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.

The survival and growth of *Bacillus cereus* and *Listeria monocytogenes*, during the manufacture of Ricotta Salata cheese

A thesis presented in partial fulfilment of the requirements for the degree of Masters in Food Technology at Massey University, Palmerston North, New Zealand

> Boyuan Su 2017

Abstract

This study was conducted with the following objectives: 1) to investigate the survival and growth of *Bacillus cereus* during the manufacture of Ricotta Salata cheese; and 2) to investigate the survival and growth of *Listeria monocytogenes* during the manufacture of Ricotta Salata cheese.

The Ricotta Salata cheese was made by heating the whole milk to 95° C, and adding citric acid to coagulate the cheese curd. The cheese curd was inoculated with 7 \log_{10} CFU/g *B. cereus* broth and 8 \log_{10} CFU/g *L. monocytogenes* broth. After moulding for 12h, Ricotta Salata cheese was stored at 4° C for 1 week. During manufacture, the physico-chemical properties [pH, water activity (a_w), and Sodium chloride (NaCl) concentration] and bacterial counts were recorded.

The pH change fluctuated between 6.00 to 6.10 on the surface and 6.00 to 5.95 in the centre; the lowest a_w was approximately 0.96 on the surface and 0.97 in the centre; and the highest NaCl concentration was 3.3% on the surface and 3% in the centre.

The survival and growth of the two *B. cereus* strains (D1 and ATCC 13061) during the manufacture of Ricotta Salata cheese were similar. The *B. cereus* grew from approximately 5 log_{10} CFU/g to a maximum of 7.7 log_{10} CFU/g of cheese curd during moulding (20h at room temperature).

The survival and growth of the two *L. monocytogenes* strains (W1 and ATCC 35152) during the manufacture of Ricotta Salata cheese were similar. The difference between the bacteria count on the surface and in the centre was very small. *L. monocytogenes* increased from 5 to 6 \log_{10} CFU/g to a maximum of 8.6 \log_{10} CFU/g during manufacture and maintained a level of around 8 \log_{10} CFU/g in the final product.

The Ricotta Salata supported the survival and growth of *B. cereus* and *L. monocytogenes* during manufacture. It is important to improve the management of process hygiene for reducing the environmental contamination. Ideally, some lethal treatments should be

applied after the packaging of the cheese, to limit the contamination of Ricotta Salata with these two bacteria.

Table of Contents

Αl	bstract	ii
Lis	st of Figures	vi
Lis	st of Tables	viii
Lis	st of Appendices	ix
1.	. Introduction	1
2.	Literature review	2
	2.1 Manufacture processes for Ricotta Salata cheese	2
	2.2 Physico-chemical parameters of Ricotta Salata cheese	4
	2.21 Temperature	4
	2.22 pH value	5
	2.23 A _w value	6
	2.24 NaCl concentration	6
	2.3 Pathogen growth in Ricotta Salata cheese	7
	2.31 B. cereus	7
	2.32 L. monocytogenes	8
	2.4 Food poisoning cases and outbreaks associated with consumption of food contaminated B. cereus and L. monocytogenes	
	2.41 B. cereus	9
	2.42 L. monocytogenes	11
	2.5 Hazard Analysis Critical Control Points (HACCP)	14
	2.51 HACCP system	14
	2.52 Hazard analysis	14
	2.53 Critical control points determination	15
	2.54 HACCP control chart	15
	2.6 Microbiological hurdle technology	15
	2.61 Heat treatment	15
	2.62 pH value	15
	2.63 A _w value	16
	2.64 NaCl concentration	16
	2.6 Shelf life and consumption of Ricotta Salata cheese	16
3.	. Materials and methods	18
	3.1 Materials	18
	3.2 Microorganism	18

3.3 Experimental design	19
3.4 Preparation of Ricotta Salata cheese	20
3.41 Pasteurised milk heating	20
3.42 Coagulation	20
3.43 Artificial inoculation	20
3.44 Moulding and draining	21
3.45 Salting	21
3.46 Aging	21
3.5 Incubation of Ricotta Salata cheese	21
3.6 Physico-chemical analysis of Ricotta Salata cheese	22
3.7 Statistical analysis	23
4. Results	23
4.1 Selected physicochemical properties of Ricotta Salata cheese	23
4.2 NaCl concentration of Ricotta Salata cheese	24
4.3 Inoculation of bacteria	25
4.4 pH change during the manufacture of Ricotta Salata inoculated with different strains	25
$4.5\ a_w$ change during the manufacture of Ricotta Salata inoculated with different strains	31
4.6 Bacteria count change during the manufacture of Ricotta Salata inoculated with different strains	34
4.7 B. cereus	37
4.8 L. monocytogenes	39
5.0 Discussion	41
References	48
Annendices	55

List of Figures

Figure No.	Title	Page
1	Process flow of traditional Ricotta Salata cheese made	3
	from whey	
2	Process flow of the manufacture of Ricotta Salata	19
	cheese made from whey	
3	The NaCl concentration of Ricotta Salata	24
4	The pH change during the manufacture of Ricotta	27
	Salata inoculated with B. cereus D1 isolated from food	
	factory at 4°C (surface) (mean±SD)	
5	The pH change during the manufacture of Ricotta	28
	Salata inoculated with B. cereus D1 isolated from food	
	factory at 4°C (centre) (mean±SD)	
6	The pH change during the manufacture of Ricotta	28
	Salata inoculated with <i>B. cereus</i> (ATCC13061) at 4° C	
	(surface) (mean±SD)	
7	The pH change during the manufacture of Ricotta	29
	Salata inoculated with <i>B. cereus</i> (ATCC13061) at 4° C	
	(centre) (mean±SD)	
8	The pH change during the manufacture of Ricotta	30
	Salata inoculated with <i>L. monocytogenes</i> W1 at 4°C	
	(surface) (mean±SD)	
9	The pH change during the manufacture of Ricotta	31
	Salata inoculated with <i>L. monocytogenes</i> W1 at 4°C	
	(centre) (mean±SD)	
10	The a _w change during the manufacture of Ricotta	32
	Salata inoculated with <i>B. cereus</i> D1 isolated from food	
	factory at 4°C (surface) (mean±SD)	
11	The a _w change during the manufacture of Ricotta	32
	Salata inoculated with <i>B. cereus</i> D1 isolated from food	
	factory at 4°C (centre) (mean±SD)	
12	The a _w change during the manufacture of Ricotta	33
	Salata inoculated with <i>L. monocytogenes</i> W1 at 4°C	
	(surface) (mean±SD)	
13	B. cereus count in the surface samples taken during	34
	the manufacture of Ricotta Salata inoculated with <i>B.</i>	
	cereus D1 over a 308h period (mean±SD).	
14	B. cereus count in the centre samples taken during the	35
	manufacture of Ricotta Salata inoculated with <i>B.</i>	
	cereus D1 over a 308h period (mean±SD).	

Figure No.	Title	Page
15	L. monocytogenes count in the surface samples taken during the manufacture of Ricotta Salata inoculated with L. monocytogenes W1 over a 308h period (mean±SD).	36
16	L. monocytogenes count in the surface samples taken during the manufacture of Ricotta Salata inoculated with L. monocytogenes (ATCC 35152) over a 308h period (mean±SD)	36
17	The pH change during the manufacture of Ricotta Salata inoculated with <i>B. cereus</i> D1 and ATCC 13061 (mean±SD)	37
18	The a_w change during the manufacture of Ricotta Salata inoculated with $B.\ cereus\ D1$ and ATCC 13061 (mean±SD)	38
19	The bacteria count change during the manufacture of Ricotta Salata inoculated with <i>B. cereus</i> D1 and ATCC 13061 (mean±SD)	39
20	The pH change during the manufacture of Ricotta Salata inoculated with <i>L. monocytogenes</i> W1 and ATCC 35152 (mean±SD)	39
21	The a_w change during the manufacture of Ricotta Salata inoculated with $L.$ monocytogenes W1 and ATCC 35152 (mean±SD)	40
22	L. monocytogenes counts taken during the manufacture of Ricotta Salata inoculated with L. monocytogenes W1 and ATCC 35152 over a 308h period (mean±SD).	41

List of Tables

Table No.	Title	Page
1	Intrinsic properties (mean±SD) and composition	6
	(%±SD) evolution of Ricotta Salata cheeses	
2	Hazard analysis chart	12
3	HACCP control chart for Ricotta Salata cheese	17
4	pH, a _w , and NaCl concentration of Ricotta Salata	26
	during manufacture (mean±SD)	

List of Appendices

Appendix	Title	Page
Figure A1	The pH change during the manufacture of Ricotta Salata inoculated with <i>L. monocytogenes</i> (ATCC 35152) at 4°C (surface) (mean±SD)	55
Figure A2	The pH change during the manufacture of Ricotta	55
rigure A2	Salata inoculated with <i>L. monocytogenes</i> (ATCC	33
	35152) at 4°C (centre) (mean±SD)	
Figure A3	The a _w change during the manufacture of Ricotta	56
	Salata inoculated with <i>B. cereus</i> (ATCC13061) at 4°C	
	(surface) (mean±SD)	
Figure A4	The a _w change during the manufacture of Ricotta	56
	Salata inoculated with <i>B. cereus</i> (ATCC13061) at 4°C	
	(centre) (mean±SD)	
Figure A5	The bacteria count change during the manufacture of	57
	Ricotta Salata inoculated with B. cereus (ATCC13061)	
	at 4°C (surface) (mean±SD)	
Figure A6	The bacteria count change during the manufacture of	57
	Ricotta Salata inoculated with B. cereus (ATCC13061)	
	at 4°C (centre) (mean±SD)	
Figure A7	The a _w change during the manufacture of Ricotta	58
	Salata inoculated with <i>L. monocytogenes</i> W1 at 4°C	
	(centre) (mean±SD)	
Figure A8	The a _w change during the manufacture of Ricotta	58
	Salata inoculated with <i>L. monocytogenes</i> (ATCC	
	35152) at 4°C (surface) (mean±SD)	
Figure A9	The a _w change during the manufacture of Ricotta	59
	Salata inoculated with L. monocytogenes (ATCC	
	35152) at 4°C (centre) (mean±SD)	
Figure A10	The bacteria count change during the manufacture of	59
	Ricotta Salata inoculated with L. monocytogenes W1	
	at 4°C (centre) (mean±SD)	
Figure A11	The bacteria count change during the manufacture of	60
	Ricotta Salata inoculated with L. monocytogenes	
	(ATCC 35152) at 4°C (centre) (mean±SD)	