Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

# Survival of *Staphylococcus aureus* During the Manufacture and Ripening of Camembert Cheese

A thesis presented in partial fulfilment of the requirements for the degree of Master of Food Technology

Massey University Palmerston North, New Zealand

> Zhetong Kang 2017

#### Abstract

Staphylococcal Food Poisoning (SFP) is the third most common cause of food poisoning internationally, caused by an enterotoxin produced by *Staphylococcus aureus*. *S. aureus* contamination in dairy products, including cheese, can lead to SFP. The survivability of *S. aureus* during the manufacture and ripening of Camembert cheese was the focus of this study. Camembert cheeses were manufactured using pasteurized milk inoculated with one of three *S. aureus* strains, comprising two reference strains ATCC 4163, ATCC 9144 and one dairy strain 172 RR. Each strain was tested in triplicate. The results showed that manufacturing and ripening of Camembert cheese reduced the risk of food safety associated with contamination with *S. aureus* with a 1.6 to 3.1 log reduction. The largest decrease occurred following drainage, which was particularly evident in 172 RR, and coincided with the lowest pH. The combined effect of culture blend (starter and secondary flora) activity and low pH are believed to contribute to the death of *S. aureus*.

First and foremost, I would like to express my deep sense of appreciation to Professor Steve Flint, my supervisor, for the valuable guidance and encouragement every week over the last year, and the critical advice on the writing of this thesis. It was and will always be an honour to be his student and I appreciated learning from him.

In the microbiology lab, I especially wish to thank Ann-Marie Jackson, Kylie Evans, and Haoran Wang for the assistance they kindly provided. Their knowledge and experience have helped me a lot during my study. In particular, I am grateful to Ann-Marie Jackson and Haoran Wang for providing the *S. aureus* strains and Baird-Parker agar plates.

I would like to thank Christine Ramsay for helping me order all experimental kits and milk. I also thank Steve Glasgow for his guidance and assistance in the chemistry lab.

Finally, I am extremely grateful to my parents for their unconditional support and encouragement.

#### Abbreviations

AOC	appellation d'origine contrôlée
АРНА	American Public Health Association
a <sub>w</sub>	water activity
CFU/g	colony forming units per gram
СМР	case inomacropeptide
g	gram
h	<u>hour (s)</u>
min	<u>minute (s)</u>
MSSA	methicillin susceptible Staphylococcus aureus
NSLAB	non-starter lactic acid bacteria
SC+	coagulase-positive staphylococci
SFP	Staphylococcal food poisoning

## Table of Contents

A	bstract	t		1
A	cknow	ledge	ements	2
A	bbrevi	ation	S	3
Т	able of	Cont	tents	4
Li	ist of Fi	igure	S	7
Li	ist of Ta	ables		10
1	Intr	oduc	tion	14
	1.1	Rese	earch questions	14
	1.2	Нур	othesis	14
	1.3	Purp	pose of Research	14
2	Lite	ratur	e Review	15
	2.1	Stap	phylococcus aureus	15
	2.1.	.1	Emergence of Staphylococcal Food Poisoning (SFP)	15
	2.1.	.2	Growth Boundaries of Staphylococcus aureus	16
	2.1.	.3	Survival and resistance	17
	2.1.	.4	Presence of Staphylococcus aureus in Cheese and food poisoning Outbre	eaks 18
	2.1.	.5	Anti-staphylococcal Activity of Starter Lactic Acid Bacteria (LAB)	19
	2.2	Carr	nembert Cheeses	20
	2.2.	.1	Background	20
	2.2.	.2	Manufacturing Process, Technology and Biochemical Changes	21
	2.2.	.3	Biochemistry of Camembert Ripening	26
	2.3	Sum	nmary and Hypothesis	35
3	Ma	terial	s and Methods	36
	3.1	Mar	nufacture of Camembert Cheeses	36

3.1.1	Materials and Equipment36
3.1.2	Experimental design
3.1.3	Sterilisation
3.1.4	Cheese Curd Preparation and <i>S. aureus</i> Inoculation37
3.1.5	Cutting, Moulding and Draining the Curds
3.1.6	Salting the cheese
3.1.7	Maturing the Cheeses
3.1.8	Flow Chart of Cheese Manufacture
3.2 Ba	cterial Enumeration40
3.2.1	Experimental Design40
3.2.2	Plate Count Method40
3.2.3	Procedure40
3.2.4	Physico-chemical Analysis41
4 Results	
4.1 Sui	rvival of S. aureus During the Manufacture and Ripening Period of Camembert
Cheese	
4.1.1	Early Stages of Cheese Manufacture43
4.1.2	Survival of <i>S. aureus</i> in different part of the cheese46
4.2 Sal	t content74
4.3 Co	ntrol groups (Cheeses Without Culture Blend)74
4.3.1	Surface of the controlled Cheeses75
4.3.2	Core of the Controlled cheeses84
5 Discuss	ion93
5.1 Re:	sults discussion93
5.1.1 cheese	Survival of <i>S. aureus</i> During the Manufacture and Ripening of Camembert 93

	5.2	Imp	portance and Methods for Preventing the Contamination of Camembert chee	ese
	with S	5. au	reus	95
6	Con	nclus	ion	98
	6.1	Lim	nitations and Further recommendations	98
7	Bibl	liogr	aphy	99
8	Арр	pend	lix1	.07
	8.1	Bac	cterial Enumeration1	.07
	8.1.	.1	Regular Camembert Cheeses1	.07
	8.1.	.2	Control groups1	.27
	8.2	Cal	culations1	.30
	8.2.	1	T-tests Results Between Strains1	.30
	8.2.	.2	Salt content	.34

# List of Figures

Figure 1 The number of reported foodborne S. aureus outbreaks out of all associated cases
during 2007 and 2016 in New Zealand ((Pattis et al., 2017)16
Figure 2 Relation between $a_w$ and pH of different cheeses (Shaw, 1981)22
Figure 3 Changes caused by the growth of P. camemberti during the ripening of Camembert
cheese (McSweeney, 2004)28
Figure 4 Changes in pH during the ripening of Camembert (P. F. Fox et al., 2004)30
Figure 5 Changes in the activities of the aspartate proteinases and the metalloproteinase
during the ripening of Camembert (Guinee & Fox, 1987; Spinnler & Gripon, 2004)32
Figure 6 Catabolism of phenylalanine (McSweeney, 2004)33
Figure 7 Pathways for the catabolism of leucine and the formation of volatile flavour
compounds (McSweeney, 2004)
Figure 8 Cutting and ladle the cheese curds before drainage
Figure 9 Survival of S. aureus at the beginning stages of Camembert cheese manufacture44
Figure 10 Changes of pH at the early stages of Camembert cheese manufacture45
Figure 11 Changes of $a_w$ at the beginning stages of Camembert cheese manufacture46
Figure 12 Survival of S. aureus at the mould rind of Camembert cheese throughout
manufacture and ripening stages48
Figure 13 Survival of S. aureus in the rind of Camembert during the ripening period49
Figure 14 Changes of pH at the mould rind of Camembert cheese throughout manufacture
and ripening50
Figure 15 Changes of pH in the rind of Camembert cheese during manufacture51
Figure 16 Changes of pH in the rind of Camembert cheese during the ripening period52
Figure 17 Changes of $a_w$ at the mould rind of Camembert cheese throughout the manufacture
and ripening period53
Figure 18 Changes of $a_w$ at the mould rind of Camembert cheese during manufacture54
Figure 19 Changes of $a_w$ at the mould rind of Camembert cheese during the ripening period
Figure 20 Survival of S. aureus at the Surface of Camembert cheese throughout manufacture
and ripening stage

Figure 21 Survival of S. aureus at the surface of Camembert cheese during manufacture57
Figure 22 Survival of S. aureus at the surface of Camembert cheese throughout ripening
period58
Figure 23 Changes of pH at the surface of Camembert cheese throughout the manufacture
and ripening period59
Figure 24 Changes of pH at the surface of Camembert cheese during manufacture60
Figure 25 Changes of pH at the Surface of Camembert cheese during ripening period61
Figure 26 Changes of $a_w$ at the surface of Camembert cheese throughout the manufacture
and ripening period62
Figure 27 Changes of $a_w$ at the surface of Camembert cheese during manufacture63
Figure 28 Changes of $a_w$ at the surface of Camembert cheese during the ripening period64
Figure 29 Survival of S. aureus at the core of Camembert cheese throughout manufacture and
ripening stage65
Figure 30 Survival of S. aureus at the core of Camembert cheese during manufacture66
Figure 31 Survival of S. aureus at the core of Camembert cheese throughout the ripening
period67
Figure 32 Changes of pH at the core of Camembert cheese throughout the manufacture and
ripening period68
Figure 33 Changes of pH at the core of Camembert cheese during manufacture69
Figure 34 Changes of pH at the core of Camembert cheese during the ripening period70
Figure 35 Changes of water activity at the core of Camembert cheese throughout the
manufacture and ripening period71
Figure 36 Changes of $a_w$ at the core of Camembert cheese during manufacture72
Figure 37 Changes of $a_w$ at the core of Camembert cheese during the ripening period73
Figure 38 Salt content in cheese samples at the 14 days of ripening74
Figure 39 Survival of S. aureus at the surface of controlled Camembert cheese (without culture
blend) throughout manufacture and ripening period75
Figure 40 Survival of S. aureus at the surface of controlled Camembert cheese (without culture
blend) during manufacture76
Figure 41 Survival of S. aureus at the surface of controlled Camembert cheese (without culture
blend) during ripening period77

Figure 42 Changes of pH at the surface of controlled Camembert cheese (without culture
blend) throughout manufacture and ripening period78
Figure 43 Changes of pH at the surface of controlled Camembert cheese (without culture
blend) during manufacture
Figure 44 Changes of pH at the surface of controlled Camembert cheese (without culture
blend) during ripening period80
Figure 45 Changes of $a_{\rm w}$ at the surface of controlled Camembert cheese (without culture
blend) throughout manufacture and ripening period81
Figure 46 Changes of pH at the surface of controlled Camembert cheese (without culture
blend) during manufacture
Figure 47 Changes of $a_{\rm w}$ at the surface of controlled Camembert cheese (without culture
blend) during ripening period83
Figure 48 Survival of S. aureus at the core of control Camembert cheese (without culture
blend) throughout manufacture and ripening period84
Figure 49 Survival of S. aureus at the core of control Camembert cheese (without culture
5
blend) during manufacture
blend) during manufacture

### List of Tables

Table 1 Conditions for the growth of <i>S. aureus</i> and production of toxin (Stewart, 2003)17
Table 2 Storage categories of products based on pH and a <sub>w</sub> (Shaw, 1981)22
Table 3 Viable Cells (CFU/g) at the beginning stages of Camembert cheese manufacture $107$
Table 4 Log Viable Cell (log CFU/g) at the beginning stages of Camembert cheese manufacture
Table 5 Viable Cells (CFU/g) at the mould Rind of Camembert cheese during manufacture and
ripening
Table 6 Log Viable Cell (log CFU/g) at the mould Rind of Camembert cheese during
manufacture and ripening109
Table 7 Viable Cells (CFU/g) at the Surface of Camembert cheese during manufacture and
ripening
Table 8 Log Viable Cell (log CFU/g) at the Surface of Camembert cheese during manufacture
and ripening110
Table 9 Viable Cells (CFU/g) at the Core of Camembert cheese during manufacture and
ripening
Table 10 Log Viable Cell (log CFU/g) at the Core of Camembert cheese during manufacture
and ripening111
Table 11 Viable Cells (CFU/g) at the beginning stages of Camembert cheese manufacture 112
Table 12 Table Log Viable Cell (log CFU/g) at the beginning stages of Camembert cheese
manufacture113
Table 13 Mean Values of all three trials of Each Strain at different manufacturing stages113
Table 14 Viable Cells (CFU/g) at the mould Rind of Camembert cheese during manufacture
and ripening114
Table 15 Log Viable Cell (log CFU/g) Viable Cells (CFU/g) at the mould rind of Camembert
cheese during manufacture and ripening114
Table 16 Mean Values of all three trials from Each Strain at different time points115
Table 17 Viable Cells (CFU/g) at the surface of Camembert cheese during manufacture and
ripening

Table 18 Log Viable Cell (log CFU/g) at the surface of Camembert cheese during manufacture
and ripening
Table 19 Mean Values of all three trials from Each Strain at different manufacturing stages
Table 20 Viable Cells (log CFU/g) at the core of Camembert cheese during manufacture and
ripening
Table 21 Log Viable Cell (log CFU/g) at the core of Camembert cheese during manufacture
and ripening
Table 22 Mean Values of all three trials from Each Strain at different manufacturing stages
Table 23 pH at the beginning stages of Camembert cheese manufacture
Table 24 Mean Values of all three trials from Each Strain at different manufacturing stages
Table 25 pH of Camembert cheese mould rind during manufacture and ripening.    120
Table 26 Mean Values of all three trials from Each Strain at different manufacturing stages
Table 27 pH at the surface of Camembert cheese during manufacture and ripening
Table 28 Mean Values of all three trials from Each Strain at different manufacturing stages
Table 29 pH at the core of Camembert cheese during manufacture and ripening122
Table 30 Mean Values of all three trials from Each Strain at different manufacturing stages
Table 31 Water activity at the beginning stages of Camembert cheese manufacture123
Table 32 Mean Values of all three trials from Each Strain at different manufacturing stages
Table 33 Water activity at the mould Rind of Camembert cheese during manufacture and
ripening124
Table 34 Mean Values of all three trials from Each Strain at different manufacturing stages
Table 35 Water activity at the mould Rind of Camembert cheese during manufacture and
ripening

Table 36 Mean Values of all three trials from Each Strain at different manufacturing stages Table 37 Water activity at the mould Rind of Camembert cheese during manufacture and Table 38 Mean Values of all three trials from Each Strain at different manufacturing stages Table 39 Viable Cells (CFU/g) at the surface of controlled Camembert cheese (without culture blend) during manufacture and ripening. .....127 Table 40 Log Viable Cells (log CFU/g) at the surface of controlled Camembert cheese (without culture blend) during manufacture and ripening. .....127 Table 41 Viable Cells (CFU/g) at the core of controlled Camembert cheese (without culture blend) during manufacture and ripening ......127 Table 42 Log Viable Cells (CFU/g) at the core of controlled Camembert cheese (without culture blend) during manufacture and ripening ......128 Table 43 pH at the surface of controlled Camembert cheese (without culture blend) during Table 44 pH at the core of controlled Camembert cheese (without culture blend) during Table 45 Water activity at the surface of controlled Camembert cheese (without culture blend) during manufacture and ripening ......129 Table 46 Water activity at the core of controlled Camembert cheese (without culture blend) during manufacture and ripening......129 Table 47 T-tests results of S. aureus between different strains at the mould rind of cheeses Table 48 T-tests results of S. aureus between different strains at the surface of cheeses...130 Table 49 T-tests results of *S. aureus* between different strains at the core of cheeses ......130 Table 50 T-tests results of S. aureus between different strains at the surface of controlled Table 51 T-tests results of S. aureus between different strains at the core of controlled cheeses (without culture blend)......131 Table 52 T-tests results of pH between different strains at the mould rind of cheeses ......131 

Table 54 T-tests results of pH between different strains at the core of cheeses132
Table 55 T-tests results of pH between different strains at the surface of controlled cheeses
(without culture blend)132
Table 56 T-tests results of pH between different strains at the core of controlled cheeses
(without culture blend)132
Table 57 T-tests results of water activity between different strains at the mould rind of
cheeses
Table 58 T-tests results of water activity between different strains at the surface of cheeses
Table 59 T-tests results of water activity between different strains at the surface of cheeses
Table 60 T-tests results of water activity between different strains at the surface of controlled
cheeses (without culture blend)134
Table 61 T-tests results of water activity between different strains at the core of controlled
cheeses (without culture blend)134
Table 62 Volume (ml) usage of ammonium thiocyanate during titration134