brine solution (Fig. 2). Concurrently the pH of the brine solution for the starter treatment dropped to 4.7 (Fig. 3). *L. monocytogenes* showed a slight increase by the end of the storage period for all other treatments in both curd and brine solution (Figs. 1 and 2). Although the brine solution was sterile at day 1, by day 3 some cells were present in the brine solution in all treatments (Fig. 2). The pH of the brine solution showed a slight increase until day 20, then it decreased with time to between 5.5 and 6.5, depending on the treatment (Fig. 3).

None of the antimicrobials had an inhibitory effect on the pathogen. After 60 d at 4°C, starter culture treatment had the lowest count and pH values for both curd and brine solution (Table 5). There was no statistically significant

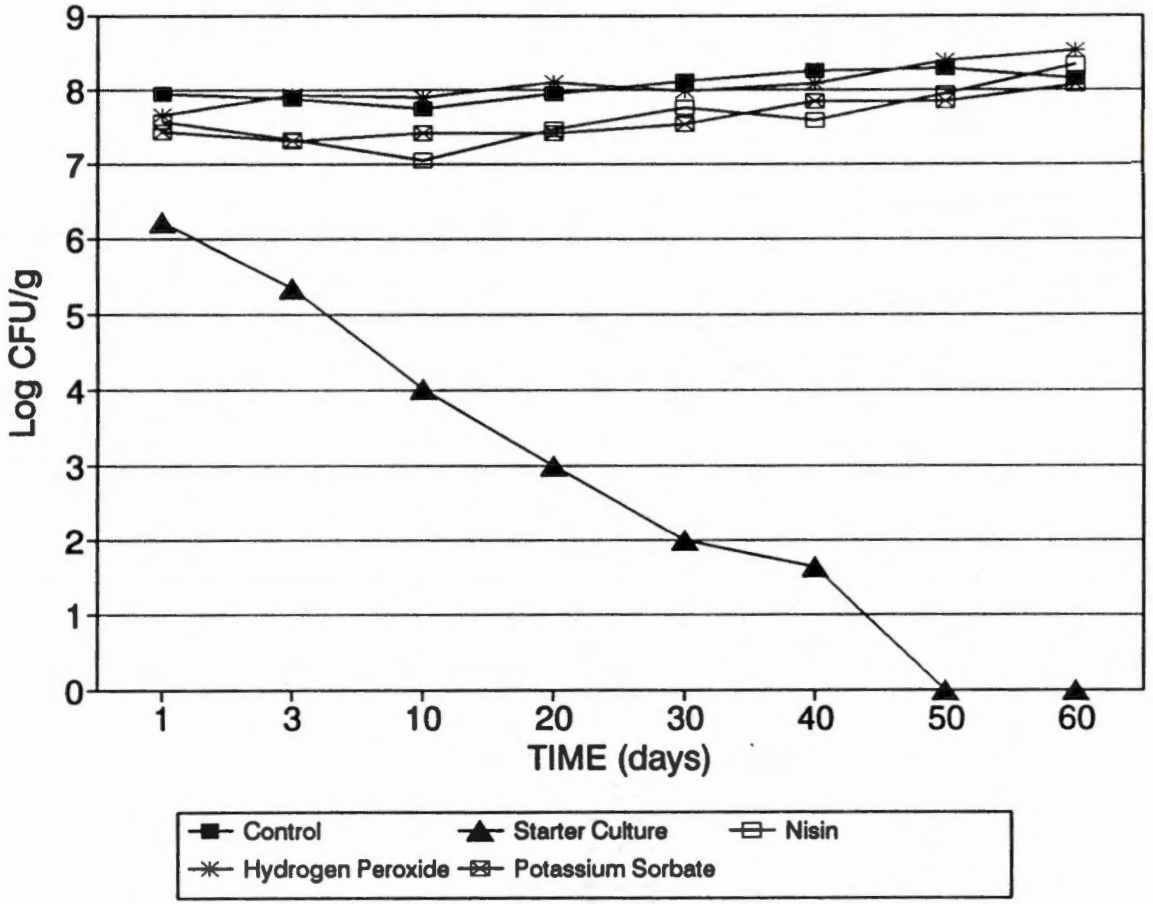


Figure 1. Behavior of *L. monocytogenes* Scott A in curd during storage of white pickled cheese at 4°C for 60 d. Cheese was made from pasteurized milk with 4% salt and preserved in 4% brine solution.

Table 5. Enumeration of *L. monocytogenes* strain Scott A in curd and brine solution of white pickled cheese made from pasteurized milk and stored at 4°C for 60 d. Values represent the mean of all storage days sampled.

Treatment	Initial inoculum[a] (Log_{10} CFU/ml)	Curd	Brine solution	
		Log_{10} CFU/g	Log_{10} CFU/ml	pH
Control	5.0	8.0^{b}	5.8^{b}	6.6^{b}
Starter culture	5.0	2.7^{d}	1.0^{d}	4.9^{c}
Hydrogen peroxide	4.9	8.0^{b}	5.8^{b}	6.6^{b}
Nisin	4.8	7.6^{c}	5.7^{bc}	6.8^{b}
Potassium sorbate	4.8	7.6^{c}	5.5^{c}	6.7^{b}

[a] Number of cells inoculated into milk prior to addition of rennet.
[b-d] Values in the same column followed by different superscripts are significantly different ($P<0.05$).

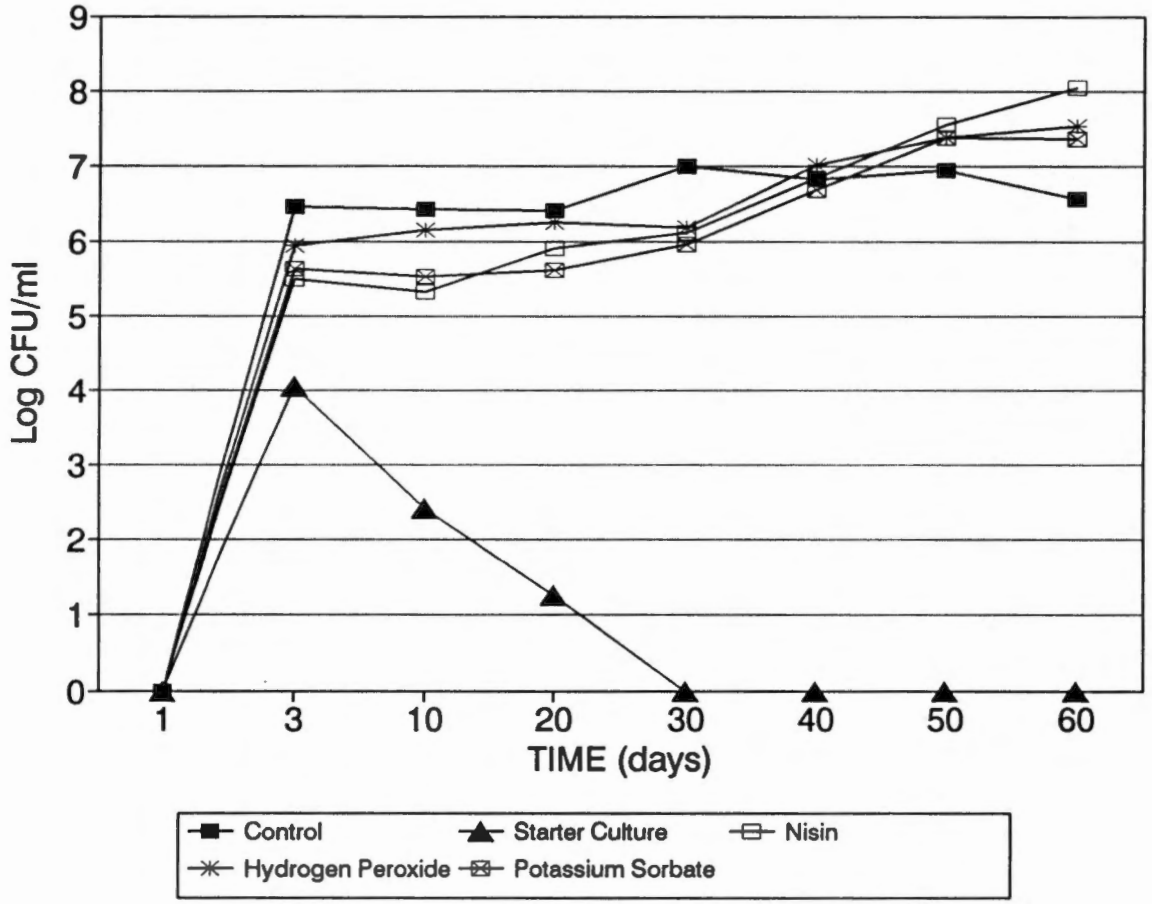


Figure 2. Behavior of *L. monocytogenes* Scott A in brine solution during storage of white pickled cheese at 4°C for 60 d.

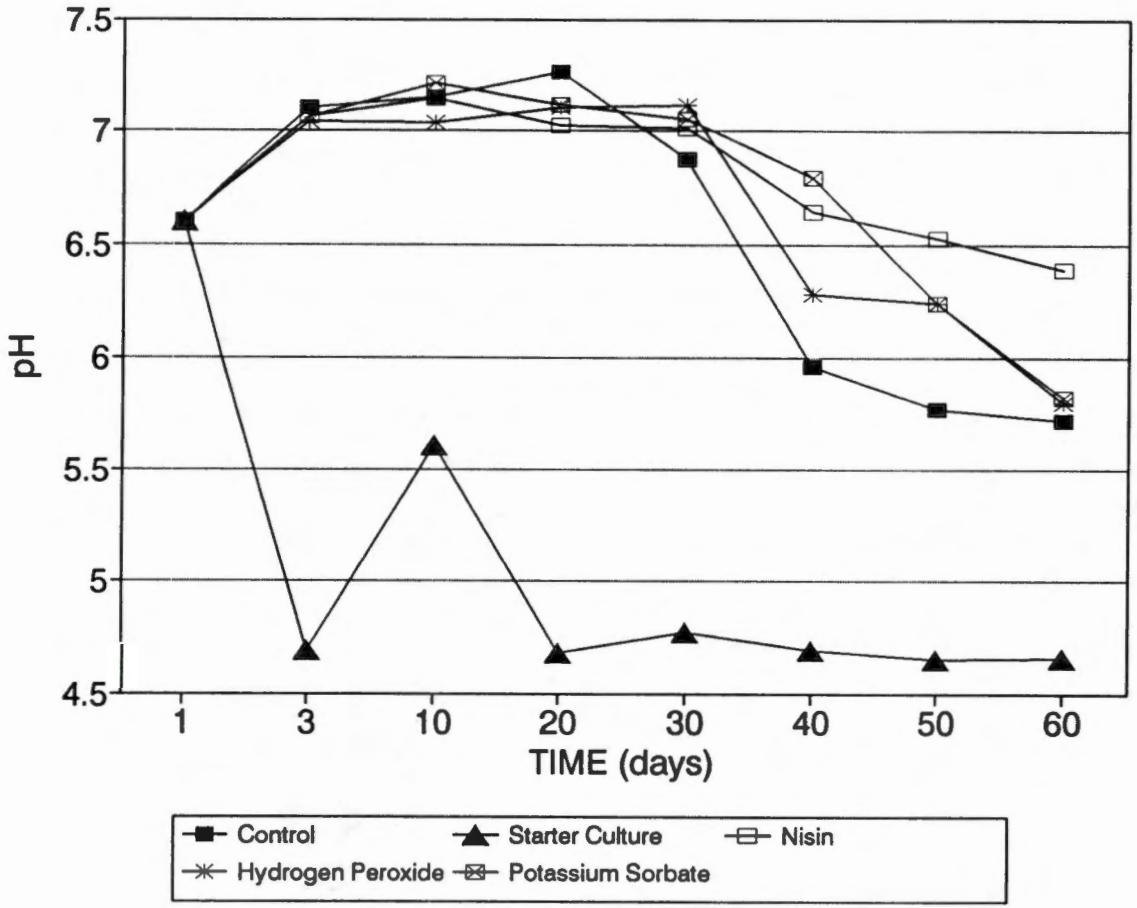


Figure 3. Change in pH of brine solution of white pickled cheese challenged with *L. monocytogenes* and stored at 4°C for 60 d.

difference between *L. monocytogenes* counts of control and hydrogen peroxide and between nisin and potassium sorbate ($P>0.05$) for either curd and brine solution (Table 5). In addition, there was no significant difference between *L. monocytogenes* count of control, hydrogen peroxide and nisin in brine solution ($P>0.05$). The pH values of all treatments other than starter culture were not significantly different ($P>0.05$) (Table 5).

Previous work indicated that *L. monocytogenes* survived for more than 90 d in Feta cheese with pH 4.3 (Papageorgiou and Marth, 1989a), more than 28 d in white brined cheese made from cow's milk (Sipka *et al.*, 1974) and for 15-60 d in white pickled cheese made from ewes' milk depending on rate of lactic acid fermentation and temperature of storage (Ikonomov and Todorov, 1964). It is clear from the present study that lowering the pH is detrimental to growth of the pathogen. Ryser *et al.* (1985) found that *L. monocytogenes* failed to grow in Cottage cheese due to lactic acid production and lowering the pH. Schaack and Marth (1988c) demonstrated that growth of *L. monocytogenes* was inhibited when the pH dropped below 4.75. In addition, because it reduced the pH of the fermented milk more, *Lactococcus lactis ssp. lactis* (5%) was more inhibitory to the pathogen than *L. lactis ssp. cremoris*. Therefore inhibition of *L. monocytogenes* by decreased pH and increased acid production was expected.

Potassium sorbate is effective against a variety of microorganisms but its effectiveness is temperature and pH dependent. El-Shenawy and Marth (1988) found that potassium sorbate completely inhibited *L. monocytogenes* at 4°C and pH 5.0. Sorbate is more effective at pH values approaching its dissociation constant (pH 4.75) where 50% is in the more effective undissociated form (Sofos and Busta, 1981). Therefore sorbate is a more effective antimicrobial in foods with low pH. In this study, since the pH was > 5.5 (Fig. 3), not much of the undissociated form existed and antimicrobial activity was decreased.

The antimicrobial effectiveness of H_2O_2 increases at higher temperatures and lower pH values (Davidson *et al.*, 1983). Catalase positive microorganisms are more resistant to hydrogen peroxide than catalase negative ones (Dominguez *et al.*, 1987). Hydrogen peroxide at concentration of 0.0495% slightly decreased the numbers of *L. monocytogenes* during the first 24 h of incubation in sterile milk, after which numbers increased, reaching counts above the initial level after 48 h (Dominguez *et al.*, 1987). In this study, although H_2O_2 was allowed to act on the bacterial cells for 30 min before decomposition by catalase, length of exposure and pH were not optimal, since pH of milk was above 6.6 (data not shown).

Like potassium sorbate, nisin is more effective against *L. monocytogenes* at pH 5.5 than pH 7.4 (Mohamed *et al.*, 1984). Nisin did not affect *L. monocytogenes* in curd or brine

solution (Figs. 1 and 2) because the pH of the cheese was not optimal for nisin activity.

Survival of *S. aureus* in curd and brine solution

S. aureus was inhibited by the starter culture (Figs. 4 and 5). In curd, populations increased slightly at day 3, after which they declined sharply. After day 30, no cells were detected by direct plating (Fig. 4). In brine solution, ca. $\log_{10}$ 3.4 CFU cell/ml were present by day 3 (Fig. 5). The population showed a sharp decrease to day 20, after which no cells were detected by direct plating (Fig. 5).

The pH of the brine solution in the starter treatment showed a sharp decrease at day 3 and remained about 4.6 through the remaining 60 d of storage. For the other treatments, there was a slight increase until day 30, after which the pH decreased slightly towards the end of the storage period (Fig. 6).

In curd, the cheese made with starter culture had the lowest mean $\log_{10}$ CFU *S. aureus*/g over time. Cheese curd made with all other treatments had *S. aureus* count in excess of $\log_{10}$ 7.0 CFU/g (Table 6). The brine solution from the treatment with starter culture also had the lowest mean *S. aureus*/ml and the lowest mean pH. Nisin, potassium sorbate, control and H_2O_2 all exceeded $\log_{10}$ 4.0 CFU/ml and had similar pH values ($P>0.05$).

S. aureus survived more than 14 d in cheese made with sterilized milk containing 8.5% NaCl and stored in varying

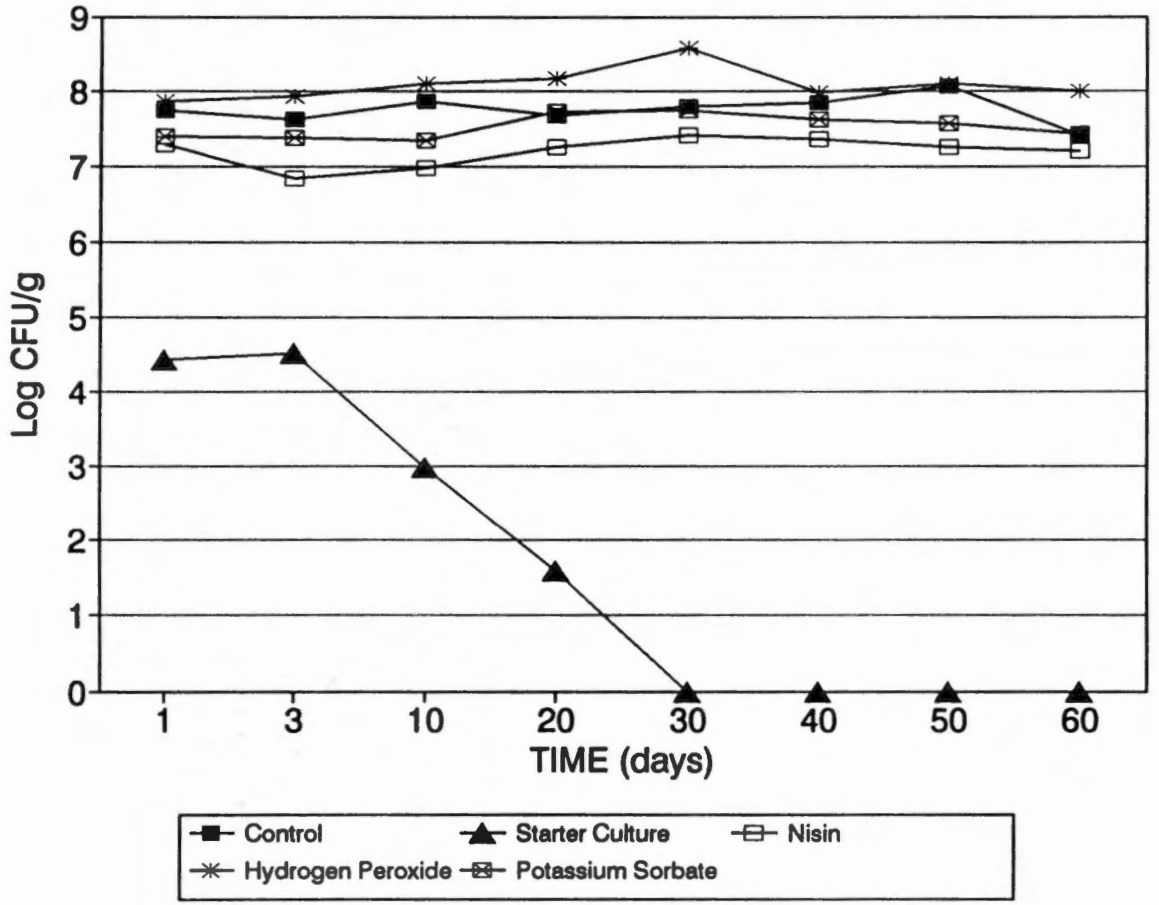


Figure 4. Behavior of *S. aureus* NRRL B-120 in curd of white pickled cheese made from pasteurized milk with 4% salt and stored in 4% brine solution at 4°C for 60 d.

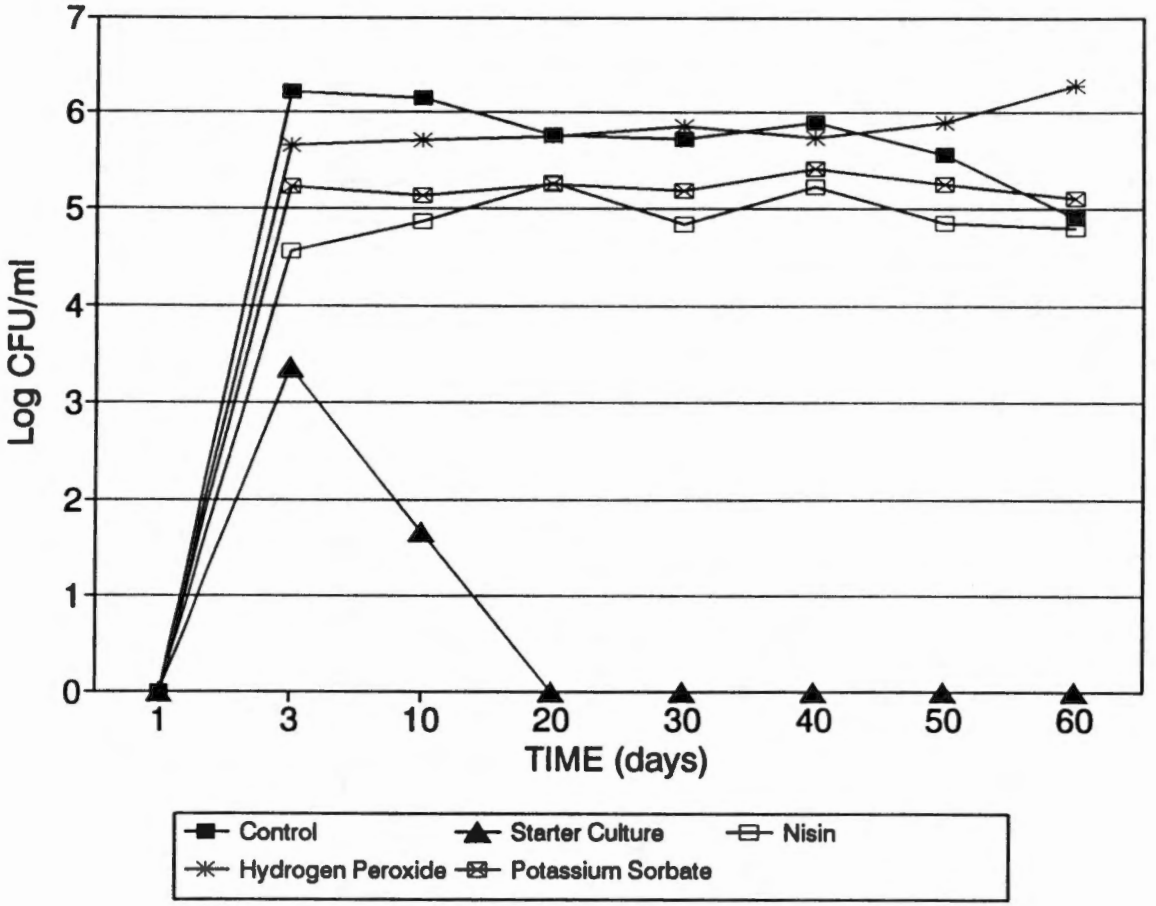


Figure 5. Survival of *S. aureus* NRRL B-120 in brine solution of white pickled cheese made from pasteurized milk and stored at 4°C for 60 d.

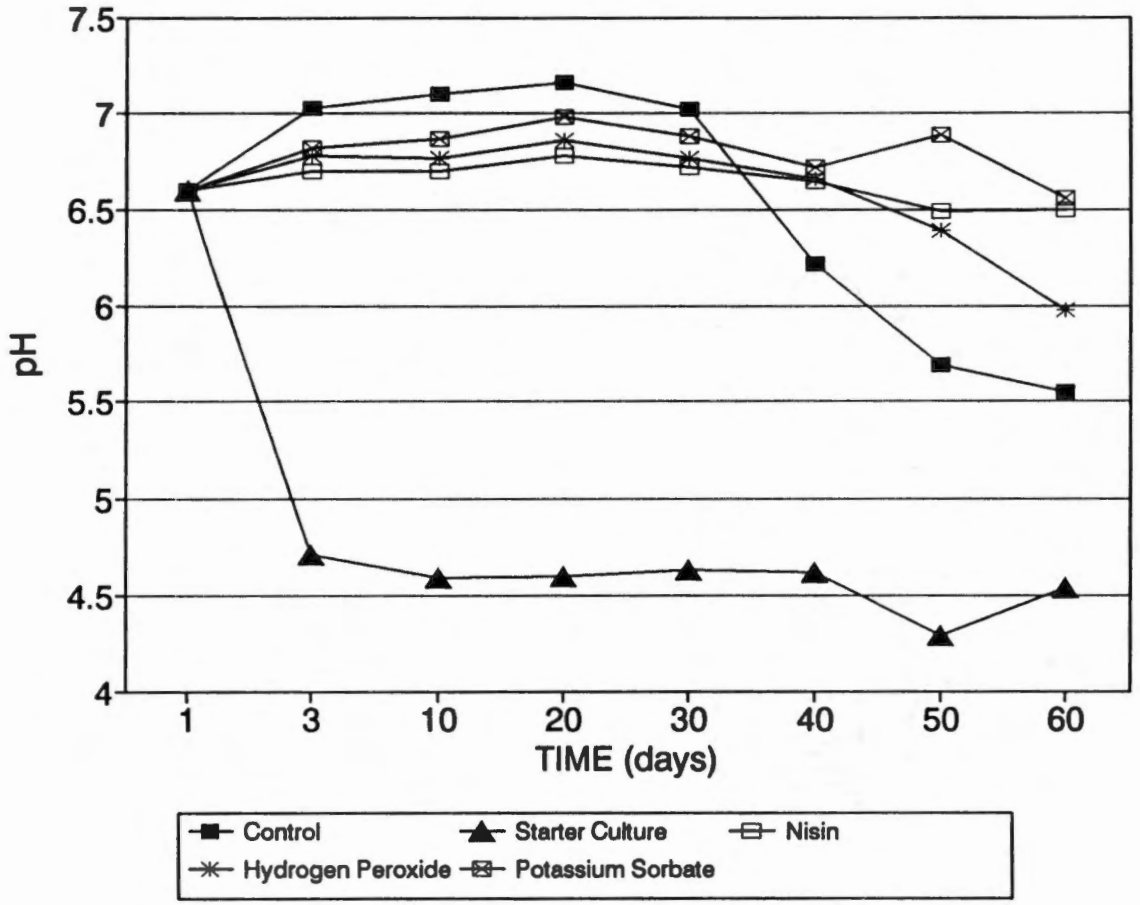


Figure 6. Change in the pH of brine solution of white pickled cheese made from pasteurized milk inoculated with *S. aureus* cells and stored in 4% brine solution at 4°C for 60 d.

Table 6. Mean[a] log_{10} of *S. aureus* NRRL B-120 and pH values of curd and brine solution for white pickled cheese made from pasteurized milk and stored at 4°C for 60 d.

Treatment	Initial inoculum[b] (Log_{10} CFU/ml)	Curd	Brine Solution	
		Log_{10} CFU/g	Log_{10} CFU/ml	pH
Control	4.3	7.7^{d}	5.0^{c}	6.5^{c}
Starter culture	4.3	1.6^{f}	0.6^{e}	4.8^{d}
Hydrogen peroxide	4.4	8.1^{c}	5.1^{c}	6.6^{c}
Nisin	4.1	7.2^{e}	4.3^{d}	6.6^{c}
Potassium sorbate	4.4	7.5^{d}	4.6^{d}	6.8^{c}

[a] Log_{10} and pH values are mean of all sampling days.
[b] Initial numbers of cells inoculated into milk prior to addition of rennet.
[c-f] Values within columns followed by different superscripts are significantly different ($P<0.05$).

concentrations of brine solution (Khalid and Harrigan, 1984). The microorganism survived more than 28 d in Brazilian Minas cheese containing starter culture made from raw or pasteurized milk (Clemente dos Santos and Genigeorgis, 1981). It also survived more than 28 d in Domiati cheese made with raw milk containing various salt levels and stored in 15% salted whey (Ahmed *et al.*, 1983). In this study, lactic acid bacteria were able to grow and produce acid that inhibited growth. Nunez *et al.* (1986) reported *S. aureus* inhibition in milk and cheese was generally due to reduced pH, but other factors such as temperature and length of storage might have been involved. This assumption was further supported by the findings of Ibrahim *et al.* (1981). They reported that, during Cheddar cheesemaking, enterotoxin was detected at the end of cheddaring in batches made with large initial inocula of *S. aureus* and/or low starter culture activity. At the end of pressing, *S. aureus* count, pH and enterotoxin concentration in the unsalted cheese were significantly lower than in salted cheese (Ibrahim *et al.*, 1981). This was attributed to the adverse effect of salt on microorganisms other than *S. aureus,* particularly lactic streptococci. Inhibition of *S. aureus* not only depends on pH but also on the type of acid added or produced. For example, Daly *et al.* (1971) reported *S. aureus* inhibition of 88%, 82%, 78% or 0% by formic, acetic, lactic or hydrochloric acid in lactic broth at pH 4.5.

In the present study, H_2O_2 had no effect on *S. aureus* survival. This disagrees with results of Naguib (1972) who found inhibition of *S. aureus* when H_2O_2 at 0.1 and 0.15% was added to raw or pasteurized milk prior to making Domiati cheese. The difference may be due to the concentration of H_2O_2 used. Naguib (1972) used 40% while 30% H_2O_2 was used in this study. In another study, Abdalla and Davidson (1990) found that H_2O_2 added to brine solution was effective when combined with starter culture, while H_2O_2 alone did not inhibit the growth of *S. aureus*. In a study of the effect of H_2O_2 on *L. monocytogenes* and *S. aureus*, Dominguez *et al.* (1987) found that *S. aureus* was initially more sensitive than *L. monocytogenes* to concentration of 0.0495% (D-value = 11.9 h). However, after 16 h *S. aureus* recovered, reaching levels similar to the original inoculum after 48 h.

No effect of potassium sorbate on *S. aureus* was observed in the present study. Similar results were obtained when potassium sorbate was incorporated into brine solution in which white pickled cheese was preserved (Abdalla and Davidson, 1990). Potassium sorbate activity increases as pH is decreased. For example, Parada *et al.* (1982) found that 0.25% potassium sorbate at pH 6.0 in synthetic media had no effect on *S. aureus*. If pH was lowered to 5.5, maximum inhibition was achieved. LaRocco and Martin (1987) reported that in synthetic medium adjusted to pH 6.3, potassium sorbate

at 0.2 or 0.3% had no effect on *S. aureus* incubated at 35°C for 48 h or 22°C for 15 d.

In the present study, nisin had no effect on *S. aureus* growth whether in curd or brine solution. This result is in agreement with the findings of Abdalla and Davidson (1990) who reported that, while nisin alone added to brine solution had no effect on *S. aureus*, it inhibited the microorganism slightly when combined with starter culture.

Survival of *S. typhimurium* in curd and brine solution

With the exception of the starter culture treatment, *S. typhimurium* was detected throughout the storage period in both curd and brine solution (Tables 7 and 8). In the starter culture treatment, *S. typhimurium* was detected up to day 20, but was not detected from day 30 (Table 7). In brine solution, no *S. typhimurium* cells were detected from day 3 (Table 8). The starter culture decreased pH rapidly in the brine solution from 6.6 at day 1 to 4.7 toward the end of storage period (Fig. 7). The pH of H_2O_2 and potassium sorbate treated samples dropped slightly below 6.6 at the end of the storage period while the pH of control and nisin were not changed (Fig. 7).

The inhibition of *S. typhimurium by starter culture* is in agreement with the finding of Hargrove *et al.* (1969). They found the rate and amount of acid produced, pH of the cheese and the size and type of starter inoculum influenced the growth and survival of salmonellae. However, growth of

Table 7. Isolation and identification of *S. typhimurium* NRRL B-4420 in curd of white pickled cheese made from pasteurized milk and stored at 4°C for 60 d.

Time (days)	Treatments				
	Control	Starter culture	Hydrogen peroxide	Potassium sorbate	Nisin
0	4.3[a]	4.7[a]	4.7[a]	4.5[a]	4.7[a]
1	+	+	+	+	+
3	+	+	+	+	+
10	+	+	+	+	+
20	+	+	+	+	+
30	+	-	+	+	+
40	+	-	+	+	+
50	+	-	+	+	+
60	+	-	+	+	+

[a] Initial number of cells inoculated into milk before addition of rennet.
+, Positive, cells were recovered by enrichment and identification.
-, Negative, no cells were recovered.

Table 8. Isolation and identification of *S. typhimurium* NRRL B-4420 and pH values in brine solution used to preserve cheese curd and stored at 4°C for 60 d during the study of the behavior of the pathogen in white pickled cheese.

Time (days)	Treatments				
	Control	Starter culture	Hydrogen peroxide	Potassium sorbate	Nisin
1	0[a]	0[a]	0[a]	0[a]	0[a]
3	+	-	+	+	+
10	+	-	+	+	+
20	+	-	+	+	+
30	+	-	+	+	+
40	+	-	+	+	+
50	+	-	+	+	+
60	+	-	+	+	+
pH[b]	6.8[c]	4.8[e]	6.6[d]	6.8[c]	6.8[c]

[a] At day 1 cheese was preserved into sterile 4% NaCl brine solution.
[b] Mean pH values over all days tested.
[c-e] Values within the same row followed by different superscripts are significantly different ($P<0.05$).
+ and -, for symbols see table 6.

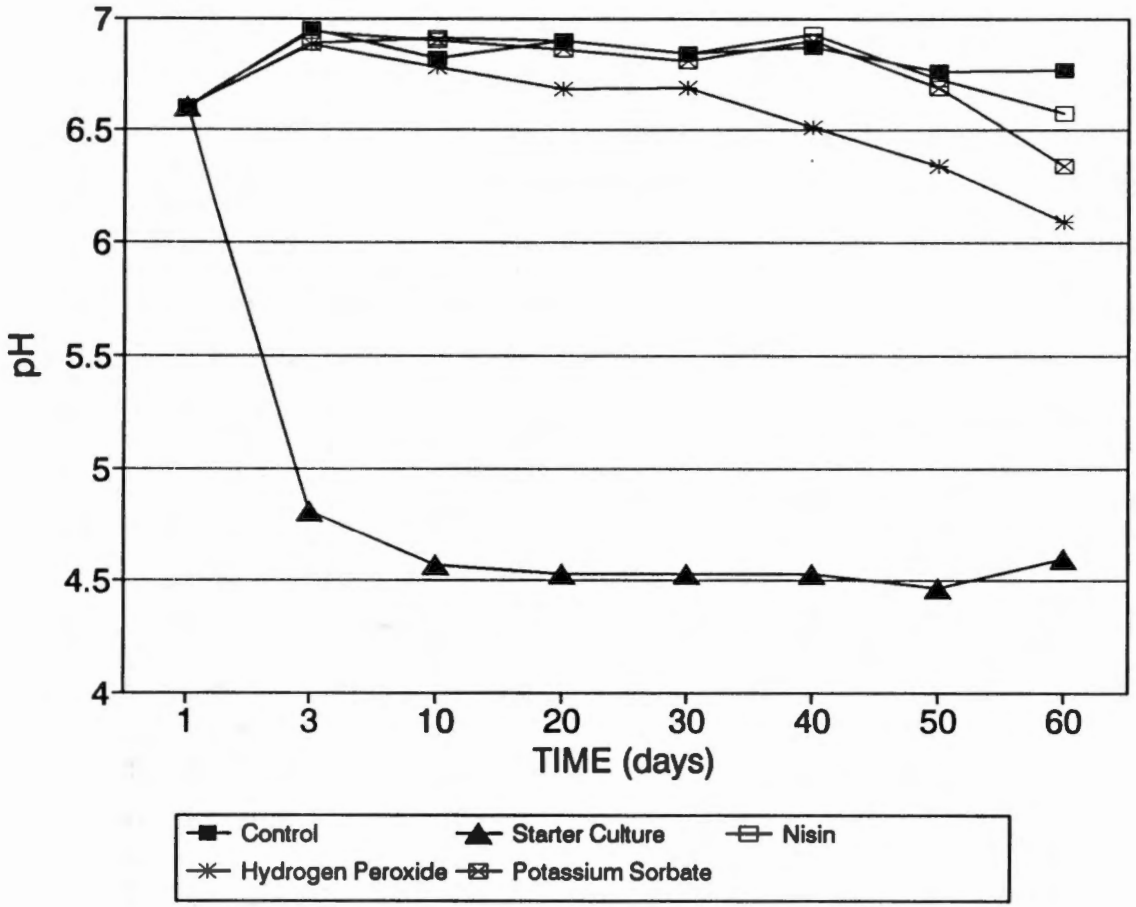


Figure 7. Change in pH of brine solution during storage of white pickled cheese at 4°C for 60 d. Cheese was made from pasteurized milk with 4% salt and preserved in 4% brine solution. Cheese was analyzed for the survival of *S typhimurium* NRRL B-4420.

salmonellae was found to be more dependent on the type of acid than on pH (Chung and Geopfert, 1970). The inhibitory effectiveness of lactic acid bacteria against *Salmonella spp.* is dependent on the bacterial strain (Park and Marth, 1972).

Potassium sorbate did not affect *S. typhimurium* in curd or brine solution probably because its activity decreases as pH is increased (Sofos and Busta, 1981). LaRocco and Martin (1981) reported that a combination of NaCl and potassium sorbate in trypticase soy broth adjusted to pH 6.3, was inhibitory to *S. typhimurium*. The most inhibitory combination was 0.3% sorbate plus 3% NaCl (LaRocco and Martin, 1981).

Inhibition of *S. typhimurium* by nisin was not achieved as the pathogen was recovered from curd and brine throughout the study period. This was expected since nisin has not been reported to be effective against gram negative bacteria, yeast or molds (Jung *et at.*, 1992). Stevens *et al.* (1991) also showed that nisin alone did not inhibit the growth of *Salmonella spp.* or other gram negative bacteria in buffer. However, simultaneous exposure to nisin and EDTA in brain heart infusion broth (BHI) caused inhibition of all *Salmonella spp.* tested (Stevens *et al.*, 1991). In another study, Stevens *et al.* (1992) concluded that the inhibitory activity of nisin could be extended to gram negative bacteria by altering the outer cell membrane by mutation or by chemical treatment with the chelating agent EDTA.

In conclusion, the use of starter culture is extremely important to safe production of white pickled cheese. The traditional way of manufacturing cheese is not considered a safe practice since foodborne pathogens can grow in starter-free cheese. The use of antimicrobials did not control the pathogens since the pH of the product was above 5.5. To activate the antimicrobials, pH of the product could be reduced either by addition of acid or starter culture. However, because some starter culture bacteria are sensitive to antimicrobials, there is probably no need to add antimicrobials. The starter culture alone was shown to be capable of inhibiting all pathogens tested when stored at 4°C for for at least.

REFERENCES

1. Abdalla, O. M. and P. M. Davidson. 1990. Effect of starter culture and antimicrobials on the growth of *Staphylococcus aureus* in Gebna Beyda cheese. Institute of Food Technologists' Annual Meeting, June 16-20, 1990, Anaheim, California.

2. Ahmed, A. A. H., M. K. Moustafa, and E. H. Marth. 1983. Growth and survival of *Staphylococcus aureus* in Egyptian Domiati cheese. J. Food Prot. 46:412-415.

3. Alla Gabo, I. H. 1986. *Studies on the composition and quality of Gibna Beyda*. M.Sc. Thesis, University of Khartoum, Khartoum, Sudan.

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

5. Chung, K. C. and J. M. Geopfert. 1970. Growth of *Salmonella* at low pH. J. Food Sci. 35:326-328.

6. Clemente dos Santos, E. and C. Genigeorgis. 1981. Survival and growth of *Staphylococcus aureus* in commercially manufactured Brazilian Minas cheese. J. Food Prot. 44:177-184.

7. Daly, C., W. E. Sandine, and P. R. Elliker. 1971. Influence of pH on inhibition of *Staphylococcus aureus* by *Streptococcus diacetilactis*. J. Dairy Sci. 54:755.

8. Dariani, N.D., K. M. Galal, S. J. Speck, and M. Loewenstein. 1980. I. Manufacturing soft pickled cheese using cow and goat milk. Dairy Field 163(6):81-84.

9. Davidson, P. M., L. S. Post, A. L. Branen, and A. R. McCurdy. 1983. Naturally occurring and miscellaneous food antimicrobials. pp. 371-419. In Branen, A. L. and P. M. Davidson (ed.), *Antimicrobials in foods*. Marcel Dekker, Inc., New York.

10. Dominguez, L., J. F. F. Garayazabal, E. R. Ferri, J. A. Vazquez, E. Gomezlucia, C. Ambrosio, and G. Suarez. 1987. Viability of *Listeria monocytogenes* in milk treated with hydrogen peroxide. J. Food Prot. 50:636-639.

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

12. El-Shenawy, M. A. and E. H. Marth. 1988. Inhibition and inactivation of *Listeria monocytogenes* by sorbic acid. J. Food Prot. 51:842-847.

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

14. Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. 312:404-407.

15. 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.

16. Ibrahim, G. F., A. K. Baldock, D. R. Bradford, and L. B. Ireland. 1981. Inhibition of *Staphylococcus aureus* growth and enterotoxin A production in Cheddar cheese produced with variable starter activity. J. Food Prot. 44:263-267.

17. Ikonomov, L. and D. Todorov. 1964. Studies of the viability of *Listeria monocytogenes* in ewes' milk and dairy products. Vet. Med. Nauki, Sofiya 1(7) 23-29. [In Dairy Sci. Abstr. (1965) 27:571].

18. Jung, D., F. W. Bodyfelt, and M. A. Daeschel. 1992. Influence of fat and emulsions on the efficacy of nisin in inhibiting *Listeria monocytogenes* in fluid milk. J. Dairy Sci. 75:387-393.

19. Khalid, A. S. and W. F. Harrigan. 1981. Incidence of food poisoning in the Sudan with special reference to *Staphylococcus aureus* in milk and white cheese (Jibna Baida). J. Appl. Bacteriol. 51:23.

20. Khalid, S. A. and F. W. Harrigan. 1984. Investigation into the effect of salt level on growth of, and toxin production by *Staphylococcus aureus* in Sudanese cheese. Lebensm-Wiss Technol. 17:99-103.

21. LaRocco, K. A. and S. E. Martin. 1981. Effects of potassium sorbate alone and in combination with sodium chloride on growth of *Salmonella typhimurium* 7136. J. Food Sci. 46:568-570.

22. LaRocco, K. A. and S. E. Martin. 1987. Effects of potassium sorbate alone and in combination with sodium chloride on growth of *Staphylococcus aureus* MF-31. J. Food Prot. 50:750-752.

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

24. Lovett, J. 1988. Isolation and identification of *Listeria monocytogenes* in dairy products. Assoc. Off. Anal. Chem. J. 71:658-660.

25. McManus, C. and J. M. Lanier. 1987. *Salmonella, Campylobacter jejuni* and *Yersinia enterocolitica* in raw milk. J. Food Prot. 50:51-55.

26. Mohamed, G. E., A. Seaman, and M. Woodbine. 1984. Food antibiotic nisin: comparative effects on *Erysipelothrix* and *Listeria*. pp. 435-442. In Woodbine, M. (ed), *Antimicrobials and agriculture*. Butterworth, London, England.

27. Naguib, K. 1972. Hydrogen peroxide treatment of milk for Domiati cheese. Aust. J. Dairy Tech. 27:56-60.

28. Nunez, M., F. J. Chavarri, B. E. Garcia, and L. E. Gaytan. 1986. The effect of lactic starter inoculation and storage temperature on the behavior of *Staphylococcus aureus* and *Enterobacter cloacae* in Burgos cheese. Food Microbiol. 3:235-242.

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

30. 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.

31. Parada, J. L., J. Chirife, and R. C. Magrini. 1982. Effect of BHA, BHT and potassium sorbate on growth of *Staphylococcus aureus* in a model system and process cheese. J. Food Prot. 45:1108-1111.

32. Park, H. S. and E. H. Marth. 1972. Behavior of *Salmonella typhimurium* in skim milk during fermentation by lactic acid bacteria. J. Milk Food Technol. 35:482-488.

33. Poelma, P. L., W. H. Andrews, and J. H. Silliker. 1984. *Salmonella*. pp. 286-326. In Speck, M. L. (ed.), *Compendium of methods for the microbiological examination of foods*. 2nd ed. American Public Health Association, Washington, DC.

34. Reilly, W. J., J. C. M. Sharp, G. I. Forbes, and G. M. Paterson. 1983. Milkborne salmonellosis in Scotland 1980 to 1982. Vet. Rec. 112:578-580.

35. Rosenow, E. M. and E. H. Marth. 1987. Growth of *Listeria monocytogenes* in skim, whole and chocolate milk, and in whipping cream during incubation at 4, 8, 13, 21 and 35°C. J. Food Prot. 50:452-459.

36. 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.

37. 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.

38. 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.

39. Schaack, M. M. and E. H. Marth. 1988a. Survival of *Listeria monocytogenes* in refrigerated cultured milks and yogurt. J. Food Prot. 51:848-852.

40. 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.

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

42. Sipka, M., S. Zakula, I. Kovincic, and B. Stajner. 1974. Secretion of *Listeria monocytogenes* in cow's milk and its survival in white brined cheese. XIX International Dairy Congr. 1E, 157. [In Food Sci. Technol. Abstr. (1975) 7:4P 642].

43. Sofos, J. N. and F. F. Busta. 1981. Antimicrobial activity of sorbate. J. Food Prot. 44:614-622.

44. Stevens, K. A., B. W. Sheldon, N. A. Klapes, and T. R. Klaenhammer. 1991. Nisin treatment for inactivation of *Salmonella* species and other gram-negative bacteria. Appl. Environ. Microbiol. 57:3613-3615.

45. Stevens, K. A., N. A. Klapes, B. W. Sheldon, and T. R. Klaenhammer. 1992. Antimicrobial action of nisin against *Salmonella typhimurium* lipopolysaccharide mutants. Appl. Environ. Microbiol. 58:1786-1788.

46. Tatini, S. R., D. G. Hoover, and R. V. F. Lachica. 1984. Methods for the isolation and enumeration of *Staphylococcus aureus*. pp. 411-427. In Speck, M. L. (ed.), *Compendium of methods for the microbiological examination of foods*. 2nd ed. American Public Health Association, Washington, DC.

47. 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.

APPENDIX

Appendix A: Results of analysis of variance for $\log_{10}$ CFU/g in cheese curd during the study of the behavior of *L. monocytogenes* in white pickled cheese.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	40	420.71005	10.51775	93.07	0.0001
Error	39	4.40744	0.11301		
Corrected Total	79	425.11749			

R-Square	C.V.	Root MSE	LOG Mean
0.989632	4.935261	0.3362	6.8116

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.04371	0.04371	0.39	0.5376
TREAT	4	339.08307	84.77077	750.11	0.0001
DAY	7	6.35362	0.90766	8.03	0.0001
TREAT*DAY	28	75.22965	2.68677	23.77	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.04371	0.04371	0.39	0.5376
TREAT	4	339.08307	84.77077	750.11	0.0001
DAY	7	6.35362	0.90766	8.03	0.0001
TREAT*DAY	28	75.22965	2.68677	23.77	0.0001

Appendix B: Results of analysis of variance for pH in brine solution during the study of the behavior of *L. monocytogenes* in white pickled cheese.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	40	61.520650	1.538016	7.57	0.0001
Error	39	7.926939	0.203255		
Corrected Total	79	69.447589			

R-Square	C.V.	Root MSE	pH Mean
0.885857	7.129704	0.4508	6.3234

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	1.242511	1.242511	6.11	0.0179
TREAT	4	40.139757	10.034939	49.37	0.0001
DAY	7	10.481839	1.497406	7.37	0.0001
TREAT*DAY	28	9.656543	0.344877	1.70	0.0630

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	1.242511	1.242511	6.11	0.0179
TREAT	4	40.139757	10.034939	49.37	0.0001
DAY	7	10.481839	1.497406	7.37	0.0001
TREAT*DAY	28	9.656543	0.344877	1.70	0.0630

Appendix C: Results of analysis of variance for $\log_{10}$ CFU/ml in brine solution during the study of the behavior of *L. monocytogenes* in white pickled cheese.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	40	636.53847	15.91346	182.00	0.0001
Error	39	3.40995	0.08743		
Corrected Total	79	639.94842			

R-Square	C.V.	Root MSE	LOG Mean
0.994672	6.201461	0.2957	4.7681

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.20100	0.20100	2.30	0.1375
TREAT	4	280.56881	70.14220	802.22	0.0001
DAY	7	267.31081	38.18726	436.75	0.0001
TREAT*DAY	28	88.45785	3.15921	36.13	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.20100	0.20100	2.30	0.1375
TREAT	4	280.56881	70.14220	802.22	0.0001
DAY	7	267.31081	38.18726	436.75	0.0001
TREAT*DAY	28	88.45785	3.15921	36.13	0.0001

Appendix D: Results of analysis of variance for $\log_{10}$ CFU/g in cheese curd during the study of the behavior of *S. aureus* in white pickled cheese.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	40	529.63799	13.24095	126.35	0.0001
Error	39	4.08700	0.10479		
Corrected Total	79	533.72500			

R-Square	C.V.	Root MSE	LOG Mean
0.992342	5.032770	0.3237	6.4322

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	1.10450	1.10450	10.54	0.0024
TREAT	4	468.75104	117.18776	1118.26	0.0001
DAY	7	8.11900	1.15986	11.07	0.0001
TREAT*DAY	28	51.66345	1.84512	17.61	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	1.10450	1.10450	10.54	0.0024
TREAT	4	468.75104	117.18776	1118.26	0.0001
DAY	7	8.11900	1.15986	11.07	0.0001
TREAT*DAY	28	51.66345	1.84512	17.61	0.0001

Appendix E: Results of analysis of variance for pH in brine solution during the study of the behavior of *S. aureus* in white pickled cheese.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	40	59.994620	1.499865	11.04	0.0001
Error	39	5.296455	0.135807		
Corrected Total	79	65.291075			

R-Square	C.V.	Root MSE	pH Mean
0.918879	5.869311	0.3685	6.2787

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	1.940645	1.940645	14.29	0.0005
TREAT	4	43.019075	10.754769	79.19	0.0001
DAY	7	5.155375	0.736482	5.42	0.0002
TREAT*DAY	28	9.879525	0.352840	2.60	0.0030

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	1.940645	1.940645	14.29	0.0005
TREAT	4	43.019075	10.754769	79.19	0.0001
DAY	7	5.155375	0.736482	5.42	0.0002
TREAT*DAY	28	9.879525	0.352840	2.60	0.0030

Appendix F: Results of analysis of variance for $\log_{10}$ CFU/ml in brine solution during the study of the behavior of *S. aureus* in white pickled cheese.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	40	462.94211	11.57355	57.69	0.0001
Error	39	7.82408	0.20062		
Corrected Total	79	470.76620			

R-Square	C.V.	Root MSE	LOG Mean
0.983380	11.44728	0.4479	3.9127

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.60552	0.60552	3.02	0.0902
TREAT	4	231.06103	57.76526	287.94	0.0001
DAY	7	179.16689	25.59527	127.58	0.0001
TREAT*DAY	28	52.10867	1.86102	9.28	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.60552	0.60552	3.02	0.0902
TREAT	4	231.06103	57.76526	287.94	0.0001
DAY	7	179.16689	25.59527	127.58	0.0001
TREAT*DAY	28	52.10867	1.86102	9.28	0.0001

Appendix G: Results of analysis of variance for pH in brine solution during the study of the behavior of *S. typhimurium* in white pickled cheese.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	40	56.290745	1.407269	66.40	0.0001
Error	39	0.826575	0.021194		
Corrected Total	79	57.117320			

R-Square	C.V.	Root MSE	pH Mean
0.985528	2.292275	0.1456	6.3510

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.036125	0.036125	1.70	0.1994
TREAT	4	46.973170	11.743292	554.08	0.0001
DAY	7	1.878240	0.268320	12.66	0.0001
TREAT*DAY	28	7.403210	0.264400	12.48	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.036125	0.036125	1.70	0.1994
TREAT	4	46.973170	11.743292	554.08	0.0001
DAY	7	1.878240	0.268320	12.66	0.0001
TREAT*DAY	28	7.403210	0.264400	12.48	0.0001

CHAPTER IV

DEVELOPMENT OF SALT-RESISTANT MUTANTS OF LACTIC STREPTOCOCCI

INTRODUCTION

White pickled cheese is usually stored under warm atmospheric conditions, causing the quality to rapidly deteriorate and the shelf-life to be limited (Fahmi and Sharara, 1950). Salt at high levels (6-20%) (Alla Gabo, 1986; Khalid and Harrigan, 1984) is added as a preservative and to prevent the formation of gas holes by undesirable microorganisms. Addition of salt to cheese has advantages: it suppresses the growth of undesirable bacteria, controls the rate of ripening by controlling growth of desirable bacteria, assists the physicochemical changes in the curd giving flavor to cheese and increases yield (Dariani *et al.*, 1980). However, high levels of salt limit the growth of naturally occurring lactic acid bacteria (Khalid and Harrigan, 1984) and causes the curd to be fragile (Abd El-Salam, 1987).

Lactic acid bacteria play an important role in the dairy industry and are responsible for producing mainly lactic acid and imparting fresh acid flavor to fermented milks (Sharpe, 1979). Lactic acid bacteria also assist in curd coagulation and texture formation during cheesemaking (Sharpe, 1979).

Cells of lactic acid bacteria resistant to 6-7% salt were produced by Karlikanova *et al.* (1978). It was, however an adaptation lasting over 200 days rather than a mutation. Strains of *Lactococcus lactis ssp. lactis, L. lactis ssp. cremoris, L. lactis ssp. diacetilactis* and *Leuconostoc dextranicum* were isolated and incorporated into starter for pickled cheese. The use of the salt-adapted cells (6-7%) ensured normal development of the microbiological processes during ripening and improved the flavor of the cheese. Since this adaptation was not permanent, further research is necessary to develop such cells.

Mesophilic lactic acid bacteria can tolerate up to 4% salt (Garvie and Farrow, 1982; Mundt, 1986), and since 6-20% salt is usually added to white pickled cheese, this research was undertaken to develop mutants that could grow and produce acid under high salt conditions.

MATERIALS AND METHODS

Media

Instant nonfat dry milk (NDM) reconstituted at 11% solids was used for culture growth. M17 broth (Terzaghi and Sandine, 1975) (BBL, Cockeysville, MD) was used for culture growth and maintenance. Plate counts were made on M17 agar plates and incubated under anaerobic conditions (BBL gas pack system, Becton Dickinson and Co., Cockeysville, MD). Sterile peptone water (0.1%) was used for serial dilution of cultures during

plating. Sterile phosphate buffer (0.1 M, pH 6.0) was used for culture washing and suspension during and after mutation and used for mutant isolation.

Isolation and Identification of Lactic Streptococci

Frozen (-70°C) starter culture (Chr. Hansen's Laboratory Inc., Milwaukee, WI) was thawed. One milliliter was transferred to 10 ml sterile NDM and incubated at 30°C for 24 h. One milliliter was transferred to 10 ml M17 broth and incubated at 30°C for 24 h. The culture was serially diluted and spread-plated on prepoured M17 agar plates and incubated at 30°C for 48 h. Colonies were picked and streaked on M17 agar plates and incubated at 30°C for 48 h. The streaking was repeated three times to isolate a pure culture. A single colony verified to be *Lactococcus* by gram staining and catalase tests, was picked and grown in M17 broth and on an agar slant. The organism was further verified by gram staining, catalase and oxidase tests and growth at 10°C, 40°C and 45°C, and in 2%, 4% and 6.5% NaCl. Biochemical tests were completed as described by Garvie and Farrow (1982). Cultures were maintained frozen (-70°C) in 20% glycerol. When needed, they were thawed and incubated in M17 broth for 24 h.

Chemicals

N-methyl-N'-nitro-N-nitrosoguanidine (Sigma Chemical Co., St. Louis, MO) was used for mutagenesis. A fresh solution in distilled water was made for each use and filter sterilized (0.45 μm pore size, Gelman Sciences, Ann Arbor, MI). Sodium

chloride (4%, 8%, 10%, 12%, and 15%, w/v) was added to broth and agar.

Mutant Isolation

A modification of the procedure reported by Khosravi and Sandine (1987) was used. A 1% (v/v) inoculum from a 24 h culture in NDM was transferred to 20 ml M17 broth and incubated at 30°C for 18 h. The culture was centrifuged at 10,000 x g for 10 min, washed with phosphate buffer and diluted to 20 ml with the buffer. Five milliliters were transferred to a sterile 250 ml Erlenmeyer flask and diluted to 10 ml with buffer. A portion was plated on M17 agar for total count (before exposure). Nitrosoguanidine was made into solution and added to the resuspended cells to give a final concentration of 100, 150, 200 or 300 μg/ml. The final volume of the suspension was maintained at 10 ml. The suspension was incubated in the dark at 30°C for 30 min with continuous agitation.

After exposure, cells were washed twice with phosphate buffer and diluted to 5 ml with M17 broth. A portion was plated on M17 agar for total count (after exposure). The culture was incubated at 30°C overnight for mutant expression. The culture was then plated on M17 agar supplemented with 4%, 8%, 10%, 12% and 15% NaCl and incubated at 30°C under anaerobic condition for up to one week and observed for the presence of colony-forming mutants.

RESULTS AND DISCUSSION

Isolation and Identification

The *Lactococcus* was isolated from a single strain commercial culture (Chr. Hansen's Laboratory Inc.). Table 9 shows the properties of the parent cell that was isolated from the commercial culture.

In the dairy industry, mesophilic starters *L. lactis ssp. lactis* and *L. lactis ssp. cremoris* singly or in combination are used in Cheddar cheesemaking (Sharpe, 1979). The commercial culture from which the bacterial cell was isolated is specific for Cheddar cheesemaking, therefore the unknown was compared to characteristics of the above two species for identification. According to the identification scheme of Garvie and Farrow (1982), the unknown bacterial cell was L. lactis ssp. cremoris. This strain differs from *L. lactis ssp. lactis* in that it does not grow in broth containing 4% NaCl, does not produce ammonia from arginine, and does not produce acid from sugars such as ribose, cellobiose, maltose and dextrin (Garvie and Farrow, 1982; Mundt, 1986). Further identification of the unknown bacterial species was done using the API Rapid Strep System (Analytab Inc., Plainview, NY). This system identified the unknown as *L. lactis ssp. cremoris* with 99% probability but there was 1% probability that it was *Streptococcus thermophilus*.

Table 9. Distinguishing features of the parent cell

Characteristics	Results
Gram stain	+ cocci
Catalase test	−
Oxidase test	+
Growth at:	
10°C	+
40°C	+
45°C	−
Growth in:	
2% NaCl	+
4% NaCl	−
6.5% NaCl	−
Growth in broth at pH 9.6	+
Hydrolysis of arginine	−
Utilization of citrate	−
Acid from:	
Arabinose	−
Ribose	−
Xylose	−
Cellobiose	−
Lactose	+
Maltose	−
Sucrose	−
Trehalose	−
Raffinose	−
Dextrin	−
Glucose	+
Mannitol	−
Sorbitol	−

+, positive for the test.
−, negative for the test.

Mutant Isolation

In preliminary studies, cells were adapted to salt by transfering to progressively higher salt concentrations. However, while cells were adapted to as high as 10% NaCl, they were not able to produce acid in milk. Therefore chemical mutation was pursued. N-methyl-N'-nitro-N-nitrosoguanidine (NTG) was used as chemical mutagen according to the modified method of Khosravi and Sandine (1987) who used it to produce β-lactamase producing mutants of *L. lactis ssp. cremoris*. In the presence of 100, 150, 200 and 300 μg/ml NTG, survival rates of 13.69, 1.76, 4.74 and 0.55, respectively were found. We were not able to produce salt resistant *Lactococcus* mutants by this procedure after five repetitions of mutant isolation.

For future research, it would be interesting to isolate and identify different strains of lactic acid bacteria from traditionally fermented food. These lactic acid bacteria then could be subjected to mutagenesis by a variety of chemical and physical mutagens (nitrous acid, 5-bromouracil, hydroxylamine, ethyl methane sulfonate, ultraviolet, gamma and x-ray radiation). In addition to production of salt-resistant mutants, mutants imparting flavor to dairy products in general might be developed.

REFERENCES

1. Abd El-Salam, M. H. 1987. Domiati and Feta type cheeses. pp. 277-309. In Fox, P. F. (ed.), *Cheese: chemistry, physics and microbiology*. Vol. 2. Elsevier Appl. Sci., N.Y.

2. Alla Gabo, I. H. 1986. *Studies on the composition and quality of Gibna Beyda*. M.Sc. Thesis, University of Khartoum, Khartoum, Sudan.

3. Dariani, N. D., K. M. Galal, S. J. Speck, and M. Loewenstein. 1980. I. Manufacturing soft pickled cheese using cow and goat milk. Dairy Field 163(6):81-84.

4. Fahmi, A. H. and H. A. Sharara. 1950. Studies on Egyptian Domiati cheese. J. Dairy Res. 17:312-328.

5. Garvie, E. I. and J. A. E. Farrow. 1982. *Streptococcus lactis* subsp. *cremoris* (Orla-Jensen) comb. nov. and *Streptococcus lactis* subsp. *diacetilactis* (Matuszewski *et al.*) nom. rev., comb. nov. Int. J. Syst. Bacteriol. 32:453-455.

6. Karlikanova, S. N., I. U. Ramazanov, and O. P. Ramazanova. 1978. Production of salt-resistant mutants of lactic acid streptococci and their use in starters for pickled cheese. XX Intern. Dairy Cong. E. 525-526.

7. Khalid, S. A. and F. W. Harrigan. 1984. Investigation into the effect of salt level on growth of, and toxin production by *Staphylococcus aureus* in Sudanese cheese. Lebensm-Wiss Technol. 17:99-103.

8. Khosravi, L. and W. E. Sandine. 1987. β-lactamase-producing mutants of *Streptococcus cremoris*. J. Dairy Sci. 70:1529-1543.

9. Mundt, J. O. 1986. Lactic acid streptococci. pp. 1065-1066. In Sneath, A. H. P., S. N. Mair, M. E. Sharpe, and G. J. Holt (ed.), *Bergey's manual of systematic bacteriology*. Vol. 2. Williams and Wilkins Co., Baltimore, MD.

10. Sharpe, M. E. 1979. Lactic acid bacteria in the dairy industry. J. Soc. Dairy Technol. 32:9-17.

11. Terzaghi, B. E. and W. E. Sandine. 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. 29:807-813.

CHAPTER V

EFFECT OF SALT ON CHEMICAL COMPOSITION AND SURVIVAL OF *L. MONOCYTOGENES* IN WHITE PICKLED CHEESE

INTRODUCTION

White pickled cheese of the Sudan is a product traditionally made from raw milk with high salt concentration ranging from 6% to 20% (Alla Gabo, 1986; Khalid and Harrigan, 1984). Although this high salt level is meant to control foodborne pathogens, it has a major influence on acid development by limiting the growth of naturally occurring lactic acid bacteria (Ahmed *et al.*, 1983; Clemente dos Santos and Genigeorgis, 1981; Ibrahim *et al.*, 1981; Khalid and Harrigan, 1984). Recent foodborne listeriosis outbreaks due to consumption of milk (Fleming *et al.*, 1985) and cheese (Linnan *et al.*, 1988) have generated an interest in defining the survival of *L. monocytogenes* in cheeses (Buazzi *et al.*, 1992; Papageorgiou and Marth, 1989a, 1989b; Ryser and Marth, 1987a, 1987b; Ryser and Marth, 1989; Yousef and Marth, 1990), milk and milk products (Doyle *et al.*, 1985; Rosenow and Marth, 1987; Schaack and Marth, 1988a, 1988b, 1988c), yogurt (Schaack and Marth, 1988a) and cream (Rosenow and Marth, 1987).

Although different food products were tested for the survival of *L. monocytogenes*, high salt foods were not studied

except for cabbage juice which was found to inhibit growth of the pathogen at salt levels of up to 5% (Conner *et al.*, 1986). Since white pickled cheese is traditionally made from raw milk with high salt levels, this study was designed to determine the effect of salt and/or lactic acid bacteria on the growth of *L. monocytogenes* in this cheese made by traditional procedures. Additionally, composition of the cheese was determined since this information is not available in the literature.

MATERIALS AND METHODS

Cheese Manufacture

Cheese was made as in chapter III except that salt (8% w/w) and $CaCl_2$ (0.2% w/w) were added to milk prior to addition of rennet. Two treatments were used, the control to which only salt, $CaCl_2$ and rennet were added and the other to which starter culture (isolated in chapter IV, 1% w/w) was added as well. The temperature following the addition of rennet was 38°C. Cheese was stored in the collected whey at 4°C. The first experiment was designed to study the chemical composition of cheese and whey, while the second experiment was designed to study the survival of *L. monocytogenes*.

Enumeration of *L. monocytogenes*

L. monocytogenes Scott A (ca. $\log_{10}$ 5.0 CFU/ml of a 24 h culture) was added to milk before rennet addition and cheese and whey were analyzed for *L. monocytogenes* and pH (Fisher

Accumet pH meter Model 825 MP with Orion Model 8163 Ross combination pH probe) at 0, 1, 3, 10, 20, 30, 40, 50 and 65 d. Enumeration of *L. monocytogenes* was done as described in chapter III.

Evaluation of Chemical Composition of Cheese

Milk (at day 0), cheese and whey (at days 1, 10, 30 and 60) were analyzed for chemical composition.

Fat (Babcock method) and salt (Mohr method) were determined according to standard methods (Richardson, 1985). The Kjeldahl method was used for protein determination as described in AOAC (1990) with the following modifications. One gram of sample was used and 15 ml H_2SO_4 were added followed by 2 tablets (Kjeltabs - 175 mg HgO and 3.5 g K_2SO_4 in each tablet). The mixture was digested for 45 min using a Kjeltec digester (Tecator 1015 digester, Fisher Scientific, Pittsburgh, PA). After the sample was cooled, 10 ml of sodium thiosulfate (80 g in 1 L distilled water) were added. Boric acid (25 ml) was used to receive the distillate which was then titrated with 0.1 N HCl. Protein (%) was calculated using a conversion factor of 6.38.

The method described in AOAC (1990) was used for the determination of titratable acidity in cheese and brine. Total solids and ash were determined as in the AOAC (1990) method except that a sample size of 2 g was used in each case. In addition, milk and cheese for total solids analyses were heated overnight in a vacuum oven at 70°C. Proteolysis in

cheese was measured according to the method of Polychroniadou (1988).

Statistical Analyses

Statistical analyses were performed using the Statistical Analysis System (SAS, 1990, Version 6.06, Cary, NC). Each experiment was repeated twice. General linear models (GLM) were used to determine the effects of day of storage, treatment (control, starter culture), replication and the interaction between day and treatment. These variables were analyzed using GLM to test the effect on fat, protein, salt, titratable acidity, total solids, ash and absorbance (A_{420}) for chemical analyses and on pH and $\log_{10}$ colony forming units (CFU)/g (ml) of curd and brine solution in the *L. monocytogenes* challenge. Means were separated using least significant difference with an $\alpha \leq 0.05$ (SAS, 1990).

RESULTS AND DISCUSSION

Chemical Composition

Raw milk had 3.0% fat, 3.31% protein, 12.19% total solids, 0.78% ash, 0.24% NaCl and 0.18% titratable acidity (Table 10).

Fat Content. Fat content in cheese curd showed a decrease over time in both control and starter culture treatments (Fig. 8). Fat content started at a value of 20.25% and 18.5% at day 1, and decreased to 14.0% and 13.0% at day 60 in control and

Table 10. Average chemical composition of milk, curd and salted whey of white pickled cheese over all sampling times.

Composition (%)	Raw Milk	Control		Starter culture	
		Curd	Whey	Curd	Whey
Fat	3.00	16.00[a]	0.10[a]	14.88[a]	0.11[a]
Protein	3.31	12.45[a]	1.12[a]	11.61[b]	1.19[a]
Total solids	12.19	37.56[a]	14.22[a]	36.03[a]	14.31[a]
Ash	0.78	8.59[a]	8.08[a]	8.80[a]	8.11[a]
Salt (8% added)	0.24	5.15[b]	8.65[a]	5.55[a]	8.66[a]
Titratable acidity	0.18	0.01[a]	0.13[b]	0.01[a]	0.14[a]
A_{420}[c]	-	0.06[a]	-	0.06[a]	-

[a-b] Values within the same row between treatments for curd or whey followed by different superscripts are significantly different ($P<0.05$).

[c] A_{420} = Absorbance at 420 nm, a measurement of proteolysis (breakdown to amino acids) in cheese.

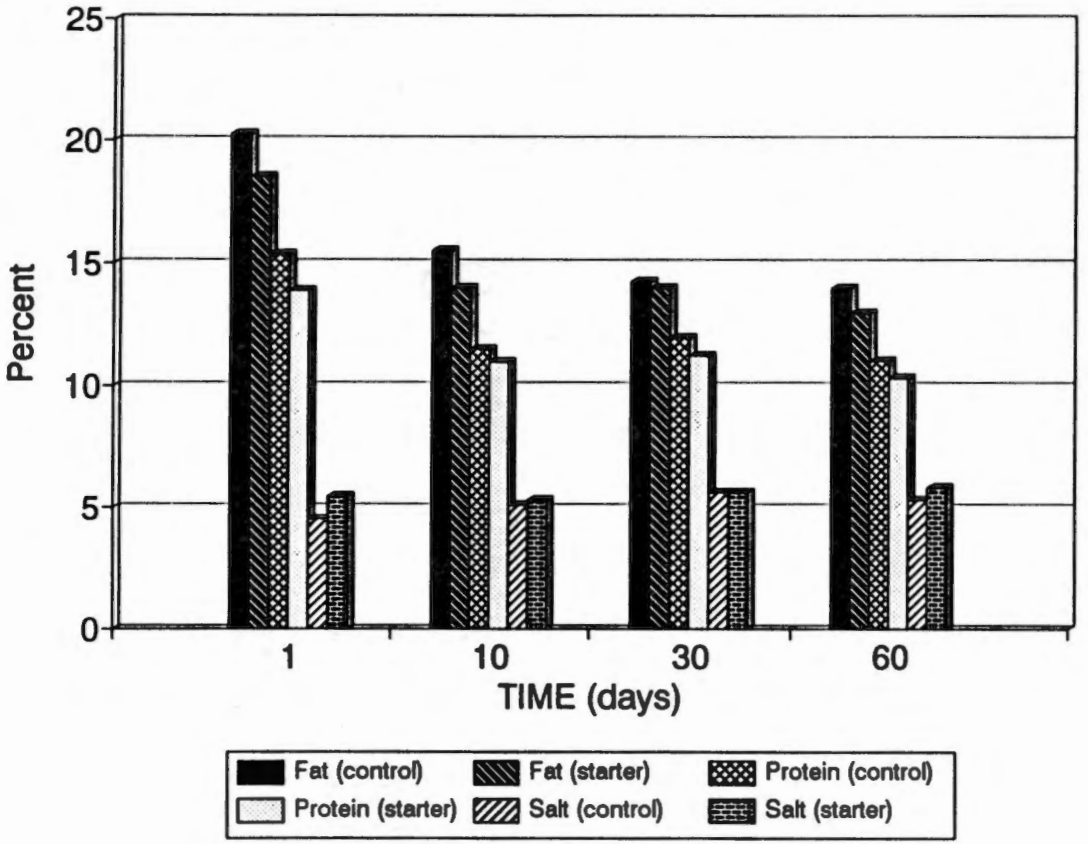


Figure 8. Change in fat, protein and salt in cheese curd during storage at 4°C for 60 d. Cheese was made from pasteurized milk with 8% salt. Starter culture (1%; *L. lactis ssp. cremoris*) was added to the starter treatment.

starter treatments, respectively (Fig. 8). In whey, fat content started at 0% in both treatments at day 1, and increased slightly to a value of 0.25% and 0.3% at day 60 in control and starter treatments, respectively (Fig. 9). Addition of starter culture did not affect ($P>0.05$) fat content (Table 10).

The decrease in fat content over time is in agreement with reports of Alla Gabo (1986) and Nofal *et al.* (1981), but does not agree with reports of Dariani *et al.* (1980) and Zaki *et al.* (1974). Fat content of whey started at 0% and increased with time. Some fat must have leaked from curd into the brine solution, which could partially explain the decrease in fat content in curd during storage.

Protein Content. Protein content in cheese curd decreased during storage from 15.35% and 13.89% to 11.02% and 10.34% in control and starter treatments, respectively (Fig. 8). In whey, protein content showed an increase from 0.85% in both treatments to 1.18% and 1.22% in control and starter treatments, respectively, at the end of the storage period (Fig. 9). Differences ($P<0.05$) between control and starter treatments in curd but not in whey were observed (Table 10).

Decreased protein content during pickling was a direct result of protein degradation leading to the formation of water soluble compounds, some of which were lost in the pickling solution leading to increased nitrogen content of

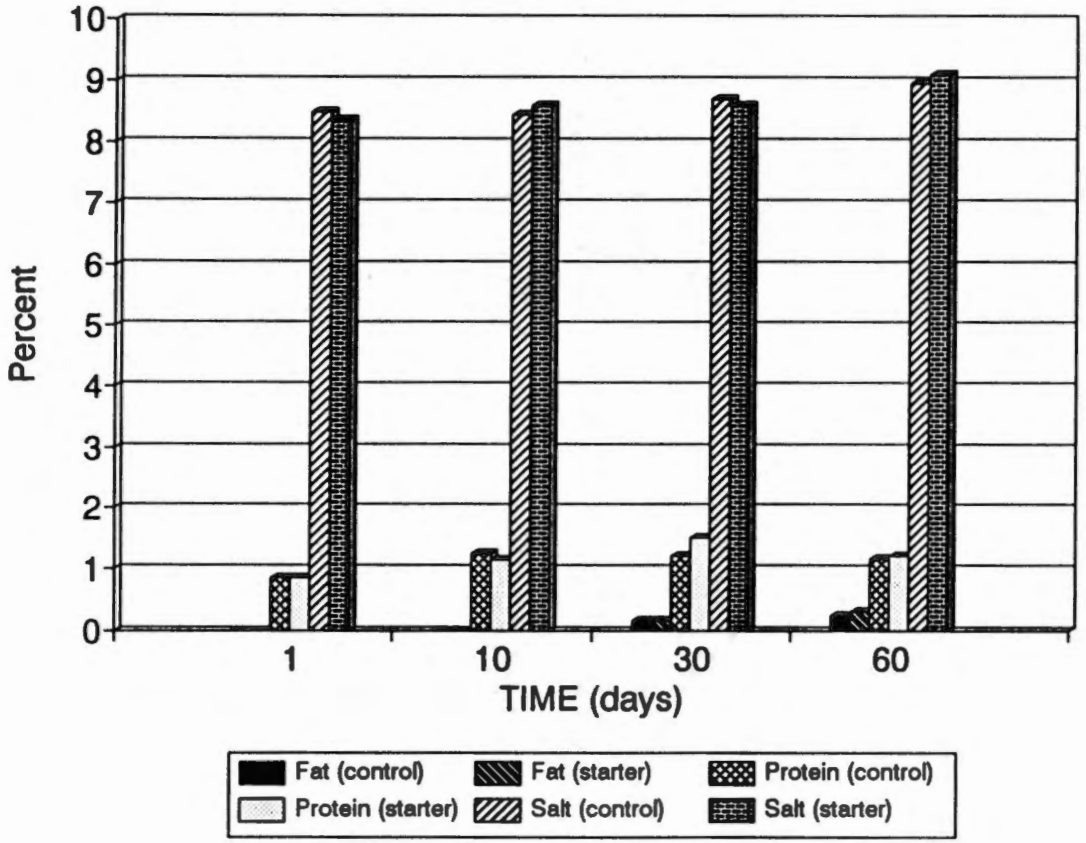


Figure 9. Change in fat, protein and salt contents of whey during storage at 4°C for 60 d of cheese made from pasteurized milk with 8% salt.

whey (Alla Gabo, 1986; Dariani *et al.*, 1980; Nofal *et al.*, 1981; Zaki *et al.*, 1974).

Salt Content. The percent salt in curd increased slightly in the control but stayed the same in the starter treatment (Fig. 8). In whey, there was a slight increase toward the end of the storage period for both treatments (Fig. 9). Mean value of salt content was higher in curd made with starter than in the control ($P<0.05$), while there was no significant difference ($P>0.05$) in whey (Table 10).

The general trend did not show a pronounced decrease or increase of salt content in either curd or whey. Previous reports (Ahmed *et al.*, 1983; Dariani *et al.*, 1980) showed a gradual decrease in salt content with advanced ripening, which was attributed to dissolution of cheese salt into the pickling whey to obtain equilibrium (Zaki *et al.*, 1974).

Total Solids. While total solids in cheese curd decreased gradually over time in both treatments (Fig. 10), in whey it decreased slightly at day 10 after which it showed a slight increase (Fig. 11). No statistically significant differences between total solids of control and starter in curd or whey ($P>0.05$) were detected (Table 10).

Total solids content is the portion of the cheese other than water. The decrease in total solids of cheese curd during pickling could be explained by degradation of total protein, dissolution of salt and fat into the pickling solution or absorption of pickling whey by curd (Dariani *et*

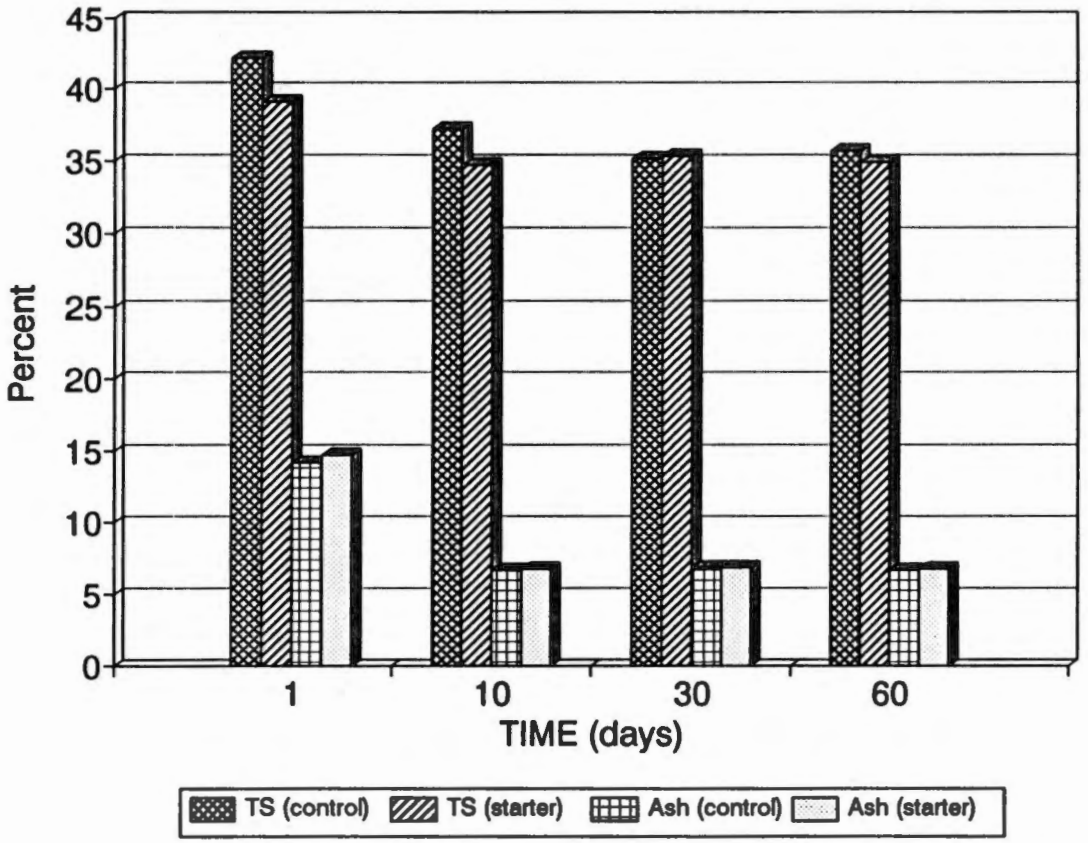


Figure 10. Change in total solids and ash contents of white pickled cheese curd during storage at 4°C for 60 d.

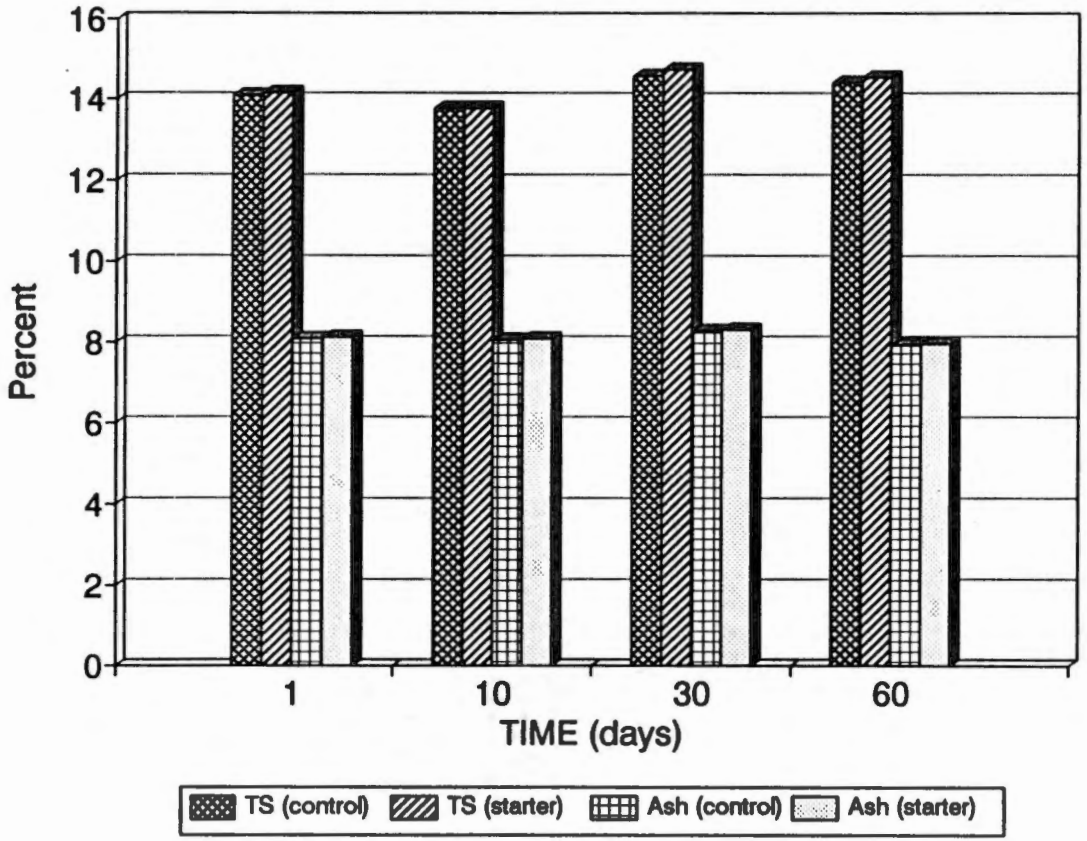


Figure 11. Change in total solids and ash contents of cheese whey from cheese made from pasteurized milk with 8% salt and stored at 4°C for 60 d.

al., 1980). The increase in total solids of whey was due to the same factors. However, other results indicated that moisture content of cheese decreased during pickling, a phenomenon attributed to curd contraction and expulsion of whey as a result of acid development (Nofal *et al.*, 1981; Zaki *et al.*, 1974).

Ash Content. The ash content of curd decreased at day 10, and remained constant throughout the storage period (Fig. 10). In whey, ash content remained around 8% throughout the storage period (Fig. 11). Addition of starter did not affect ($P>0.05$) ash content of curd or whey (Table 10).

Ash content constitutes the inorganic part of the solid matter. During pickling, ash content followed a trend somewhat similar to total solids, indicating that ash content in curd and whey was very much affected by diffusion of salt from curd into whey.

Protein Degradation. Protein breakdown into amino acids was measured to evaluate the effect of proteases. During the 60 d ripening period, absorbance changed slightly (Fig. 12). All values were small (<0.1 absorbance units), indicating only small quantities of free amino groups maintained in the curd. The concentration of free amino acids was the same ($P>0.05$) in both treatments (Table 10). Nitrogen content of curd decreased during ripening while that of whey increased (Figs. 8 and 9). Perhaps free amino groups were diffusing into whey and we were unable to measure them in the curd.

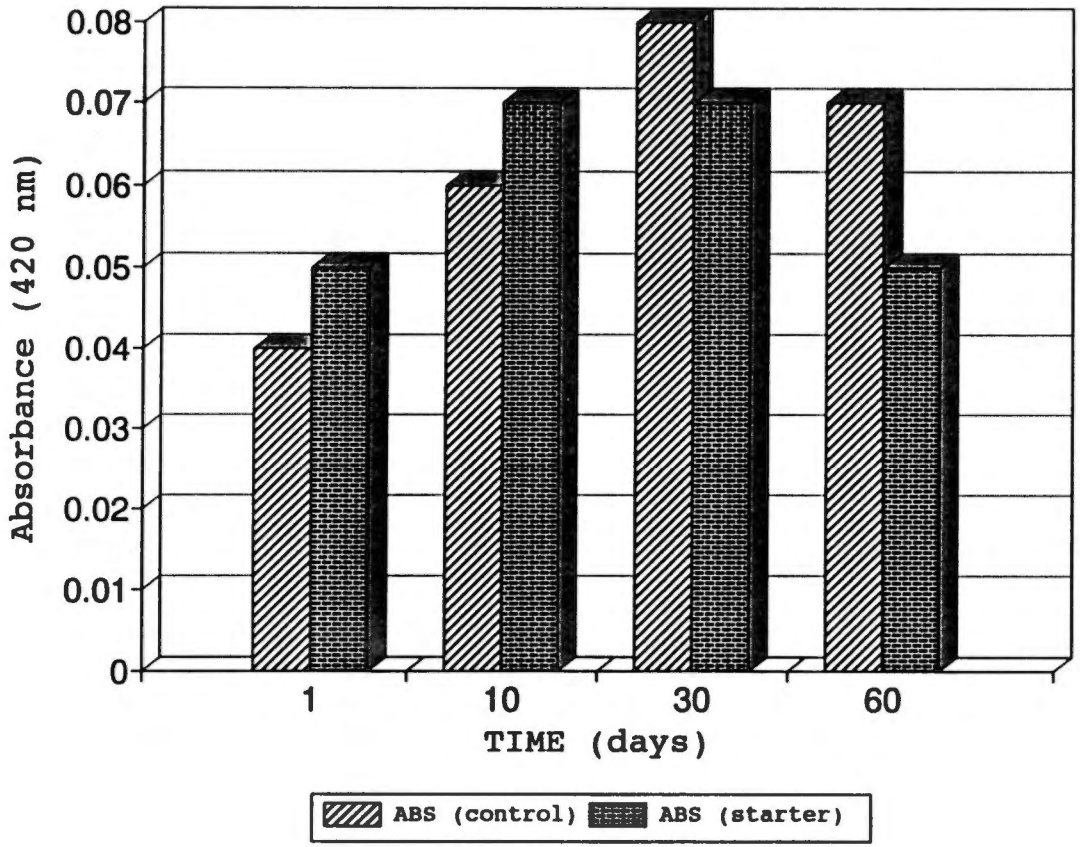


Figure 12. Proteolysis (A_{420}) in cheese curd during storage at 4°C for 60 d. ABS=absorbance at wavelength 420 nm.

Heat treatment of milk and high salt level inactivated the natural microflora as well as starter bacteria added to milk (Zaki *et al.*, 1974) resulting in reduced proteolysis. Similar results were found in the present study where milk was pasteurized and high salt levels inhibited starter bacteria.

Acidity. The acidity in curd did not change during the storage period in either treatment and remained at 0.01% from day 1 to day 60 (Fig. 13). In whey, acidity slightly increased from day 1 to a maximum at day 30, then decreased toward the end of the storage period (Fig. 13). Whey from cheese made with starter culture had higher mean acidity value ($P<0.05$) than the control (Table 10).

In cheese made in the same way but challenged with *L. monocytogenes*, pH of cheese curd increased irregularly until day 30, then decreased at day 50 after which it increased to a maximum value at the end of the storage period (Fig. 14). The pH of the cheese made with starter was always below that of the

control. The pH of whey increased steadily over time with a slight increase and a slight decrease observed at day 30 and 50 respectively (Fig. 15). The mean pH value for the starter culture treatment was lower ($P<0.05$) than control in both curd and whey (Table 11).

Lactic acid bacteria are unable to grow in high salt levels thus low acidity resulted (Khalid and Harrigan, 1984). The strain used in the present study was found to grow in salt

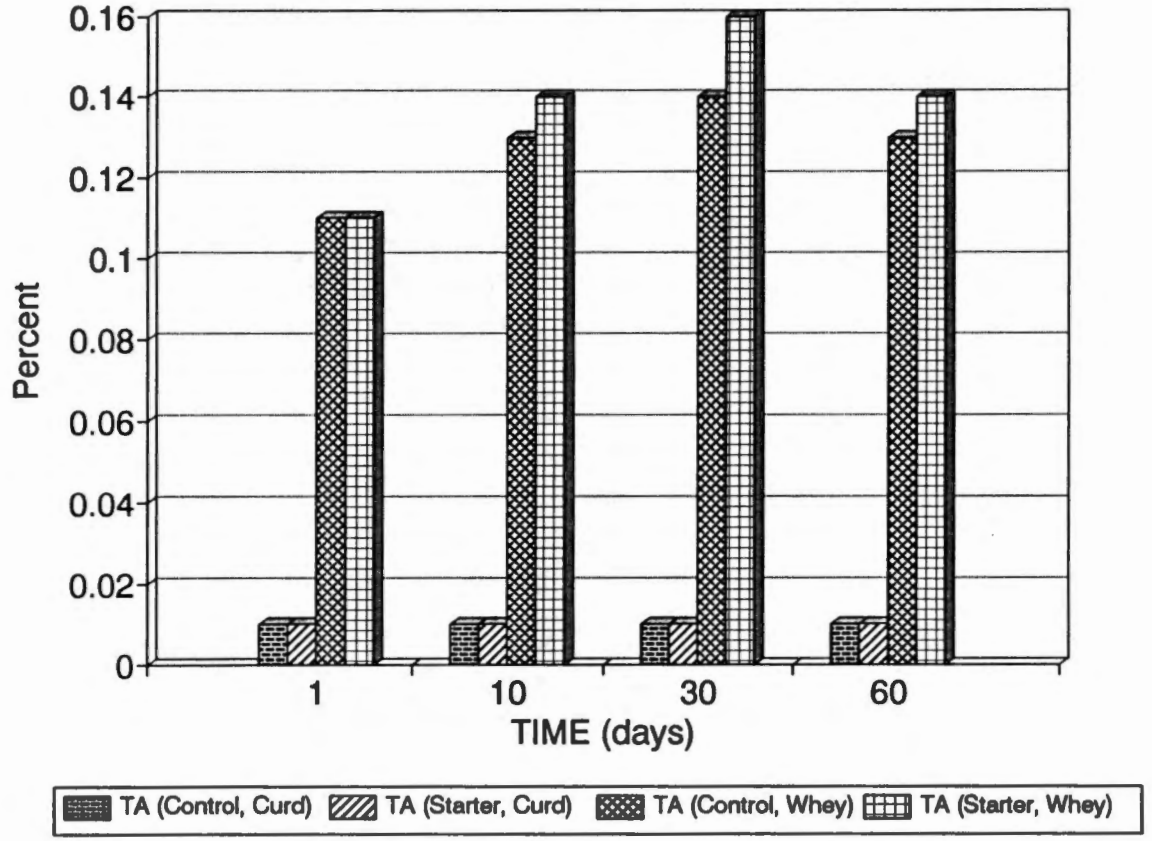


Figure 13. Change in titratable acidity of curd and whey of white pickled cheese during storage at 4°C for 60 d. Cheese was made from pasteurized milk with 8% salt.

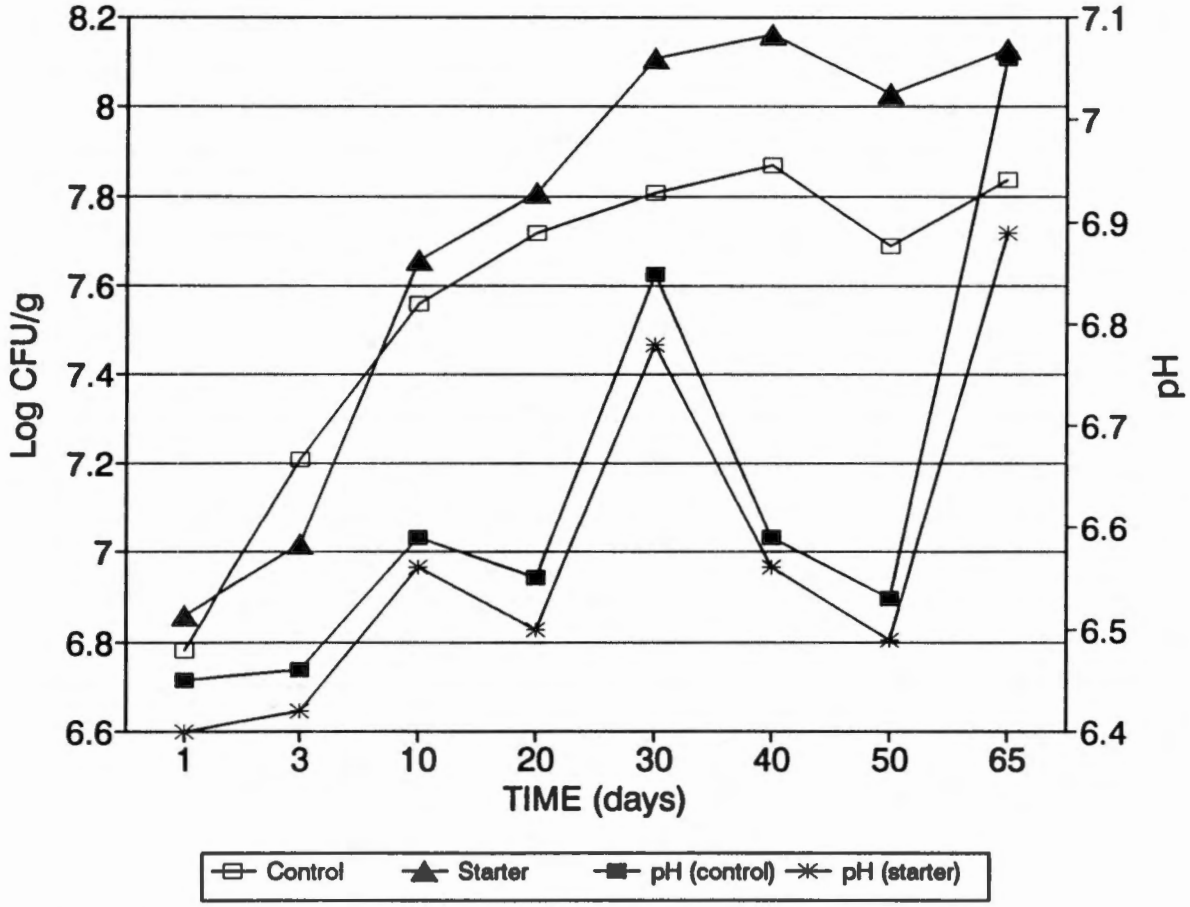


Figure 14. Survival of *L. monocytogenes* Scott A and pH in white pickled cheese curd made from pasteurized milk with 8% salt and stored at 4°C for 65 d. Starter culture (*L. lactis ssp. cremoris*) at 1% was added to the starter treatment.

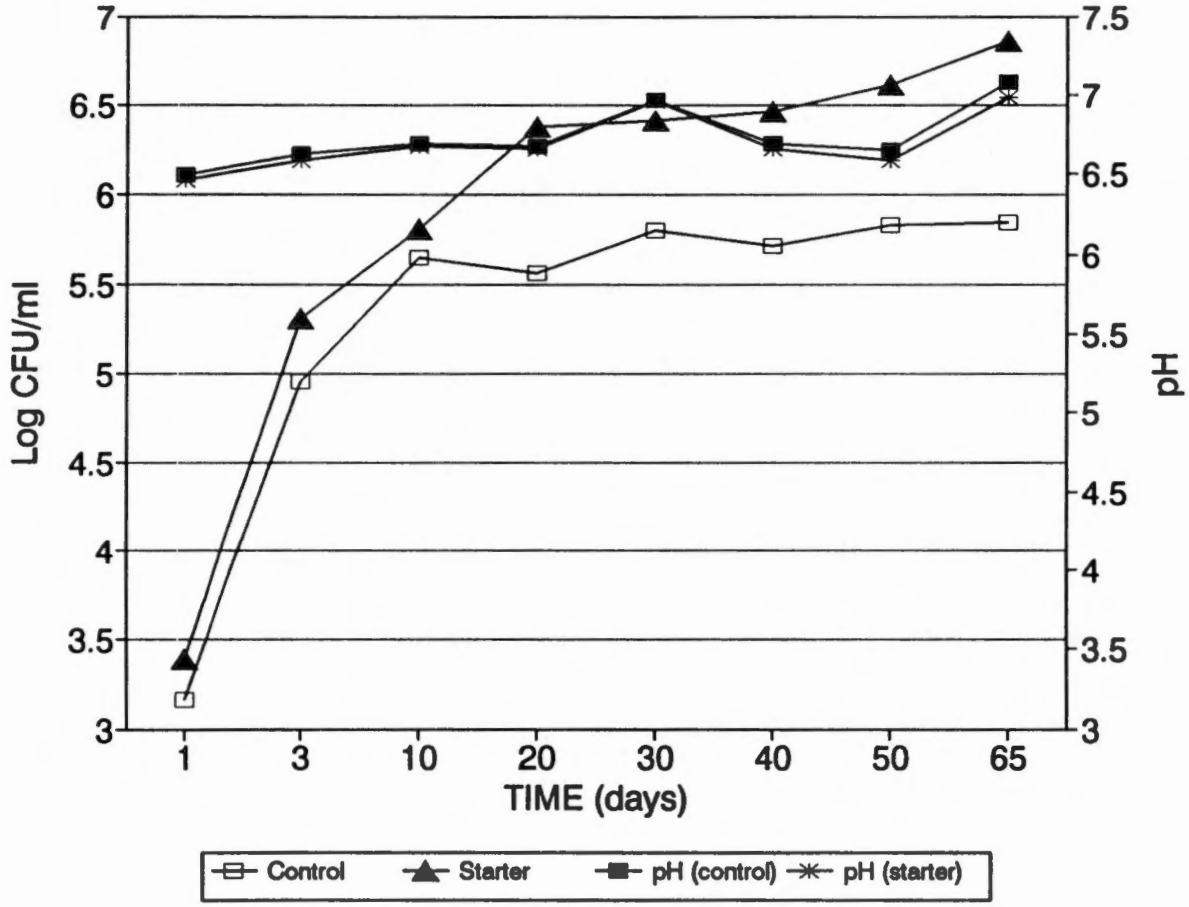


Figure 15. Behavior of *L. monocytogenes* Scott A and pH in cheese whey made from milk with 8% salt and stored at 4°C for 65 d.

Table 11. Mean log_{10} CFU/g (ml) of *L. monocytogenes* Scott A and pH for curd and whey of white pickled cheese made from pasteurized milk with 8% salt.

Treatment	Initial inoculum (Log_{10} CFU/ml)	Curd[a]		Whey[a]	
		Log_{10} CFU/g	pH	Log_{10} CFU/ml	pH
Control	5.0	7.6^{c}	6.63^{b}	5.6^{c}	6.73^{b}
Starter culture	5.1	7.7^{b}	6.57^{c}	5.9^{b}	6.69^{c}

[a] Log_{10} CFU/g (ml) and pH values represent the mean of all sampling days.
[b-c] Values within each column followed by different superscripts are significantly different ($P<0.05$).

content up to 4% (Chapter IV). Pasteurization inactivated the naturally occurring microflora in milk. There is an inverse relationship between salt content and acid production (Dariani *et al.*, 1980; Khalid and Harrigan, 1984; Zaki *et al.*, 1974). In cheese made from 4% salt and preserved in 4% brine solution, lactic acid bacteria were able to grow and produce acid such that they inhibited growth of some foodborne pathogens (Abdalla and Davidson, 1990; Chapter III).

Survival of *L. monocytogenes* in Cheese Curd and Whey

L. monocytogenes in cheese curd showed a similar trend in control and starter treatments (Fig. 14). Numbers (log_{10} CFU/g) increased steadily until day 40, after which a slight decrease occurred at day 50, and an increase was seen at the end of the storage period.

During cheese manufacture, cells diffused into the whey so that by day 1, approximately log_{10} 3.2 and 3.4 CFU/ml were detected in control and starter treatments, respectively (Fig. 15). Numbers increased sharply until day 10, after which numbers in the control remained constant throughout the storage period while numbers in starter treatment increased steadily until the end of storage (Fig. 15). Significantly higher number of cells were detected in curd and whey of the starter treatment ($P<0.05$) than the control (Table 11).

Salt did not inhibit *L. monocytogenes* in this study. The pathogen has been shown to survive for 10 d or one year in salt concentrations of 30.5% or 10% respectively, in other

foods depending on temperature and pH in other foods (Lovett, 1989). In cabbage juice, *L. monocytogenes* was able to grow at 5°C in the presence of 5% NaCl (Conner *et al.*, 1986). Two recent outbreaks of listeriosis were associated with the consumption of pasteurized milk and Mexican-style cheese made from pasteurized milk contaminated with unpasteurized milk (Fleming *et al.*, 1985; Linnan *et al.*, 1988). Soft cheeses are considered to be effective vehicles for *L. monocytogenes* because they are treated with brine solution during production or storage which leads to high concentration of NaCl that may inhibit competing microorganisms particularly, lactic acid bacteria (Linnan *et al.*, 1988). In addition, soft cheeses never acquire strong acidic environment to discourage bacterial growth since they are not aged (Linnan *et al.*, 1988). Traditionally, white pickled cheese is made with raw milk and high salt level (6-20%). If contaminated with *L. monocytogenes*, white pickled cheese could be unsafe.

Salt suppressed starter culture growth in this study thereby preventing acid production. High NaCl content inhibited growth of lactic acid bacteria (Ahmed *et al.*, 1983; Clemente dos Santos and Genigeorgis, 1981; Ibrahim *et al.*, 1981). Additionally, the presence of starter culture in 8% NaCl appeared to enhance *L. monocytogenes* growth (Figs. 14 and 15). However, the use of 4% NaCl in cheese with added starter and preserved in 4% brine solution inhibited *L. monocytogenes* (Chapter III). In order to produce a safe cheese of this

type, pasteurization, starter addition and use of 4% salt is recommended. An alternative to use of low salt levels is to develop mutant starter organisms that are able to grow at high salt levels. Even without pasteurization, cheese made with 4% salt and starter culture may be safe if stored for at least 60 d (Chapter III).

REFERENCES

1. Abdalla, O. M. and P. M. Davidson. 1990. Effect of starter culture and antimicrobials on the growth of *Staphylococcus aureus* in Gebna Beyda cheese. Institute of Food Technologists' Annual Meeting, June, 16-20, 1990. Anaheim, California.

2. Ahmed, A. A. H., M. K. Moustafa, and E. H. Marth. 1983. Growth and survival of *Staphylococcus aureus* in Egyptian Domiati cheese. J. Food Prot. 46:412-415.

3. Alla Gabo, I. H. 1986. *Studies on the composition and quality of Gibna Beyda*. M.Sc. Thesis, University of Khartoum, Khartoum, Sudan.

4. AOAC. 1990. *Official methods of analysis*, 15th ed. Association of Official Analytical Chemists, Washington, DC.

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

6. Clemente dos Santos, E. and C. Genigeorgis. 1981. Survival and growth of *Staphylococcus aureus* in commercially manufactured Brazilian Minas cheese. J. Food Prot. 44:177-184.

7. Conner, D. E., R. E. Brackett, and L. R. Beuchat. 1986. Effect of temperature, sodium chloride and pH on growth of *Listeria monocytogenes* in cabbage juice. Appl. Environ. Microbiol. 52:59-63.

8. Dariani, N. D., K. M. Galal, S. J. Speck, and M. Loewenstein. 1980. II. Manufacturing soft pickled cheese using cow and goat milk. Dairy Field 163(7):70-72.

9. Doyle, M. P., L. M. Meske, and E. H. Marth. 1985. Survival of *Listeria monocytogenes* during the manufacture and storage of nonfat dry milk. J. Food Prot. 48:740-742.

10. Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. 312:404-407.

11. Ibrahim, G. F., A. K. Baldock, D. R. Bradford, and L. B. Ireland. 1981. Inhibition of *Staphylococcus aureus* growth and enterotoxin A production in Cheddar cheese produced with variable starter activity. J. Food Prot. 44:263-267.

12. Khalid, S. A. and F. W. Harrigan. 1984. Investigation into the effect of salt level on growth of, and toxin production by *Staphylococcus aureus* in Sudanese cheese. Lebensm-Wiss Technol. 17:99-103.

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

14. Lovett, J. 1989. *Listeria monocytogenes*. pp. 283-310. In Doyle, P. M. (ed.), *Foodborne bacterial pathogens*. Marcel Dekker, Inc., N.Y.

15. Nofal, A., M. Elhami, H. El-Gazzar, and A. Abu-El-Kheir. 1981. Studies on the acceleration of manufacturing Domiati cheese. III. Effect of storage on the yield and properties of cheese manufactured by the suggested method. Agric. Res. Rev. 59:301-311.

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

17. 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.

18. Polychroniadou, A. 1988. A simple procedure using trinitrobezenesulphonic acid for monitoring proteolysis in cheese. J. Dairy Res. 55:585-596.

19. Richardson, G. H. (ed.). 1985. *Standard methods for the examination of dairy products*. 15th ed. American Public Health Association. Washington, DC.

20. Rosenow, E. M. and E. H. Marth. 1987. Growth of *Listeria monocytogenes* in skim, whole and chocolate milk, and in whipping cream during incubation at 4, 8, 13, 21 and 35°C. J. Food Prot. 50:452-459.

21. 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.

22. 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.

23. 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.

24. SAS/STAT User's Guide, version 6, 4rth. ed., Vol. 2, Cary, NC: SAS Institute Inc., 1989. 846 pp.

25. Schaack, M. M. and E. H. Marth. 1988a. Survival of *Listeria monocytogenes* in refrigerated cultured milks and yogurt. J. Food Prot. 51:848-852.

26. 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.

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

28. Yousef, A. E. and E. H. Marth. 1990. Fate of *Listeria monocytogenes* during the manufacture and ripening of Parmesan cheese. J. Dairy Sci. 73:3351-3356.

29. Zaki, M. H., N. H. Metwally, E. M. Gewaily, and L. A. El-Koussy. 1974. Domiati cheese stored at room temperature as affected by heat treatment of milk and different salting levels. Agric. Res. Rev. 52:217-231.

APPENDIX

Appendix A: Results of analysis of variance for fat content in cheese curd.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	92.750000	11.593750	8.38	0.0056
Error	7	9.687500	1.383929		
Corrected Total	15	102.437500			

R-Square	C.V.	Root MSE	Fat Mean
0.905430	7.620437	1.1764	15.438

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.562500	0.562500	0.41	0.5441
TREAT	1	5.062500	5.062500	3.66	0.0974
DAY	3	85.812500	28.604167	20.67	0.0007
TREAT*DAY	3	1.312500	0.437500	0.32	0.8136

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.562500	0.562500	0.41	0.5441
TREAT	1	5.062500	5.062500	3.66	0.0974
DAY	3	85.812500	28.604167	20.67	0.0007
TREAT*DAY	3	1.312500	0.437500	0.32	0.8136

Appendix B: Results of analysis of variance for protein content in cheese curd.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	41.079625	5.134953	11.12	0.0024
Error	7	3.232875	0.461839		
Corrected Total	15	44.312500			

R-Square	C.V.	Root MSE	Protein Mean
0.927044	5.647933	0.6796	12.033

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.265225	0.265225	0.57	0.4733
TREAT	1	2.890000	2.890000	6.26	0.0409
DAY	3	37.400150	12.466717	26.99	0.0003
TREAT*DAY	3	0.524250	0.174750	0.38	0.7718

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.265225	0.265225	0.57	0.4733
TREAT	1	2.890000	2.890000	6.26	0.0409
DAY	3	37.400150	12.466717	26.99	0.0003
TREAT*DAY	3	0.524250	0.174750	0.38	0.7718

Appendix C: Results of analysis of variance for salt content of cheese curd.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	2.3508000	0.2938500	5.77	0.0161
Error	7	0.3565750	0.0509393		
Corrected Total	15	2.7073750			

R-Square	C.V.	Root MSE	Salt Mean
0.868295	4.217656	0.2257	5.3512

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.1482250	0.1482250	2.91	0.1318
TREAT	1	0.6480250	0.6480250	12.72	0.0091
DAY	3	1.0334750	0.3444917	6.76	0.0178
TREAT*DAY	3	0.5210750	0.1736917	3.41	0.0822

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.1482250	0.1482250	2.91	0.1318
TREAT	1	0.6480250	0.6480250	12.72	0.0091
DAY	3	1.0334750	0.3444917	6.76	0.0178
TREAT*DAY	3	0.5210750	0.1736917	3.41	0.0822

Appendix D: Results of analysis of variance for titratable acidity of cheese curd.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F***
Model	8	0	0	.	.
Error	7	0	0		
Corrected Total	15	0			

R-Square	C.V.	Root MSE	TA Mean
0.000000	0	0	0.0100

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0000000	0.0000000	99999.99	0.0001
TREAT	1	0.0000000	0.0000000	99999.99	0.0001
DAY	3	0.0000000	0.0000000	99999.99	0.0001
TREAT*DAY	3	0.0000000	0.0000000	99999.99	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0000000	0.0000000	99999.99	0.0001
TREAT	1	0.0000000	0.0000000	99999.99	0.0001
DAY	3	0.0000000	0.0000000	99999.99	0.0001
TREAT*DAY	3	0.0000000	0.0000000	99999.99	0.0001

*** All readings of curd for both treatments had same values.

Appendix E: Results of analysis of variance for total solids content of cheese curd.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	102.02440	12.75305	4.63	0.0290
Error	7	19.29630	2.75661		
Corrected Total	15	121.32070			

R-Square	C.V.	Root MSE	TS Mean
0.840948	4.512006	1.6603	36.797

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	6.100900	6.100900	2.21	0.1804
TREAT	1	9.394225	9.394225	3.41	0.1074
DAY	3	79.738650	26.579550	9.64	0.0070
TREAT*DAY	3	6.790625	2.263542	0.82	0.5223

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	6.100900	6.100900	2.21	0.1804
TREAT	1	9.394225	9.394225	3.41	0.1074
DAY	3	79.738650	26.579550	9.64	0.0070
TREAT*DAY	3	6.790625	2.263542	0.82	0.5223

Appendix F: Results of analysis of variance for ash content of cheese curd.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	179.46093	22.43262	228.98	0.0001
Error	7	0.68578	0.09797		
Corrected Total	15	180.14670			

R-Square	C.V.	Root MSE	Ash Mean
0.996193	3.600784	0.3130	8.6925

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.26523	0.26523	2.71	0.1439
TREAT	1	0.16810	0.16810	1.72	0.2316
DAY	3	178.84400	59.61467	608.51	0.0001
TREAT*DAY	3	0.18360	0.06120	0.62	0.6214

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.26522	0.26522	2.71	0.1439
TREAT	1	0.16810	0.16810	1.72	0.2316
DAY	3	178.84400	59.61467	608.51	0.0001
TREAT*DAY	3	0.18360	0.06120	0.62	0.6214

Appendix G: Results of analysis of variance for proteolysis (measured as absorbance at 420 nm wavelength) of cheese curd.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.0016711	0.0002089	8.09	0.0061
Error	7	0.0001807	0.0000258		
Corrected Total	15	0.0018518			

R-Square	C.V.	Root MSE	ABS[a] Mean
0.902413	8.511742	0.0051	0.0597

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0001046	0.0001046	4.05	0.0841
TREAT	1	0.0000107	0.0000107	0.42	0.5398
DAY	3	0.0011740	0.0003913	15.16	0.0019
TREAT*DAY	3	0.0003818	0.0001273	4.93	0.0379

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0001046	0.0001046	4.05	0.0841
TREAT	1	0.0000107	0.0000107	0.42	0.5398
DAY	3	0.0011740	0.0003913	15.16	0.0019
TREAT*DAY	3	0.0003818	0.0001273	4.93	0.0379

[a] ABS= absorbance at 420 nm.

Appendix H: Results of analysis of variance for fat content in whey.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.2450000	0.0306250	3.33	0.0653
Error	7	0.0643750	0.0091964		
Corrected Total	15	0.3093750			

R-Square	C.V.	Root MSE	Fat Mean
0.791919	90.25695	0.0959	0.1062

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0306250	0.0306250	3.33	0.1108
TREAT	1	0.0006250	0.0006250	0.07	0.8018
DAY	3	0.2118750	0.0706250	7.68	0.0129
TREAT*DAY	3	0.0018750	0.0006250	0.07	0.9752

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0306250	0.0306250	3.33	0.1108
TREAT	1	0.0006250	0.0006250	0.07	0.8018
DAY	3	0.2118750	0.0706250	7.68	0.0129
TREAT*DAY	3	0.0018750	0.0006250	0.07	0.9752

Appendix I: Results of analysis of variance for protein content in whey.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.7155000	0.0894375	7.73	0.0070
Error	7	0.0810000	0.0115714		
Corrected Total	15	0.7965000			

R-Square	C.V.	Root MSE	Protein Mean
0.898305	9.333672	0.1076	1.1525

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0324000	0.0324000	2.80	0.1382
TREAT	1	0.0182250	0.0182250	1.57	0.2498
DAY	3	0.5737500	0.1912500	16.53	0.0015
TREAT*DAY	3	0.0911250	0.0303750	2.62	0.1323

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0324000	0.0324000	2.80	0.1382
TREAT	1	0.0182250	0.0182250	1.57	0.2498
DAY	3	0.5737500	0.1912500	16.53	0.0015
TREAT*DAY	3	0.0911250	0.0303750	2.62	0.1323

Appendix J: Results of analysis of variance for salt content in whey.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.9448750	0.1181094	10.95	0.0025
Error	7	0.0755000	0.0107857		
Corrected Total	15	1.0203750			

R-Square	C.V.	Root MSE	Salt Mean
0.926008	1.199761	0.1039	8.6563

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0000000	0.0000000	0.00	1.0000
TREAT	1	0.0009000	0.0009000	0.08	0.7811
DAY	3	0.8710250	0.2903417	26.92	0.0003
TREAT*DAY	3	0.0729500	0.0243167	2.25	0.1694

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0000000	0.0000000	0.00	1.0000
TREAT	1	0.0009000	0.0009000	0.08	0.7811
DAY	3	0.8710250	0.2903417	26.92	0.0003
TREAT*DAY	3	0.0729500	0.0243167	2.25	0.1694

Appendix K: Results of analysis of variance for titratable acidity in whey.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.0044500	0.0005562	20.10	0.0004
Error	7	0.0001938	0.0000277		
Corrected Total	15	0.0046438			

R-Square	C.V.	Root MSE	TA Mean
0.958277	3.989416	0.0053	0.1319

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0000562	0.0000562	2.03	0.1970
TREAT	1	0.0005062	0.0005062	18.29	0.0037
DAY	3	0.0037687	0.0012562	45.39	0.0001
TREAT*DAY	3	0.0001188	0.0000396	1.43	0.3127

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0000562	0.0000562	2.03	0.1970
TREAT	1	0.0005062	0.0005062	18.29	0.0037
DAY	3	0.0037687	0.0012562	45.39	0.0001
TREAT*DAY	3	0.0001188	0.0000396	1.43	0.3127

Appendix L: Results of analysis of variance for total solids content in whey.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	1.8366000	0.2295750	31.53	0.0001
Error	7	0.0509750	0.0072821		
Corrected Total	15	1.8875750			

R-Square	C.V.	Root MSE	TS Mean
0.972994	0.598163	0.0853	14.266

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0240250	0.0240250	3.30	0.1122
TREAT	1	0.0306250	0.0306250	4.21	0.0794
DAY	3	1.7686750	0.5895583	80.96	0.0001
TREAT*DAY	3	0.0132750	0.0044250	0.61	0.6309

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0240250	0.0240250	3.30	0.1122
TREAT	1	0.0306250	0.0306250	4.21	0.0794
DAY	3	1.7686750	0.5895583	80.96	0.0001
TREAT*DAY	3	0.0132750	0.0044250	0.61	0.6309

Appendix M: Results of analysis of variance for ash content in whey.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.2590000	0.0323750	6.49	0.0116
Error	7	0.0349000	0.0049857		
Corrected Total	15	0.2939000			

R-Square	C.V.	Root MSE	Ash Mean
0.881252	0.872531	0.0706	8.0925

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0001000	0.0001000	0.02	0.8914
TREAT	1	0.0030250	0.0030250	0.61	0.4615
DAY	3	0.2556500	0.0852167	17.09	0.0013
TREAT*DAY	3	0.0002250	0.0000750	0.02	0.9972

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0001000	0.0001000	0.02	0.8914
TREAT	1	0.0030250	0.0030250	0.61	0.4615
DAY	3	0.2556500	0.0852167	17.09	0.0013
TREAT*DAY	3	0.0002250	0.0000750	0.02	0.9972

Appendix N: Results of analysis of variance for pH of cheese curd during the study of the behavior of *L. monocytogenes* in white pickled cheese made with 8% salt.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	16	1.0607375	0.0662961	228.61	0.0001
Error	15	0.0043500	0.0002900		
Corrected Total	31	1.0650875			

R-Square	C.V.	Root MSE	pH Mean
0.995916	0.257899	0.0170	6.6031

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0004500	0.0004500	1.55	0.2320
TREAT	1	0.0300125	0.0300125	103.49	0.0001
DAY	7	1.0168875	0.1452696	500.93	0.0001
TREAT*DAY	7	0.0133875	0.0019125	6.59	0.0011

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0004500	0.0004500	1.55	0.2320
TREAT	1	0.0300125	0.0300125	103.49	0.0001
DAY	7	1.0168875	0.1452696	500.93	0.0001
TREAT*DAY	7	0.0133875	0.0019125	6.59	0.0011

Appendix O: Results of analysis of variance for $\log_{10}$ CFU/g of cheese curd during the study of the behavior of *L. monocytogenes* in white pickled cheese made with 8% salt.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	16	5.9989000	0.3749312	9.32	0.0001
Error	15	0.6036875	0.0402458		
Corrected Total	31	6.6025875			

R-Square	C.V.	Root MSE	LOG Mean
0.908568	2.626048	0.2006	7.6394

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0528125	0.0528125	1.31	0.2699
TREAT	1	0.2112500	0.2112500	5.25	0.0369
DAY	7	5.5087875	0.7869696	19.55	0.0001
TREAT*DAY	7	0.2260500	0.0322929	0.80	0.5979

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0528125	0.0528125	1.31	0.2699
TREAT	1	0.2112500	0.2112500	5.25	0.0369
DAY	7	5.5087875	0.7869696	19.55	0.0001
TREAT*DAY	7	0.2260500	0.0322929	0.80	0.5979

Appendix P: Results of analysis of variance for pH of whey during the study of the behavior of *L. monocytogenes* in white pickled cheese made with 8% salt.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	16	1.0147750	0.0634234	76.43	0.0001
Error	15	0.0124469	0.0008298		
Corrected Total	31	1.0272219			

R-Square	C.V.	Root MSE	pH Mean
0.987883	0.429201	0.0288	6.7116

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.0009031	0.0009031	1.09	0.3134
TREAT	1	0.0132031	0.0132031	15.91	0.0012
DAY	7	0.9947469	0.1421067	171.26	0.0001
TREAT*DAY	7	0.0059219	0.0008460	1.02	0.4567

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.0009031	0.0009031	1.09	0.3134
TREAT	1	0.0132031	0.0132031	15.91	0.0012
DAY	7	0.9947469	0.1421067	171.26	0.0001
TREAT*DAY	7	0.0059219	0.0008460	1.02	0.4567

Appendix Q: Results of analysis of variance for $\log_{10}$ CFU/ml of whey during the study of the behavior of *L. monocytogenes* in white pickled cheese made with 8% salt.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	16	33.460900	2.091306	19.23	0.0001
Error	15	1.631487	0.108766		
Corrected Total	31	35.092387			

R-Square	C.V.	Root MSE	LOG Mean
0.953509	5.734971	0.3298	5.7506

Source	DF	Type I SS	Mean Square	F Value	Pr > F
REP	1	0.177013	0.177013	1.63	0.2215
TREAT	1	0.720000	0.720000	6.62	0.0212
DAY	7	32.110487	4.587212	42.18	0.0001
TREAT*DAY	7	0.453400	0.064771	0.60	0.7503

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP	1	0.177012	0.177012	1.63	0.2215
TREAT	1	0.720000	0.720000	6.62	0.0212
DAY	7	32.110487	4.587212	42.18	0.0001
TREAT*DAY	7	0.453400	0.064771	0.60	0.7503

CHAPTER VI

CONCLUSIONS

Objectives of these studies were twofold: to determine the effect of white pickled cheese processing conditions on the chemical and microbial quality of the cheese, and to develop salt-resistant mutants of lactic streptococci for use in high salt white pickled cheese.

When cheese was made with 4% NaCl and preserved in 4% (w/w) brine solution, starter culture produced acid that inhibited growth of *L. monocytogenes, S. aureus* and *S. typhimurium*. In cheese made with 8% NaCl, high salt levels inhibited growth of salt-sensitive lactic acid bacteria such that they were not able to produce acid, and therefore *L. monocytogenes* was able to grow. Additionally, antimicrobials nisin, H_2O_2 and potassium sorbate did not affect pathogens under conditions evaluated.

The use of starter culture was a key factor in white pickled cheesemaking. The use of 4% salt initially, followed by preserving in 4% brine solution is recommended. Traditionally, white pickled cheese is made from raw milk with high salt levels (6-20%). This practice will not produce a safe product if milk is contaminated. High salt does not inhibit pathogenic microorganisms, and also inactivates naturally occurring microflora in milk. Salt-sensitive lactic

acid bacteria are particularly affected by high salt levels. If lactic acid bacteria grow, they may inhibit pathogens in cheese during pickling. The antimicrobials used in this study did not inhibit the pathogens because these antimicrobials are not highly active at pH of the product evaluated. To achieve antimicrobial activity, the pH of the product should be decreased. This could be accomplished by addition of starter culture or by direct addition of acids. However, some starter bacteria are sensitive to some antimicrobials and their incorporation into cheese together with antimicrobials without knowledge of their effect will likely not be advantageous. Since starter culture alone can inhibit pathogenic microflora if stored at least 50 d, there is little value for the added cost of antimicrobials in manufacture of white pickled cheese. Addition of antimicrobials will only be economical if cultures of lactic acid bacteria are not available and direct addition of acid is necessary.

Experiment were conducted to produce salt-resistant mutants of lactic streptococci. A single strain of Lactic streptococci isolated from commercial culture, was found to be *L. lactis ssp. cremoris*. After five replications, mutants were not produced. For future research, it may be necessary to isolate and identify different strains of lactic acid bacteria from traditionally fermented food products. Then mutation could be induced to these strains using a variety of chemical and physical mutagens. In addition to salt-

resistance, other flavor characteristics may be pursued. To produce a safer product, that still meets consumer desires, starter cultures capable of tolerating high salt levels and producing lactic acid should be developed. Consumer education programs could be conducted to convince the consumer to use lower salt cheese so that presently available starter cultures can be added.

VITA

Mohamed Osman M. Abdalla was born in 1960 in Bent Elhaj, Sudan, in an agricultural environment where he entered Quranic school (Khalwah) under his father's supervision. Upon graduation from high school, he entered the University of Khartoum, Faculty of Agriculture and received an honored Bachelor of Science degree in Agricultural Biochemistry in 1983. In 1984, he joined the Ministry of Education as a high school teacher and in 1985, he accepted a job as a teaching assistant at the University of Khartoum, Institute of Animal Production. In May 1985, he started his graduate program in the Institute of Animal Production and completed the work for the Master of Science in Dairy Science in May, 1987. In December, 1987, he was awarded a scholarship from the University of Khartoum to study abroad. In June, 1988, he continued his graduate program in the Department of Food Science and Technology at the University of Tennessee, Knoxville and was awarded the Ph.D. in December, 1992. He is a member of The Muslim Students' Association; Institute of Food Technologists; International Association of Milk, Food and Environmental Sanitarians; and Gamma Sigma Delta.