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EFFECT OF LAURIC ACID AND MONOLAURIN ON THE MULTIPLICATION OF *LISTERIA MONOCYTOGENES* AND *LISTERIA INNOCUA* AT 10 °C IN BI-PHASIC SYSTEMS

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A Thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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This work is dedicated to my friends and family whose support and love, especially over the Summer of 1997, have given me the encouragement to complete this research.

Summary

The effect of monolaurin (0.35 mM) and lauric acid (5.0 mM) alone and in combination has been tested on growth of three cheeseborne strains of *Listeria monocytogenes* and two strains of *L. innocua*. The *Listeria* spp. were grown in Tryptose Soya Yeast Glucose Broth (TSGYB) in shake culture at 10 °C and an initial pH of 7.0. The additives were dissolved in butter oil 10 % (w/v). Lauric acid (5.0 mM) increased the doubling time of the five *Listeria* spp. by 3 – 8 h at 10 °C. Monolaurin by itself was found to slightly increase or decrease the doubling time depending on the microbial strain. Monolaurin had an augmentative effect when combined with lauric acid in the presence of butter oil, where the doubling time increased between 10 to 15 h depending on the strain.

Inhibition of *Listeria* spp. was seen in the model bi-phasic broth system. A model food system was developed to test the antimicrobial properties of lauric acid and monolaurin, where the fat soluble additives were dissolved in cream and milk with 3.6% (w/v) fat. The milk was reconstituted from skim milk and cream 40 % (w/v) fat containing lauric acid or monolaurin. This milk was used to make a soft-ripened cheese of the Brie Camembert type. Two strains of *L. innocua* were added to the reconstituted milk. During production of the soft-ripened cheese, a draining table was designed to comply with COSHH regulations so that the whey containing *L. innocua* could be removed and disposed of by heating for 30 min at 121 °C.

In cheeses without lauric acid or monolaurin the population of *L. innocua* increased from 10^3 g^{-1} to 10^7 cfu g^{-1} on the surface of the cheese. The counts in the centre of the cheese were less at 10^5 cfu g^{-1} after ripening for 28 d at 10 °C. Addition of 0.9 mM monolaurin reduced the count to 10^5 cfu g^{-1} after 28 d ripening at 10 °C on the surface of the cheese. The effect of increasing the initial draining time at ambient temperature from 24 h to 48 h reduced the population to 0 after 28 d ripening at 10 °C. Unlike experiments in broth culture, addition of lauric acid changed the aroma of the Camembert-type cheese to give a blue cheese aroma. This was due to the conversion of lauric acid to a methyl ketone (2-undecanone) by the starter fungus *Penicillium camembertii*. Due to lack of stability of lauric acid in this system, lauric acid was omitted from the reconstituted milk in further experiments. During production of cheese, lactose was converted to mainly lactic acid by metabolism of the lactic acid starter. The presence of lactic acid combined with the added monolaurin resulted in a significant reduction in the population of *L. innocua* particularly when the draining time was increased from 24 h to 48 h.

The unusual approach in this study was to dissolve the biocide in the non-aqueous phase, butter oil in the experiments in broth culture and in cream in the model cheese experiments. An untrained taste panel detected monolaurin (0.9 mM) in soft-ripened cheese. Some respondents liked the 'mature' taste whilst others described it as 'farmyard like'. In food systems the use of antimicrobials which result in an increase in the lag phase or a reduction in the overall population of pathogens, has a significant role in promoting the microbiological safety of a product which is eaten without further heat treatment.

Advanced studies, conferences and publications undertaken in connection with this research

Advanced Courses

1. Attendance at a series of computer courses for staff which were held at Sheffield Hallam University in 1995 (Word 6, Powerpoint, Excel, Access, the Internet).

2. The following courses were taken for credit, combined studies course in Statistics, Mathematics and Information Technology, Cell Biology, and the Biochemistry courses given by Department of Biomedical Sciences, Sheffield Hallam University (Sheffield, January to June 1996). The overall grades were, 85 %, 70 % and 72 % respectively.

3. A workshop was taken on Teaching and Learning in Higher Education, which was given by the School of Education, Sheffield Hallam University (Sheffield, May 1996).

4. A two week course in Chromatography (part of a MSc course), given by the Department of Chemistry, University of Warwick , was taken in September 1995. The overall grade was 84 %.

Conferences

1. Attendance at the winter Symposium which were organised by Society for Applied Bacteriology, Royal Society of Medicine, London, January 1995, 1996 and 1998.

2. † Summer Meeting, Symposium: **The Biology of Streptococci and Enterococci**, organised by Society for Applied Bacteriology, at the University of Bradford, Bradford, 15 – 18 July 1996.

3. †**Lipids in Health and Nutrition**, conference organised by Royal Society for Chemistry (Oils and Fats group), at Sheffield Hallam University, Sheffield. 9 – 10 September 1996.

4. † **Microbiology for the Hospitality Industry**, organised by the Society for Applied Bacteriology, 14 – 15 Belgrave Square, London. 13 March 1997.

† A studentship was awarded to attend these conferences.

Other

1. Visit to Stilton cheese factory to see how Blue cheese was made, at Melton Mowbray on 17 April 1996.

Publication

Pauline A. Burgess, Andrew Page and Judith L. Kinderlerer (1996) Effect of lauric acid and monolaurin on growth of cheeseborne strains of *Listeria monocytogenes* and *Listeria innocua*, poster presented at the Summer Conference, organised by the SAB, University of Bradford, 15 – 18 July. Awarded first prize for the best poster, Organon Teknika Prize.

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List of Abbreviations

°C	Degrees centigrade
%	Percent
µL	Microlitre
µm	Micrometre
α-MAN	α-Mannoside
ATP	Adenosine Triphosphate
BHI	Beef Heart Infusion broth
cm	Centimetre
Cl^-	Chloride ion
d	days
DAG	Diacylglycerol
DIM	Differentiate between *L. innocua* and *L. monocytogenes*
EC	European Community
ESC	Aesculin
Fe^{3+}	Ferric iron
g	Gram
H^+	Hydrogen ion
h	Hour
HACCP	Hazards Analysis of Critical Control Points
HBA	Horse Blood Agar
mM	Millimolar
L	Litre
LA	Lauric acid
L. innocua A7	NCIMB 1350, isolated from Brie cheese by JLK
L. innocua NCTC (11288)	Obtained from national collection of type cultures by JLK
L. monocytogenes A3	NCIMB 13449 (Isolated from Fourme d'Ambert cheese)
L. monocytogenes A13	Isolated from Brie cheese by JLK
L. monocytogenes LB	NCIMB 1351 (Isolated from Lanark Blue Cheese by P. Finner)
L. monocytogenes BL/88/1b	Obtained from Dr B. M. Lund, IFR
MAG	Monoacylglycerol
MCFA	Medium Chain Fatty Acid
MIC	Minimum Inhibitory Concentration
min	Minute
mL	Mililitre
ML	Monolaurin
mm	Milimetre
NaCl	Sodium chloride
NADH	Nicotinamide Adeine dinucleotide (reduced form)
NBP	Neutralised Bacteriological Peptone
ND	Not detected
OA	Oxford Agar
P. camembertii	*Penicillium camembertii*
pH	Logarithmic index for hydrogen ion concentration
pKa	Negative logarithm of equilibrium constant
P. roquefortii	*Penicillium roquefortii*
SD	Standard Deviation

T	Temperature
t	Time
TSA	Tryptose Soya Agar
TSB	Tryptose Soya Broth
TSGYA	Tryptose Soya Glucose Yeast Agar
TSGYB	Tryptose Soya Glucose Yeast Broth
v/v	Volume / volume
w/v	Weight / volume

CHAPTER ONE

'The goal of food microbiology is to minimise or prevent microbial growth rather than optimise it, as often occurs in biotechnology. Consequently, effects of the inhibitory environmental factors, like preservatives, have been investigated more intensively'.
(Baranyi and Roberts 1994)

Introduction

Section 1. The incidence and importance of *Listeria* spp. in soft mould-ripened cheese

1.1 *Listeria* spp.

Listeria spp. are small, short, Gram positive rods, 0.4-0.5 μm in diameter and 0.5-2 μm in length, with rounded ends and 4 peritrichous flagella. The organisms are motile. Mobility occurs at 20 °C but not at 25 °C and above (Lovett 1989). Motility is characterised by a cork-screw like tumbling movement when viewed with the light microscope. Currently seven species of listeria are recognised, *Listeria monocytogenes, L. innocua, L. ivanovii, L. seeligeri, L. welshimeri, L. grayi* and *L. murrayi* (Schuchat *et al.*1991). There are at least 13 serotypes in this genus. Serotyping is based on the serological grouping of flagella (H) antigens and somatic (0) antigens (Schuchat *et al.* 1991). Of the 13 serotypes 1, 2a, 1b, 2b and 4b are responsible for most human cases of the disease listeriosis (Jones 1990).

Table 1.1. The effect of temperature, pH and salt content on growth of *L. monocytogenes*

	Temperature (°C)	pH	Salt (%)
Range	1 – 45	5 – 9	10
Optimum	30– 37		

(adapted from Lund 1990)

Listeria spp. are catalase positive, oxidase negative and produce cytochromes a, b and d (Feresu and Jones 1988). They ferment glucose and other sugars mainly to give L(+)-lactic acid without the production of gas. *Listeria* spp. are methyl red positive, Voges-Proskauer positive and indole negative. Aesculin and sodium hippurate are hydrolysed by this species (Seeliger and Jones 1986).

Listeria monocytogenes is an important pathogen in the food industry. *Listeria monocytogenes* can grow over a wide range of environmental conditions, in particular at refrigeration temperatures of 4 - 5°C (Table 1.1). Serotypes 1 a and 1 b have been isolated in new born babies, whereas serotype 4 b is more common in those infected after birth (Ryser and Marth, 1991). Around seventy percent of food-borne outbreaks of listeriosis are associated with serotype 4 b (Seeliger and Jones 1986).

1.2 Occurrence of *Listeria monocytogenes* and *L. innocua* in the environment

Listeria monocytogenes is wide-spread in the environment; it is found in dust, soil, fresh and salt water, sewage, decaying vegetation and in animal feeds including silage. The organism is found in fresh and processed foods, such as milk, cheese, poultry, red meat, meat products, fish and various fruit and vegetables (Jones 1990, Farber and Peterkin 1991). In milk products, *L. innocua* is always found together with *L. monocytogenes.* The early work on the survival of *L. monocytogenes* in milk subjected to pasteruisation led to the hypothesis that the microbe could hide in leucocytes and as such would not be affected by the heat treatment (Bahk and Marth, 1990). This observation has not been substantiated in later work.

There have been an increasing number of cases of listeriosis reported world-wide (Anon 1997). This may be due to the increase in awareness of *L. monocytogenes* as a pathogen or to improvements in the methods for the isolation and identification of the organism. Listeriosis is a notifiable disease, this means that all recorded cases of listeriosis in England and Wales are collated by the Public Health Laboratory Service and the statistics are published in the Communicable Disease Reports (CDR).

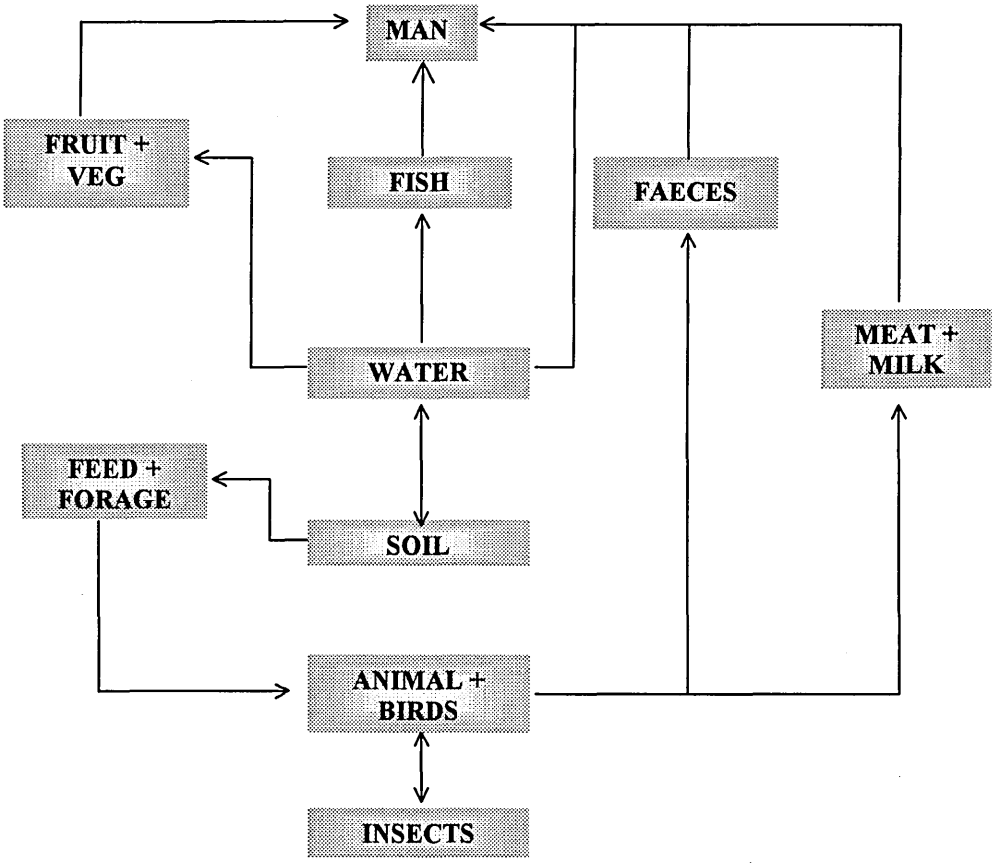


Figure 1.1. Route for transmission of *L. monocytogenes* from the environment to man

Figure 1.1 gives the route of transmission of *L. monocytogenes* in the biosphere and through this to people. This Figure demonstrates a classic faecal-oral route for the transmission of the pathogen. *Listeria monocytogenes* is commonly found in the faeces of ruminants, such as sheep and cows (Jones 1990). This figure indicates that milk can be a vehicle of transmission of *L. monocytogenes* and *L. innocua*.

Analyses of the factors contributing to the presence of the organism in milk are as follows. Faeces may contaminate the udder and the organism can pass into the milk. The organism may be present in the milking parlour and pass into the milk through cross-contamination.

Cheese can be contaminated with *L. monocytogenes* through using either (a) raw milk contaminated with *L. monocytogenes*, (b) inadequately pasteurised milk, (c) pasteurised milk to which contaminated raw milk has been added (Lovett *et al.* 1990), or (d) the cheese can be contaminated with *L. monocytogenes* by cross-contamination during ripening from the shelves on which the cheeses have been placed (Loncarevic 1995). In the manufacture of cheese raw materials can be a vehicle of re-contamination of the process plant. High standards of hygiene in the milking parlour, processing plant and ripening room are essential to prevent contamination by *L. monocytogenes*. Correct cleaning procedures are essential as the organism can adhere to food preparation surfaces and survive in biofilms making its removal by cleaning and disinfecting difficult (OH and Marshall 1995). Routine monitoring of the critical control points is essential during cheese manufacture and is usually part of a Hazard Analysis of Critical Control Points (HACCP) system. No microbiological sampling plan can ensure the absence of *L. monocytogenes* in soft mould-ripened cheese (Gilbert 1996).

1.3 Listeriosis

Listeriosis is an extremely dangerous disease with a high mortality rate for susceptible individuals. It is caused by the ingestion of *L. monocytogenes. Listeria monocytogenes* was first recognised as a human and animal pathogen by Murray *et al.* (1926). *Listeria monocytogenes* was described as *Bacterium monocytogenes* but was reclassified later as

L. monocytogenes in 1940 by Pirie (Farber and Peterkin 1991). Food-borne listeriosis is caused by the consumption of food contaminated with *L. monocytogenes* (Farber and Peterkin 1991).

Symptoms of listeriosis occur from 1 to 90 days after infection, typically one week after the initial ingestion of contaminated food. The delay in the onset of symptoms makes it difficult for the individual to remember which foods, if any, were the foodborne vehicle. The initial symptoms are diarrhoea, fever and malaise (Dalton *et al.* 1997). After several weeks other symptoms such as meningo-encephalitis and septicaemia may occur. In pregnant women foetal infection can occur via the transplacental route which can result in abortion, still-birth of the foetus or premature labour (Bahk and Marth 1990). The mortality rate for susceptible individuals is thirty percent (Faber and Peterkin 1991), although it can be as high as seventy percent (Milner, 1995). Penicillin, Ampicillin or Erythromycin can be used in treatment of the disease (Bahk and Marth 1990). Most healthy humans can fight off infection with only minor symptoms (stomach upset or 'flu').

Since the 1980's there have been many sporadic and some epidemic cases of listeriosis caused by consumption of chilled food products particularly soft-ripened cheeses (Genigeorgis *et al.* 1991, Stillmunkes 1993 and McLauchlin *et al.* 1991). The epidemic cases of listeriosis known to be caused by milk and cheese are listed in Table 1.2. Only individuals who are immuno-compromised such as the pregnant or those with AIDS or cancer are susceptible to infection with *L. monocytogenes* leading to the development of listeriosis. The minimum infective dose of the organism is not known, although Ryser and Marth (1987) suggest that the infective dose may be as low as 10^2 cfu g^{-1} for

susceptible individuals. A susceptible individual will have a condition leading to suppressed T-cell

Table 1.2. Number of cases of listeriosis due to consumption of milk and soft-ripened cheeses (1983 –1995)

Year	Area	Cases	Mortality rate (%)	Food vehicle	Serotype	Reference
1983	Boston Massachusetts, USA.	41	34	Pasteurised milk	4b	Fleming *et al.* 1985 Farber & Peterkin 1991 FDA 1995
1983-87	Switzerland	122	29	Vacherin Mont d'Or Cheese	4b	Bille 1990 IFST 1995
1985	Los Angeles County, California, USA	142	34	Mexican Style Soft Cheese	4b	Linnan *et al.* 1988 Farber & Peterkin 1991
1995	France	20	20	Brie de Meaux Soft Cheese	4b	IFST 1995

mediated immunity. During pregnancy the bacterium can infect the placenta and subsequently can be transferred to the amniotic fluid and the foetus (Jones 1990).

Listeria monocytogenes is able to survive inside non-active cells of mononuclear phagocytes. During bacterial invasion of the cell, phagocytes are activated forming free toxic radicals. *Listeria monocytogenes* produces the enzyme superoxide dismutase which contributes to increasing the virulence of the pathogen and guards it against the toxic radicals produced by phagocytic response (Farber, 1993). In addition, constituents of the cell wall of the organism are thought to be involved in its pathogenicity, e.g., proteins, water-soluble polysaccharides, carbohydrates and lipids (Bahk and Marth 1990). Haemolysin (listeriolysin) is also thought to be involved with pathogenicity (Rocourt 1994).

1.4 Epidemiology of *Listeria monocytogenes*

In the period pre-1986 there were three cases of listeriosis per million people in England and Wales. From 1986 to 1989 there was an upsurge in the number of reported cases to give a rate of nine cases per million people (Anon 1997). The increase in the number of cases of listeriosis between 1987 and 1989 was caused by consumption of pâté produced by one manufacturer in Belgium and imported into the United Kingdom (McLauchlin *et al.* 1991). In 1989 the Department of Health issued a warning to pregnant women and those who were immuno-compromised to avoid the consumption of high-risk uncooked foods such as pâté, cook-chill products and soft-ripened cheese. Since then there has been a steady decrease in the number of cases of listeriosis in England and Wales (Anon 1997). Currently in England and Wales there are 1.6 cases of listeriosis per million people (Anon 1997). Figure 1.2 shows the number of cases of listeriosis over the last 15 years for England and Wales.

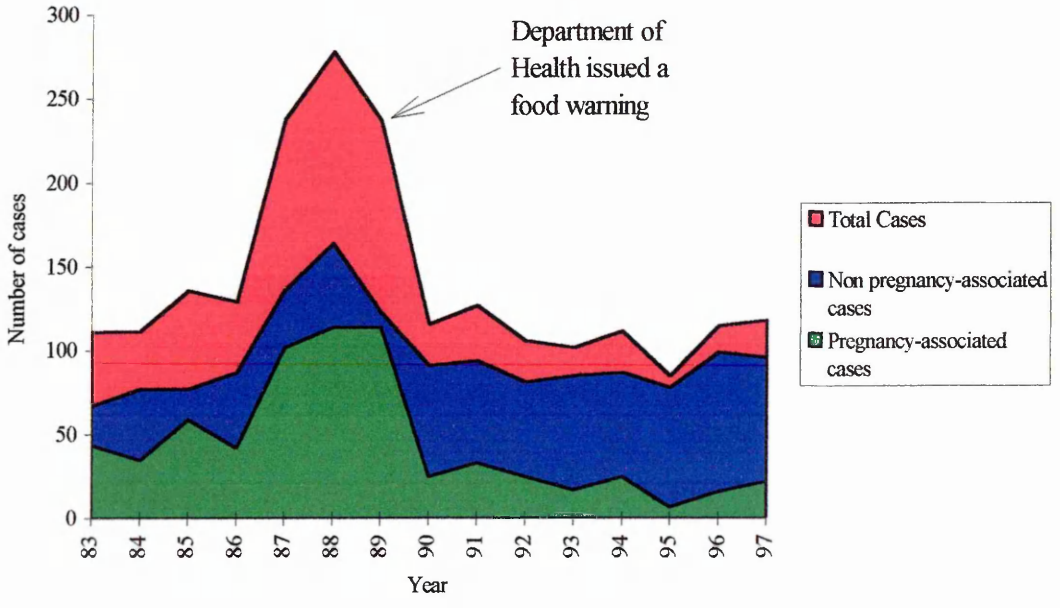


Figure 1.2. Cases of listeriosis in England and Wales (1983 to 1997) adapted from Anon (1997)

Table 1.3. *Listeria monocytogenes* and *L. innocua* in surface mould-ripened and blue veined cheese (Kinderlerer *et al.* 1996)

Cheese type	Date purchased	Outlet[1]	Pasteurised milk	Lm[3]	JLK No.	No positive
Brie de Pays (3 half cheeses)	7/92	A	- - -	+[2] - -	A7	2/6 - -
Brie de Pays (3 half cheeses)	12/92	A	- - -	+ + +	A13 A14 A15	3/6
Brie (whole cheese)	8/92	C	+ + +	- - -		0/6
Brie (3 pieces)	8/92	D	+ + +	- - -		0/6
Brie de Meaux (whole cheese)	8/92	B	- - -	- - -		0/6
Vacherin d'Or (half cheese)	11/92	E	+ +	- -		0/4
Vacherin Mont d'Or (whole cheese)	1/93	C	+ +	- -		0/4
Bleu d'Auvergne (3 pieces)	7/92	A	+ + +	- - -		0/6
Bleu d'Auvergne (whole cheese)	7/92	B	+ + +	- - -		0/6
Bleu d'Auvergne (3 pieces)	8/92	D	+ + +	- - -		0/6
Fourme d'Ambert (3 pieces)	6/92	A	+ + +	- - +	 A3	1/3
Fourme d'Ambert (3 pieces)	6/92	A	+ + +	+ - -	A4	1/3
Fourme d'Ambert (whole cheese)	6/92	B	+ + +	- - -		0/3

[1]Cheeses where obtained from (A) a national supermarket chain, (B) a cheese importer based in London, (C) a retail delicatessen in Sheffield, (D) a large retail store in Manchester, (E) a specialist cheese shop in London. [2] This isolate was *Listeria innocua.* [3] All the isolates of *Listeria monocytogenes* were serotype 1/2.

1.5 Occurrence of *Listeria monocytogenes* and *L. innocua* in soft mould-ripened cheese

In a survey of milk and cheese products in England and Wales 8.2 % of soft-ripened cheese were contaminated with *L. monocytogenes* and *L. innocua* (Greenwood *et al.* 1991). *Listeria innocua* is used as an indicator organism for the presence of *L. monocytogenes* as both organisms are found together in dairy products (O'Donnell 1991). In a small survey by Kinderlerer *et al.* 1996 (Table 1.3), low numbers of *L. monocytogenes* and *L. innocua* were found in mould-ripened cheese, particularly in those cheeses produced during the winter months and which were made from unpasteurised milk. The presence of listerias in these cheeses may be due to consumption of poorly fermented silage by the cows as part of their winter rations. It is known that listerias grow in poorly fermented silage (Kinderlerer 1996). Table 1.4 demonstrates the level of contamination of mould-ripened cheese produced in England and Wales. It has been suggested that the use of raw milk in the manufacture of these cheeses has led to the presence of *L. monocytogenes* after production and ripening (Bell and Kyriakides 1997).

Table 1.4. Occurrence of *L. monocytogenes* in mould-ripened cheese

Year	Area	Sample no.	*L. monocytogenes* (%)	Reference
1987	U.K.	222	10.4	Pini and Gilbert (1988)
1988-89	England and Wales	1135	5.9	Greenwood *et al.* (1991)
1987-90	NW and Yorkshire	131	0	Greenwood *et al.* (1991)
1991-92	N. Bristol	251	4.0	MacGowen *et al.* (1994)
1995	PHLS Results	1437	1.1	PHLS
1997	UK	65	10.8	Kinderlerer *et al.* (1997)

There are two types of mould-ripened cheese; soft-ripened cheese, ripened with *Penicillum camembertii* (i.e. Brie and Camembert) and the blue mould-ripened cheese ripened with *P. roquefortii.* In soft-ripened cheese such as Brie and Camembert the mould grows on the outside of the cheese to form a dense fungal mat. In the blue-veined cheeses the mould grows along the veins inside the cheese. The blue colour is due to presence of blue-green conidiospores of the fungus (Kinderlerer *et al.* 1996). Examples of blue veined cheeses are Stilton, Roquefort and Blue d'Auvergne.

1.6 Growth of *Listeria monocytogenes* in soft mould-ripened cheeses

Considerable research has been undertaken with experimental mould-ripened cheeses made with milk to which *L. monocytogenes* or *L. innocua* was added. This research is summarised in Table 1.5. From this work it was found that Brie and Camembert pose a greater risk as a possible food substrate for the growth of *L. monocytogenes* as they have a significantly higher final pH at the end of the ripening period (Ryser and Marth 1987 and Sulzer and Busse 1993).

Table 1.5. Growth of *L. monocytogenes* in experimental blue and soft-ripened cheese during ripening at 10 °C ± 5 °C

Cheese type	Ripening time (d)	Initial count (Log_{10} cfu g^{-1})	Final Count (Log_{10} cfu g^{-1})	Reference
Blue	70	3.2	2.7	Papageorgiou and Marth 1989
Camembert	70	2.9	4.7	Ryser and Marth 1987
Camembert	45	1.0	6.0	[1]Sulzer and Busse 1993
Camembert	40	1.5	8.0	Back 1993
Camembert	22	4.4	6.4	Genigeorgis *et al.* 1991
Camembert	20	2.0	8.0	Wan *et al.* 1997
Blue	25	2.5	1.8	[1]Schaffer *et al.* 1995

[1] Approximate numbers read from graph. The population of *L. monocytogenes* in Camembert-type cheese refer to the outside of the cheese.

The first step in the manufacture of mould-ripened cheese is fermentation of lactose by homo and heterofermentative lactic acid bacteria to give lactic acid and an array of flavour compounds (Urbach 1997). Production of lactic acid causes the pH to drop from 6.5 to around 5.0 (Fox and McSweeny 1997). In a hostile environment such as non mould-ripened cheese, *L. monocytogenes* would be unlikely to grow due to the low pH (5.2) and the high salt content (5 %). In contrast the final pH of Brie and Camembert cheeses is between 6.9 and 7.0 and the water activity is high (Marcos *et al.* 1991). The pH of blue mould-ripened cheeses is slightly lower, i.e. Roquefort, pH 6.2 and Bleu de Beste, pH 6.9 (Marcos *et al.* 1991). In soft-ripened Camembert-type cheese when the mould is added to the curds there is an increase in pH. The increase in pH is due to fungal growth resulting in the metabolism of lactic acid, the production of free amino acids and ammonia from the degradation of casein (Lenoir 1984).

The water activity (a_w) of the cheese is dependent on its salt, fat, protein and mineral content. In soft-ripened cheese the a_w is around 0.97 (Marcos *et al.* 1991). Mould-ripened cheeses are ripened in special rooms where the temperature and humidity are kept constant. Most mould-ripened cheeses are ripened at a relative humidity of 95 % and a temperature of between 8 – 14 °C (Scott 1986). Under these conditions the mould will sporulate (Scott 1986). Unlike the non mould-ripened cheeses if any bacterial pathogens are present they can grow due to the high pH. It appears that growth of *L. monocytogenes* is accelerated when cultured with *P. camembertii* in an aqueous system in the presence of protein and in the absence of sugars (McIntyre and Griffths 1992 and Kinderlerer 1996). It is believed that the mould produced a specific metabolite from degradation of casein that stimulates the growth of *L. monocytogenes*. *Penicillum roquefortii* and *P. camembertii* degrade fat present in cheese to produce free

fatty acids, glycerol, methyl ketones and secondary alcohols (Kinderlerer *et al.* 1996, Molinard and Spinner 1996).

Papageorgiou and Marth (1989) suggest that the high salt content, free medium chain length fatty acids (C6:0 – C14:0), methyl ketones and secondary alcohols inhibit the multiplication of *L. monocytogenes* in the Blue mould-ripened cheese. Although the pH of blue mould-ripened cheese is high many workers have commented that the growth of *L. monocytogenes* is controlled in blue veined cheeses, Danielsson-Tham *et al.* (1992), Bougle (1994), Papageorgiou and Marth (1995), Sulzer and Busse (1993), Ryser and Marth (1987), Back (1993), I.F.S.T. (1995). Kinderlerer *et al.* (1996), in an investigation to find natural antimicrobial compounds in mould-ripened cheese, demonstrated that there was an increased concentration of lauric acid and mystric acid in the vicinity of the blue conidiospores in the veins of Blu d'Auvergn.

Caprylic (C8:0), capric (C10:0) and lauric acid (C12:0) are called medium chain length fatty acids (MCFA). Kinderlerer *et al.* (1996) suggested that localised concentrations of these acids inhibited growth of any listerias which could be present. A continuation of their work at Sheffield by Liu (1998) demonstrated that milk fat contains approximately 15 % medium chain length fatty acids (Table 1.6) calculated on a molar basis. Medium chained length fatty acids, if present in the free state (non-glycerides) could provide a reservoir of antimicrobial compounds if they were present in a food such as a mould-ripened cheese.

Evidence for the antimicrobial activity of medium chain length fatty acids was demonstrated by Hatton and Kinderlerer (1991) who showed that these acids inhibited germination of conidia spores of *P. crustosum,* a species closely related to

Table 1.6. Fatty acid composition of two samples of butter oil (adapted from Liu 1998)

Fatty acid	Carbon number	Composition (mol %)	
		1995	1996
Caproic acid	6:0	4.96 ± 0.36	4.26 ± 0.4
Caprylic acid	8:0	2.15 ± 0.11	2.33 ± 0.17
Capric acid	10:0	3.6 ± 0.13	3.97 ± 0.18
Lauric acid	**12:0**	**3.81 ± 0.08**	**3.94 ± 0.10**
Mysristic acid	14:0	10.84 ± 0.19	11.08 ± 0.15
9, Tetradecenoic	14:1 (9c)	0.86 ± 0.02	0.87 ± 0.02
Methyltetradecanoic	15:0	1.08 ± 0.02	1.11 ± 0.01
Palmitic acid	16:0	25.05 ± 0.39	25.92 ± 0.18
Palmitoleic acid	16:1 (9c)	1.45 ± 0.04	1.35 ± 0.06
Stearic acid	18:0	11.48 ± 0.10	10.08 ± 0.28
Oleic acid	18:1 (9c)	26.64 ± 0.28	25.23 ± 0.49
11, Octadecenoic	18:1 (11c)	3.23 ± 0.51	2.63 ± 0.26
Linoleic acid	18:2 (9c,12c)	2.7 ± 0.04	1.89 ± 0.07
α-Linolenic acid	18:3 (9c, 12c, 15c)	0.69 ± 0.05	0.68 ± 0.02
	Total 6:0 – 12:0	**14.52 ± 0.41**	**14.50 ± 0.39**
	Total fatty acids	98.01	94.19

c = cis. Results are the mean ± standard deviation (sd) of 3 observations

P. roquefortii. In order to remove these toxic fatty acids fungal species can convert medium chain length fatty acids to the methyl ketone, one carbon atom less (Hatton and Kinderlerer 1991). It has been suggested by these authors that this conversion is a detoxification reaction. Medium chain length fatty acids also inhibit the growth of bacteria. A number of research workers have shown that many fatty acids including lauric acid inhibit growth of Gram positive bacteria (Kabara and Eklund 1991 and Wang and Johnson 1997).

1.7 Medium chain length fatty acids as food preservatives

Early work carried out by Neiman (1954) summarises the preservative effect of free fatty acids on Gram positive bacteria. Kabara has discussed the antimicrobial effect of

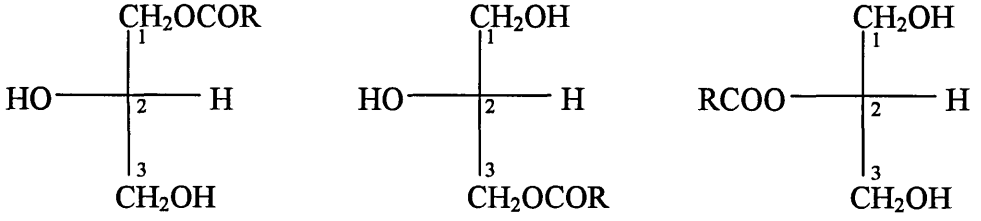
free fatty acids, monoacylglycerols and other fatty acid esters in numerous reviews (Karbara 1979, 1981, 1984 a, 1984 b, 1993, Kabara and Eklund 1991). Medium Chain Fatty Acids containing 6 to 12 carbon atoms, inhibit the growth of *Listeria* spp. as well as other bacteria (Kabara and Eklund 1991). In order for the fatty acid to be taken up by the microbial cell and exert an effect on growth, the acid has to be undissociated otherwise the charge on the fatty acid will inhibit passage through the cell membrane (Kabara and Eklund 1991).

Kinderlerer *et al.* (1996) tested the ability of MCFAs caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0) and myristric (C14:0) on the growth of *L. monocytogenes* in a bi-phasic system where the MCFAs were dissolved in butter oil. Lauric acid was more inhibitory than the other acids. Lauric acid is sparingly soluble in water but soluble in fat and alcohol, the pk_a of lauric acid is around 4.8 (Freese *et al.* 1973). The American workers have dissolved lauric acid in alcohol (Wang and Johnson 1997, 1992, Oh and Marshall 1995, 1993, Rohani and Griffiths 1994), whilst the Sheffield group dissolved lauric acid in butter oil and allowed the acid to diffuse into the aqueous phase.

1.8 Monoacylglycerols as food preservatives

Fatty acids are found in most living organisms as the triglyceride. The degradation products of triglycerides, the mono and diglycerides and free fatty acids are known to have antimicrobial properties and are found in low concentrations in most living organisms. They can be produced by degradation of triglycerides by lipase enzymes. Work mainly in the United States has demonstrated that monoglycerides, particularly monolaurin, are antimicrobial under certain conditions (Karabara and Eklund 1991).

Monoacylglycerols (MAGs) are amphilic molecules, the molecule has hydrophobic and hydrophilic groups, Figure 1.3. They are commonly used to stabilise water in oil emulsions, i.e. margarine, and in the promotion of aeration of an emulsion, i.e. in ice cream. Acylation of one of the free hydroxyl groups (R group, Figure 1.3), with an acid, such as lauric acid, makes the molecule more stable. A process of transesterification occurs in the presence of an acid allowing the fatty acid to migrate from the α to the β position. Fatty acid monoesters of glycerol exist in two isomeric forms; α- monoacylglycerols, the R group either on carbon 1 or 3 and the β-monoacylglycerols, the R group on carbon 2 (Gunstone 1996). The inhibitory action of a MAG increases at a low pH of 5.0 (Oh and Marshall 1993). Monolaurin and coconut MAGs can act as preservatives and inhibit growth of *L. monocytogenes* in refrigerated foods (Wang and Johnson 1997, Oh and Marshall 1993). Temperature and concentration of other antimicrobials and emulsifiers affect the degree of inhibition as well.



1- and 3-monoacly-*sn*-glycerols
α-monoacylglyceride

2-monoacyl-*sn*-glycerol
β-monoacylglyceride

Figure 1.3. Alpha and beta monoacylglycerides (adapted from Gunstone 1996)

Monolaurin is a bi-functional additive, it can act both as a preservative and as an emulsifier. The pioneering research using monolaurin as a preservative was conducted by Moustafa and Agin (1980). Monolaurin is a food-grade glycerol monoester of lauric acid. It has been approved by the United States Food and Drug Administration (FDA)

as a food emulsifier (21 CFR GRAS 182.4505, FDA 1955), Table 1.7. Monolaurin accounts foe seventy five percent of the world production of food emulsifiers (Krog 1996). Monoglycerides and monolaurin have no known toxicity (WHO 1974). Table 1.6 shows the levels of monoglycerols which are permitted as additives in food.

Table 1.7. Maximum permitted levels of monoglycerols allowed in food

Country	Additive	Serial number	Uses	Food	Level
UK and EC	Mono and diglycerides	E471	Emulsifier	Cocoa and chocolate products, breads, cream, quick cook rice, non-emulsified oils and fats	*Quantum satis* 10 g / l
USA	Monolaurin (C12:0)	[1]21 CFR 182.4505	Preservative Emulsifier	Most foods	Level 1
	Mono and diglycerides (C12 – C18)	21 CFR 184.1505	Emulsifier	UK and EC	Level 1

USA Legislation: FDA Regulations (4.1.95 ed). UK legislation: Butterworths Law of Food and Drugs 1997. Regulation implemented European parliament and Council directive (95/2/EC, OJ No 161, 18.3.95 pl) on food additives other than colours and sweeteners with Council Directive 89/107/EEC, OJ No L40, 11.2.89). Level 1: at a level not excess of the amount reasonably required to produce its intended effect.1 Generally Recognised as Safe (GRAS), FDA code.

Certain naturally occurring MAGs inhibit the growth of *Listeria* spp, Table 1.8. Wang and Johnson (1992) found that monolaurin did not inhibit *L. monocytogenes* in whole milk. These authors explain this by suggesting that the presence of fat globules interacted with the monoacylglycerol. Interaction of bacteria, foods and antimicrobials are complex. Monolaurin forms complexes with macromolecules found in food, including starch, proteins and lipids and in so doing looses its activity (Shibasaki 1982, Bala'and Marshall 1996 b and Wang and Johnson 1992). Monolaurin is an expensive chemical and when used in high concentrations in food can result in a soapy flavour (Wang and Johnson 1992).

Table 1.8. Minimum inhibitory concentration of monolaurin, lauric acid and coconut MAGs against *L. monocytogenes*

Medium	Temperature (°C)	Monolaurin ($\mu g\ ml^{-1}$)	Lauric acid ($\mu g\ ml^{-1}$)	Coconut MAG ($\mu g\ ml^{-1}$)	Reference
Beef slurry, Camembert, seafood salad	37	250	-	500	Wang and Johnson (1997)
Bi-phasic system	10	-	233	-	Kinderlerer *et al.* (1996)
Cat fish fillets	Not stated	50	-	-	Verhaegh *et al.* (1996)
Commercial medium	25-35	16	-	-	Bal'a and Marshall (1996 b)
Cat fish medium	25-35	64-128	-	-	
Italian Stecchino cheese	5	200	-	-	Stecchini *et al.* (1995)
Biofilms on stainless steel	65 (5 min)	50	-	-	Oh and Marshall (1995)
Tryptose Soya Broth	35	10	>1000	-	OH and Marshall (1993)
BHI Broth	37	96	-	-	Rohani and Griffiths (1993)
BHI broth	37	25	-	10	Wang *et al.* (1993)
Skimmed milk	4	200	100-200	-	Wang and Johnson (1992)

BHI – Beef Heart Infusion

1.9 Intrinsic factors controlling growth of pathogens in cheese

There are a number of intrinsic factors which influence the growth of pathogens in cheese. The pH value of cheese varies from pH 4.7 up to pH 5.0 in acid cheeses such as Mature Cheddar cheese (Scott 1986). At these pHs there is little growth of pathogenic bacterial can take place (Johnson *et al.* 1990). The pH of mould-ripened cheeses increases from pH 5.0 initially during production, to values in excess of 7.0 at the end of

ripening (Marcos *et al.* 1991). At these pHs any pathogenic bacteria which may be present could grow. Low pH is an intrinsic factor in the control growth of pathogenic bacteria in most cheeses. This effect of these controls has been lost in mould-ripened cheese due to the high pH.

The water activity (a_w) of cheese affects the growth of pathogenic bacteria. The a_w of cheese is dependent on salt concentration, fat and protein content. The a_w of white mould-ripened cheese is higher than that of Blue mould-ripened cheese (Marcos *et al.* 1991). At the higher a_ws of the Camembert-type cheese mould and pathogenic bacteria can grow.

Moulds are aerobic and do not grow in the absence of oxygen. *Listeria monocytogenes* is a facultative anaerobic organism. In the Camembert-type cheese the oxygen concentration is high on the outside of the cheese. Diffusion of oxygen in such a viscous type of cheese will be difficult and there will be an oxygen gradient from the outside to the inside of the cheese. In Blue-type cheeses, there will be high concentrations of oxygen in the veins, as these cheeses are pricked to allow diffusion of oxygen into the cheese. This difference in oxygen tension between the outside and inside of the Brie or Camembert type of cheeses may account for the larger population of listerias which can develop on the surface of the cheese compared to the interior of the cheese (Johnson and Doyle *et al.* 1990).

1.10 Growth of *Listeria monocytogenes* in emulsions and bi-phasic systems

An emulsion is a system in which two immiscible phases (oil and water) are bound into one continuous phase which is kept stable by use of an emulsifier (Coupland and

McClements 1997). An emulsifier is an amphoteric molecule such as a phospho-lipid which has a structure with a polar head and a non-polar (hydrocarbon) tail (Kirk and Sawyer 1991 a). Oil droplets dispersed in an aqueous phase are termed an oil-in-water emulsion (e.g. mayonnaise, cream and milk), water droplets dispersed in an oil phase is termed a water-in-oil emulsion (e.g. margarine butter and spreads). Emulsifiers align themselves on the surface of droplets during homogenisation. These molecules form a protective membrane, preventing the droplets from aggregating (Coupland and McClements 1997). The structure of an emulsion is shown in Figure 1.4.

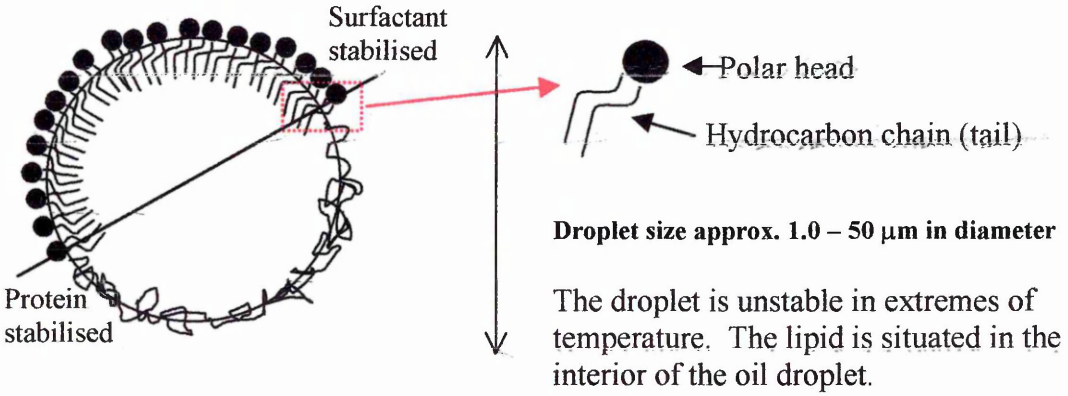


Figure 1.4. Structure of an oil droplet in an oil-in-water emulsion (adapted from Coupland and McClements 1996)

There are three parts of an emulsion, the interior of the droplet, the continuous phase and the interfacial region. The interface is a narrow region surrounding each droplet that consists of a mix of oil, water and emulsifier molecules (Coupland and McClements 1997). The size of oil droplets in an oil-in-water emulsion affects the growth rate of *L. monocytogenes* (Brocklehurst *et al.* 1995). In less viscous emulsions no inhibition is seen. This may be attributed either to the restricted diffusion of nutrients and oxygen or to the accumulation of metabolic end products.

Section 2. Microbial growth and predictive modelling of *L. monocytogenes*

1.11 Bacterial Growth

Growth is defined as the orderly increase of all chemical components of an organism (Birge 1992). When bacteria adapt to their environment they are said to be in a state of balanced growth (Stanier *et al.* 1987). The increase in biomass of a population of bacteria is proportional to the increase of all the other measurable properties of that culture. The rate of growth of a bacterial culture can be measured by any component of that population. This rate mimics a first order autocatalytic chemical reaction (Stanier *et al.* 1987 and Birge 1992).

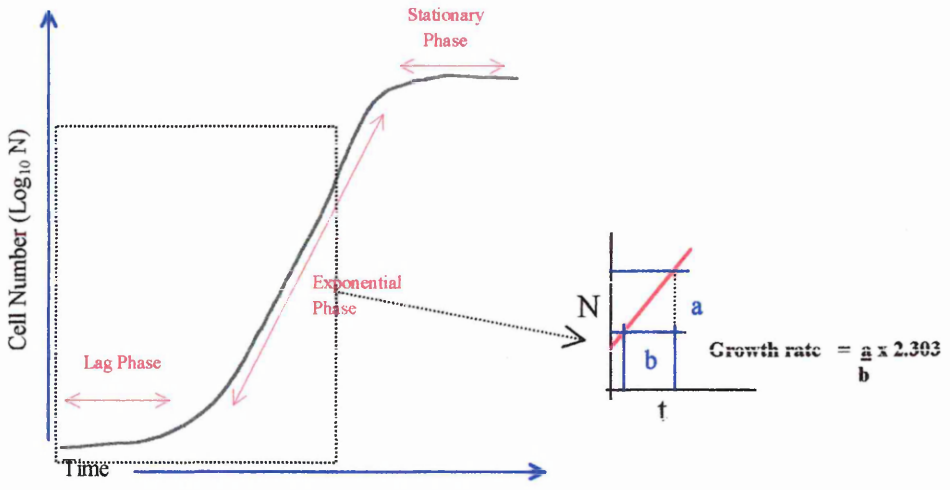


Figure 1.5. Bacterial growth in batch culture

A typical growth curve is sigmodial in shape and can be divided into three phases: lag, exponential phase of growth, stationary phase, Figure 1.5. In the lag phase cells are adjusting their metabolism to prepare for a new cycle of growth. If an actively growing inoculum is used the lag phase will be short. During the exponential phase of growth,

the cells are growing and dividing at the maximum rate possible for the medium and incubation conditions. The rate at which the population is increasing is measured in this phase by calculating certain constants, shown below. The growth rate of a culture dividing by binary fisson can be expressed as the doubling time (t_d) or as the specific growth rate constant (μ). The t_d is the time required for the population to increase by a factor of two. The growth constant μ is a measure of the speed of cellular growth in the exponential phase. These constants can be defined as:

$$n = \frac{\text{Log } N - \text{Log } N_0}{\text{Log } 2} \quad (1.1)$$

$$\nu\, h^{-1} = \frac{\mu}{0.693} \quad (1.2)$$

$$\mu\, h^{-1} = \text{Slope} \times 2.303 \quad (1.3)$$

$$t_d\, h = \frac{0.693}{\mu} \quad (1.4)$$

n = number of divisions

N_0 = initial number of cells

N = number of cells after time t

ν = number of divisions per hour

μ = growth rate

t_d = doubling time

1.12 Modelling the growth of *L. monocytogenes*

Zwietering *et al.* (1990), Little and Knøchel (1994) and Whiting (1995) have published a number of models in an attempt to define microbial growth mathematically in order to predict the growth of microbes under specific environmental conditions. This approach

has been termed predictive modelling. There are 3 levels of predictive modelling; the primary level, describes changes in microbial populations with time, the secondary level shows how the primary model varies with environmental conditions and the tertiary model combines all these with a software package to calculate microbial behaviour.

Successful predictive models for the growth of *L. monocytogenes* with respect to NaCl, pH, temperature and $NaNO_3$ have been described (McClure *et al.* 1997 and Fernadez *et al.* 1997). A predictive model has been developed for *L. monocytogenes* in skimmed milk and validated in dairy products, i.e. Camembert cheese (Murphy *et al.* 1996). Most predictive models do not take into account the extracellular, physiochemical environment and intracellular conditions encountered in a biological system. The bacterial growth curve has been studied by Baranyi and Roberts (1994). Growth rate and maximum population density were used in this model. This enabled the authors to predict variations in parameters of the growth curve as a function of growth conditions using a polynomial equation.

The Baranyi and Roberts model was found to be the most appropriate program to fit data obtained during this study. This model was used to determine the doubling time and growth constant from a bacterial growth curve (Baranyi and Roberts 1994). This was because the program calculates a lag phase if it is present and does not force a lag phase into the data like other models such as the Gompertz equation.

1.13 Aims of research

The aims of this study were to determine the effects and interactions of lauric acid and monolaurin and their combination on preventing growth of *Listeria monocytogenes* and *L. innocua* in a bi-phasic broth culture and in a model soft-ripened cheese. All experiments were carried out at 10 °C. Lauric acid and monolaurin were dissolved in butter oil for the broth experiments and in cream for the cheese experiments. The results for the broth experiments were analysed to determine the lag phase (if present), the growth rate and the doubling time using a programme written by Baranyi and Roberts (1994).

Materials and Methods

2.1 Chemicals

Lauric acid (LA), (Sigma No. L4250) and monolaurin (ML), (Sigma No. M1765) were obtained from Sigma Chemicals (Poole, BH17 7NH), and were Sigma Grade. Cobalt (II) sulphide 7-hydrate, sodium hydroxide and anhydrous glucose (AR) were obtained from Merck Chemicals (Leicestershire, CE 14 4XN). Chloride meter standard (200 mg Cl^{-1}) and acetic acid buffer were obtained from Ciba-Corning Diagnostics Ltd, Essex CO9 2DX. Sodium chloride was obtained from Fisons Scientific, Loughborough LW11 ORG. Butter oil was a gift from Mr Brian Jeffrey, Unilever Research, Colworth House. Liquid microbial rennet (Chymogen) was obtained from Chr. Hansens Laboratory, Reading RG2 0QL, and was the pure standardisation solution of coagulation enzymes produced by fermentation of *Aspergillus niger* var, *awamori*.

2.2 Media

Tryptose Soya Broth (TSB), Oxoid CM 129, Yeast Extract (YE), Oxoid L 21, Neutralised Bacteriological Peptone (NBP), Oxoid L 34, Horse Blood Agar (HBA), Blood Agar Base No. 2, Oxoid CM 127, defibrinated horse blood, Oxoid SR 50, Oxford Agar (OA), Oxoid CM 856, Listeria selective supplement, Oxoid SR 140 and Agar Bacteriological No. 1, Oxoid L 11 were obtained from Oxoid (Basingstoke RG24 0PN). The Api Listeria identification system was obtained from Bio Mérieux, France 10 300.

2.3 Starter culture

Freeze dried Rediset mesophilic aroma starter culture type LD was obtained from Chr. Hansens Laboratory, Reading, RG2 OQL. Type L contained citrate positive *Leuconostoc lactis*. *L. mesenteriodes* subsp. *cremoris* and Type D contained *Lactococcus lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis*. Dried Skimmed Milk powder was obtained from Cadbury, Bourneville.

2.4 *Listeria* stains

Three cheeseborne strains of *L. monocytogenes* were obtained. Strain A3, (NCIMB 13449) and strain A13 were isolated by J. L. Kinderlerer from Brie and Fourme d'Ambert respectively (Hatton and Kinderlerer 1991). *Listeria monocytogenes* LB (NCIMB 1351) was isolated by P. Finner at the PHLS in Sheffield from Lanark Blue cheese which was purchased in December 1995. This strain has been deposited in the National Culture Collection of Industrial micro-organisms as NCIMB 1351. Two strains of

L. innocua, strain A7 (NCIMB 1350) was isolated by J. L. Kinderlerer from Brie cheese made with unpasteurised milk (Kinderlerer *et al.* 1991) and *L. innocua* NCTC (11288) was the type culture which was originally isolated from calf brain.

2.4.1 Maintenance of strains

Stock cultures were grown on slopes in 5 mL Bijoux bottles containing 2.5 mL Tryptose Soya Agar, (TSA) and incubated at 30 °C for 24 h and then the cultures were stored at -70 °C under liquid paraffin until required. Running cultures were stored at 4 °C and subcultured at 4 week intervals on TSA.

2.4.2 Preparation of inoculum

Tryptose soya Glucose Yeast Broth (TSGYB) was prepared from Tryptose Soya Broth which contained additional glucose to give 10 g L^{-1} and Yeast Extract (3 g L^{-1}). Tubes containing 10 mL TSGYB were inoculated using a 10 µL sterile plastic loop with the relevant bacterial strain. The cultures were incubated for 24 h at 30 °C and then sub-cultured into fresh TSGYB and incubated for a further 20 h at 30 °C. Dilution (1:10,000) were prepared in 0.1 % NBP by successive decimal dilutions (1 mL + 9 mL).

2.5 Identification of *Listeria monocytogenes* and *innocua*

2.5.1 Gram stain

All 6 strains were cultured for 18 h in TSB. A bacterial smear was made on a clean microscope slide from the 18 h culture and a Gram stain was performed. *Listeria monocytogenes* and *L. innocua* were Gram positive (purple) rods or cocco-bacilli, 0.4 - 0.5 µm in diameter and 0.5 - 2 µm in length, with rounded ends.

2.5.2 β-Haemolysis

Listeria cultures taken from slopes stored at 5 °C and grown in 10 mL TSGYB for 18 – 20 h at 30 °C. An inoculum (10 µL) was streaked onto HBA and plates were incubated for 24 h at 30 °C, β-haemolysis was detected as a yellow fluorescent colour, *Listeria monocytogenes* gave a positive β-haemolytic reaction on HBA. *Listeria innocua* did not break down haemoglobin so that there was no yellow colour on the red agar.

2.5.3 Catalase test

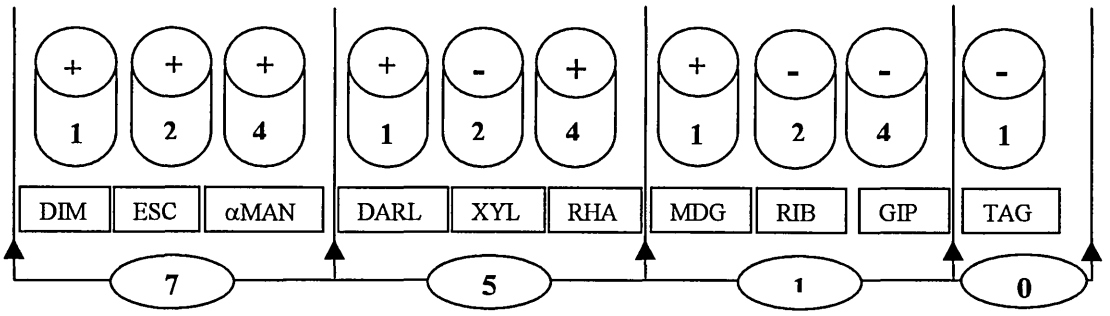
All six strains of *Listeria* (*L. innocua* A7 and NCTC 11288, *L. monocytogenes* BL/88/1b, A3, A13 and LB) were cultured for 18 h in TSB, and 10 µL was streaked

onto HBA plates which were incubated for 24 h at 30 °C. Material from an individual colony on each plate was picked off and placed on a microscope slide with 5 µL sterile water and spread to create a film. Hydrogen peroxide, 50 volume (10 µL) was placed in contact with the film, a positive reaction was indicated by effervescence of oxygen. All strains of *L. monocytogenes* and *L. innocua* were catalase positive.

2.5.4 API Listeria system for the identification of *Listeria* spp.

An inoculum was prepared from streaks grown on HBA. Plates were incubated for 24 h at 30 °C and separate colonies (0.2-1.5 mm in diameter, translucent and dewdrop like) were picked off and inoculated into the suspension medium. The inoculum was distributed into each ampoule of the test strip and incubated for 24 h at 30 °C.

The differentiation of *L. monocytogenes* and *L. innocua* was confirmed with the DIM reagent. A drop of Zym b (DIM) reagent was added to the ampoule and left for 3 min. A colour change from red to orange indicated *L. monocytogenes.* Hydrolysis of aesculin (ESC) to give aesculetin was observed by the formation of a black colour when complexed with iron (Fe^{3+}). Metabolism of α-mannoside (α-MAN) reaction was indicated by a yellow colour. The remaining tests, D-arabitol (DARL), D-xylose (XYL), rhamnose (RHA), α-methyl-D-glucoside (MDG), ribose (RIB), glucose-1-phosphate (G1P) and tagatose (TAG) represented fermentation of the respective sugars to lactic acid. The presence of lactic acid was detected by the production of a yellow colour due to the change of colour of the pH indicator. The absence of a yellow colour was recorded as a negative result. Speciation was achieved by the production of a numerical profile (depending on individual reactions), which was compared to reference values (Figure 2.1.).



Numerical Profile: **7 5 1 0** Identification: ***L. innocua***

Figure 2.1. API LISTERIA identification strip

2.6 Examination of *Listeria monocytogenes* using transmission electron microscopy (TEM)

Inocula were prepared as described in 2.4.1. and 200 µL of the 1:10,000 dilution was used to inoculate sterile 50 mL TSGYB in a 250 mL Erlenmeyer flask. Sterile butter oil containing lauric acid (equivalent to 1.015 g L^{-1}) broth or 5.05 mM was added to each of three flasks. The cultures were incubated at 10 °C in a refrigerated water-bath (Grant 5550D, Shaking bath) at 100 rpm. Samples were taken at 72 h (logarithmic phase) and at 144 h (stationary phase). Cells taken from the logarithmic phase were centrifuged to increase the number, those in the stationary phase were not. Specimens were mounted on copper grids with a cellulose support film which were sputter coated with pure carbon. All grids were negatively stained with a saturated aqueous solution of uranyl acetate. The electron microscope (JEOL 1200 EX, Tokyo, Japan) was operated at an acceleration voltage of 80 KV. Each sample was examined under the TEM and photographed. Measurements were taken from each electron micrograph, the cell size was measured using the calibration bar and the cell dimensions were calculated in µm. Cell volume was calculated as $\pi r^2 L$ assuming the cell was a cylinder, where r was half

the cell width and L the cell length. All strains were examined for the presence of flagella.

2.7 Temperature calibration and temperature control of the refrigerated incubator (Vindon Ltd, Oldham OL3 55Y)

2.7.1 Temperature measurement

Thermocouples type K (RS Components Ltd, Northants NN17 9RS) were placed in the incubator and were linked to a Digitron type S10 (Digitron Instrumentation, Herts, SG13 7AW). This was to monitor the temperature of the incubator during each experiment. The first thermocouple was placed in the geometric centre of a 125 mL Erlenmeyer flask. The flask contained 50 mL of TSGYB and was positioned in the centre of the New Brunswick Gyrotory Shaker, Model G2 (New Brunswick Scientific Co., NJ, USA). The shaker was placed in the centre of the incubator. The second thermocouple was placed in an empty flask. The third and fourth thermocouples measured air temperature and were attached to the top of the centre flask and onto the incubator wall. The temperature of the contents of the 125 mL Erlenmeyer flask was measured by placing a thermocouple in the geometric centre of a flask containing 50 mL broth and in the centre of the incubator. In later experiments, the thermocouples were linked to a 1000 series Squirrel meter/logger Grant type CM-U-V3-0 (Grant Instruments, Cambridge, B2 5QZ). All the data was downloaded to a PC at the end of each experiment.

2.7.2 Calibration of the refrigerated incubator to give an internal flask temperature of 10 °C

Sterile TSB (50 mL) was added to each of the ten 125 mL Erlenmeyer Flasks. The weights of the flasks, empty and with TSB were recorded at the start of each experiment. The flasks were placed on a New Brunswick Gyrotory Shaker (G2) with a shaker speed of 200 or 75 rpm. The shaker was placed inside the incubator and the dial set to give an internal temperature of 10 ° C (Figure 2.2.). Initially the shaker speed was set at 200 rpm for 12 d. The flasks were weighed every 4 d to establish any loss of water. In the subsequent experiments the shaker speed was reduced to 75 rpm and the flasks were weighed at the beginning and end of each run. These experiments were run for 18, 7, 6 and 7 days respectively (Table 2.1).

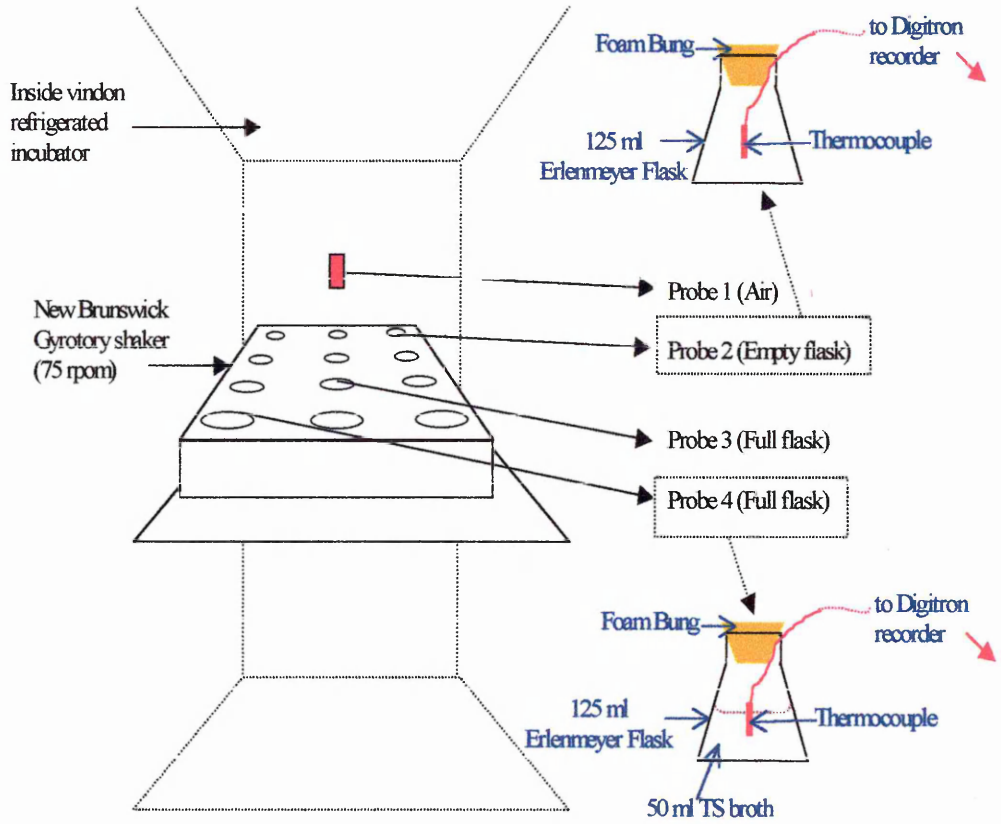


Figure 2.2. Position of the thermocouples in the refrigerated incubator

The temperature of the broth inside the flasks and the air temperature were recorded throughout the experiment. This was achieved by placing a type K thermocouple (RS Components ltd, Northants, NN17 9RS) in the geometric centre of the liquid in the flask and another inside the incubator next to the shaker. The thermocouple was linked to a Digitron type S10 Data Logger (Digitron Instrumentation, Hertfordshire, SG13 7AW) interfaced to a Psion Organiser II. Transfer of water onto the refrigeration coils inside the incubator caused weight loss from broth in the flask.

Table 2.1. Shaker speed, duration and original air temperature during calibration of flasks

Test No.	Date	Vindon setting	Temperature (± SD ° C)		Shaker Speed	Oil	Test Duration	Weighing interval
			Air	Flask	(rpm)		(d)	(d)
1	8.12.94	7.77	10.0 ± 0.2	NT	200	-	12	4
2	22.12.95	7.77	10.0 ± 0.3	11.0 ± 0.6	75	-	18	18
3	9. 01.95	7.67	9.0 ± 0.3	10.5 ± 0.3	75	-	7	7
4	24.01.95	7.62	8.5 ± 0.2	10.0 ± 0.2	75	-	6	6
5	30 01.95	7.62	8.6 ± 0.2	10.0 ± 0.2	75	+	7	7

2.8 Growth of cheeseborne strains of *Listeria monocytogenes* and *Listeria innocua* in a model bi-phasic system in broth culture

The method was adapted from Kinderlerer and Lund (1992). Tryptose Soya Glucose Yeast Broth was filter sterilised using a Nalgene bottle top filter unit (Nalgene Company, New York, USA) coupled to a Milipore vacuum pump. A 0.2 μm pore size 47 mm cellulose nitrate filter membrane (Whatman, Maidstone, N.718004) was over laid with a 0.45 μm membrane (Millipore, Ireland). The initial pH was adjusted to 7.0

with 0.2 M HCl and tested with a Philips type CE2 semi-micro pH probe (diameter 4.5 mm and stem 80 mm) attached to an Unicam Ion Selective pH Meter, 9640, (Unicam Analytical Systems, Cambridge CB1 2PX). The sterile broth was dispensed with the Accustep fluid management system (Tri-Continent Scientific Inc.) using 25 mL Syringes (No.640), into twelve sterile 125 mL Erlenmeyer flasks which were sealed with foam tops.

The butter oil (21 g) was put in a 125 mL Erlenmeyer flask which was closed with a foam bung and covered with aluminium foil, the flask was sterilised by heating at 121 °C for 15 min and stored at 5 °C. Before use the oil was re-melted in a waterbath type KD (Grant Instruments Ltd, Cambridge CB2 5QZ) at 40 °C for 20 min. Lauric acid, (0.213 g), monolaurin (0.020 g) or both were added aseptically to the oil. The butter oil was mixed (Whirlimixer, Chem Lab Instruments Ltd, London) for 30 s and replaced into the water bath for a further 10 min to dissolve the lauric acid and monolaurin. Each 125 mL Erlenmeyer flask contained 50 mL of TSGYB broth, to which 5 g of butter oil alone or with monolaurin, lauric acid, or both were added aseptically. The flasks were re-closed, inoculated with 200 µL of the of the 1:10,000 dilution of the relevant bacterial strain. Concentration of lauric acid (5 mM) and monolaurin (0.35 mM) was calculated with respect to volume of the aqueous phase. Flasks were set up in triplicate.

Twelve Erlenmeyer flasks (150 mL) containing 50 mL TSGYB were inoculated with 200 µL of the (1:10,000) dilution of the relevant culture, see section 2.4.2. Two experiments were undertaken for each strain. In the first experiment, six flasks contained broth and inoculum but no butter oil. Three were used to obtain an initial count and discarded after use. The remaining three were used as a control (C). Butter

oil was added to a further six flasks of which three contained lauric acid (LA) and three did not (O). These were used to measure the effect of the oil solvent and LA on the growth of the *Listeria* spp. In the second experiment, three flasks without oil were used to obtain the initial count. Three flasks contained the broth culture and butter oil (O), these were used as a control. Monolaurin and oil solvent were added to the remaining six flasks, three with and three without LA. The flasks were incubated at 10 °C in a refrigerated incubator (Vindon) on a New Brunswick Shaker (G2) at 75 rpm. A continuous record of the temperature was kept through out each experiment, section 2.1.4.

The viable count was determined at 0, 24, 48, 72, 96, 120, 144, 168 and 192 h by appropriate decimal dilution in 0.01 % peptone (Oxoid L34), and 0.85 % NaCl. The tubes were kept in ice water in a Dewar flask before inoculation. The final two of the dilutions were surface spread plated (100 µL or 50 µL), in triplicate onto dried TSGYA plates. Plates were incubated at 30 °C and counted after 48 h. Results were expressed as $\log_{10}$ cfu mL^{-1}. At 192 h the final pH was determined. This was repeated for each strain of listeria.

2.8.1 Motility

Each strain of listeria was tested for motility after being exposed to monolaurin and lauric acid for 192 h at 10 °C. Motility was determined using the hanging drop technique. A drop of test culture was placed on a glass cover slip and inverted over a thin ring of Vaseline on a glass microscope slide. The slides were examined using an optical microscope. *Listeria* spp. exhibit a typical 'tumbling' motility at 21 °C but not at 37 °C. The control for no movement was prepared by boiling the listeria culture for

1 min to kill the organism. The rate of motility was determined using a 0 – 5 scale, 0 exhibiting no movement (Brownian motion) and 5 the rate of the untreated listerias.

2.9 Growth of *Listeria innocua* in a model cheese system

2.9.1 Development of cheese making equipment to comply with COSHH regulations

In this experiment, the ripening cabinet was required to hold at least 20 cheeses. In order to use a rectangular plastic desiccator inside the refrigerated incubator it was decided to reduce the original mould size from 12.9 cm, internal diameter, to 7.2 cm and the height from 11 cm to 7.5 cm so that all the cheeses could be ripened at one time. The moulds had to be made from durable material that could survive autoclaving for 30 min at 121 °C, and this material had to be non-toxic. Trials were conducted with several materials. Polypropylene piping, with an external diameter 9 cm and a 0.9 cm thick wall and with 20 drainage holes was found to be suitable. A fully autoclavable stainless steel cheese draining table was designed and built. The table could be sterilised in the autoclave and fitted into the laminar flow cabinet. The heights of the legs on the table could be adjusted to allow the whey to flow out of the drainage hole into the Demijohn, (Figure 2.3, 2.4 and 2.5).

The cheese table allowed the aseptic drainage of the contaminated cheese whey from the table to a Demijohn underneath the laminar flow cabinet. The Demijohn was sealed with non-absorbent cotton wool, covered with aluminium foil and surrounded by bio-hazard tape, (Figures 2.6). Once full, the Demijohn was detached from the piping, and the contaminated cheese whey sterilised by autoclaving at 121 °C for 30 min and then discarded.

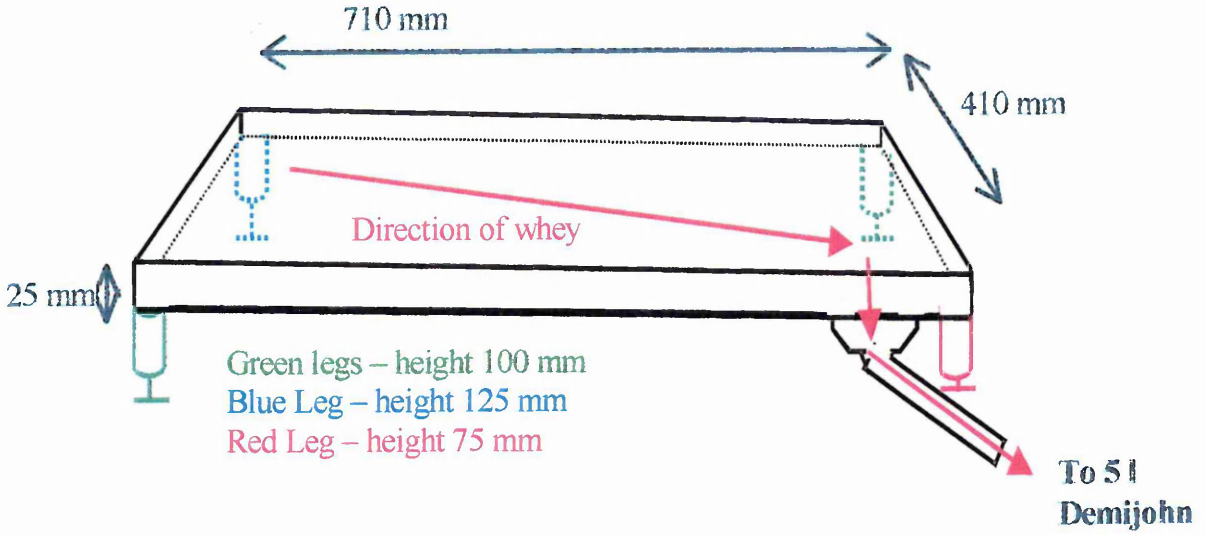


Figure 2.3. Design of the cheese table to allow collection and disposal of the whey

Figure 2.4. Empty cheese mould on the cheese table in the laminar flow cabinet

Figure 2.5. Cheese moulds on draining table filled with cheese curds, showing collection of whey

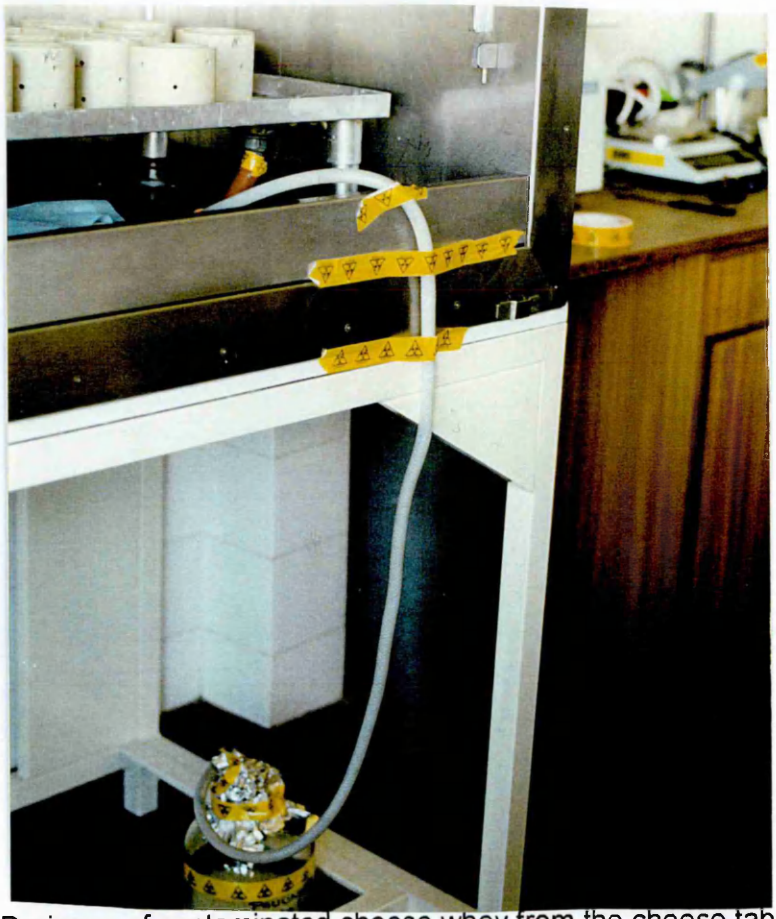


Figure 2.6. Drainage of contaminated cheese whey from the cheese table to a Demijohn on the floor

2.9.2 Preparation of *Listeria innocua* and starter culture

Listeria innocua, strains A7 and NCTC 11288 were examined in separate trials. Each strain was prepared as described in section 2.1.3. A 1:1,000 decimal dilution (1 mL culture + 9 mL 0.1 % peptone) of the culture was made. This was used for the initial inoculum into the milk to give approximately 10^3 cfu of *Listeria* spp. mL^{-1}.

The starter culture was prepared the night before cheese production. Skimmed milk (5 % w/v, Cadbury) was prepared in a sterile 100 mL Duran bottle in sterile distilled water which was boiled by heating in a microwave, (Menumaster, System 80/56, Kitchen Equipment Ltd, Sheffield S9), set on medium heat for 30 s. The milk was cooled and Chr. Hansens Redi-Set Mesophillic Aromatic Starter Culture (Type LD) sprinkled onto the surface of the milk, which was left overnight (15.5 h) at ambient temperature before inoculation into the milk.

2.9.3 Preparation of experimental mould-ripened cheese

All equipment used for cheese making was sterilised by autoclaving for 20 min at 121 °C. Waste was disposed of by autoclaving for 30 min at 121 °C. The cheese table, mats and moulds were designed to survive autoclaving at 121 °C for 20 min to comply with COSHH regulations. The draining table was made to fit the laminar flow cabinet and had controlled whey drainage into a Demijohn (Figure 2.6).

Pasteurised skimmed milk (0.1 % fat) and double cream (48 % fat) were refrigerated at 5 °C and used within 12 h of purchase. Each batch of Camembert-type cheese was made in 10 L sterile stainless steel buckets containing 5 L of milk, (4.63 L of skimmed milk and 0.37 L of double cream). The fat content of the milk was determined using the Gerber analysis (total fat content was 3.7 %). A flow diagram of the method used ro prepare the cheese is given in Figure 2.7.

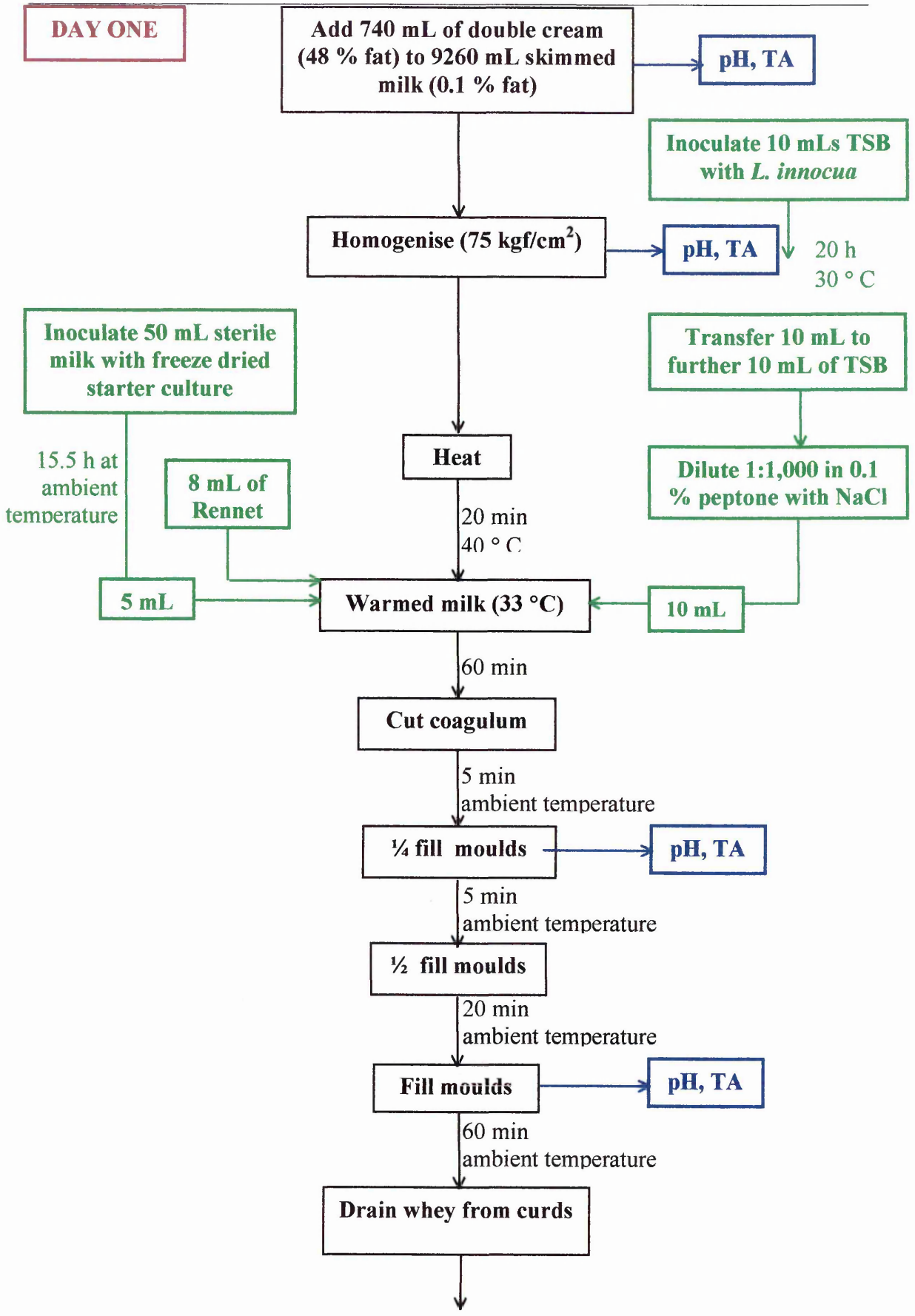


Figure 2.7. (a) Flow diagram to show the production of experimental mould-ripened cheese

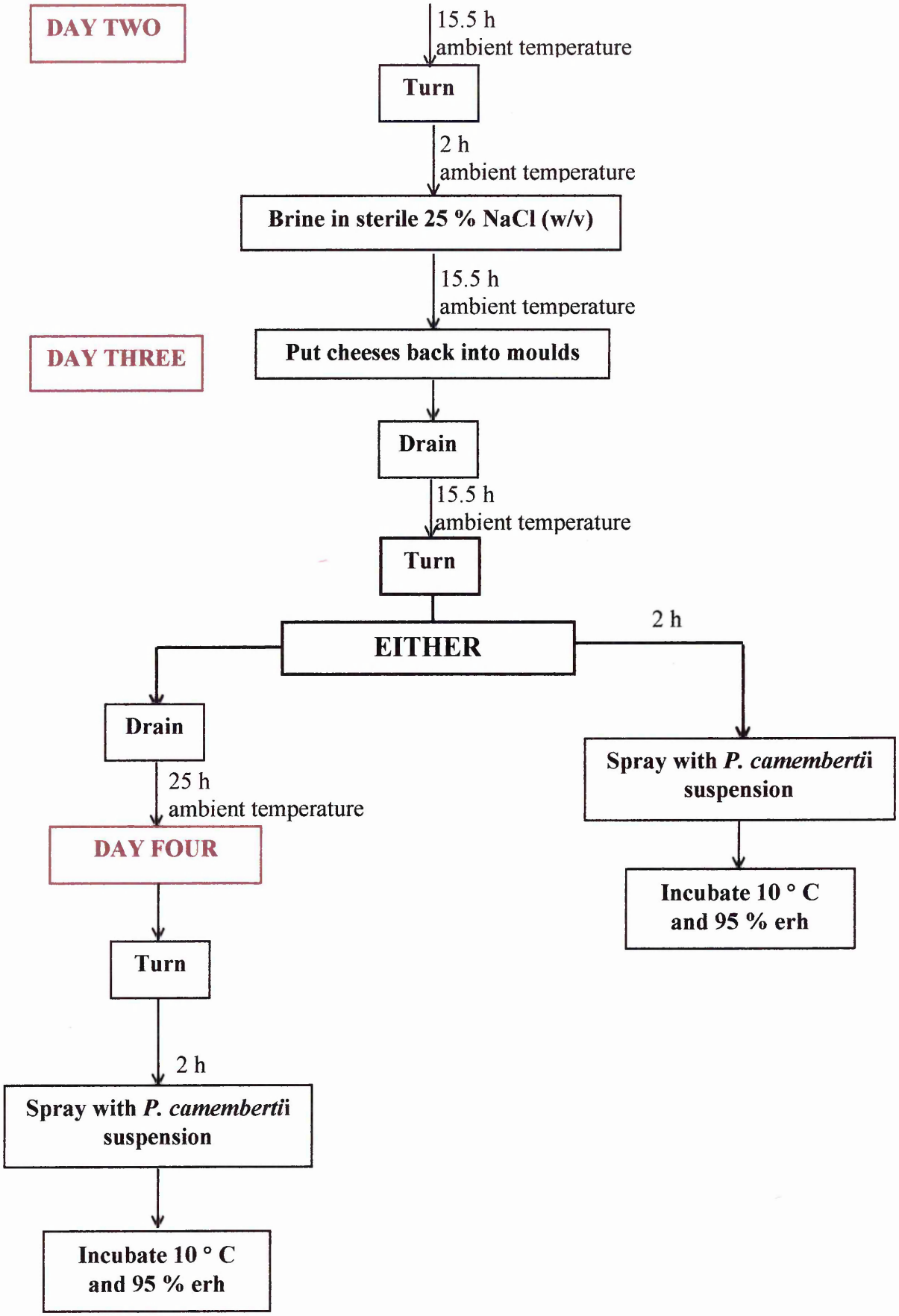


Figure 2.7. (b) Flow diagram to show the draining of experimental mould-ripened cheese (continued)

The double cream was warmed in a waterbath to 40 °C, monolaurin and / or lauric acid were dissolved in the warmed cream and mixed. The final concentration of lauric acid and monolaurin were 5.04 mM and 0.33 mM with respect to the original 5 L of mixed skim milk and cream for the first batch. In subsequent batches lauric acid was eliminated and the concentration of monolaurin increased to approximately 0.9 mM or 0.25 g L^{-1} milk. The cream and milk were mixed together and homogenised at 75 kgf per cm^2, with a homogeniser (Armfield Ltd, Ringwood, Hampshire). Buckets containing milk were left in warm water in a 40 °C water bath, for 20 min to raise the temperature of the milk to 33 °C ± 2 °C. Rennet (Chr. Hansens Laboratory Chymogen), starter culture and *L. innocua* (A7 or NCTC 11288) were added at the rate of 0.8 mL L^{-1}, 0.5 mL L^{-1} and 1 mL L^{-1} of milk respectively. The milk was mixed for 1 min after each addition with a sterile cheese-spoon and left for 70 min. The coagulum was cut twice with a sterile cheese knife into long vertical strips, left to right and top to bottom after which it was left for 5 min. At this point 2 x 10 g of the coagulum was taken aseptically from each batch and used to determine the initial count. The samples were left in stomacher bags at 0 °C in ice water mixture, before decimal dilution and microbiological analysis.

The moulds and mats were assembled on the cheese table in a laminar flow cabinet. Using a sterile cheese spoon the curds were cut for a third time, 2.5 cm from the coagulum surface, producing 2.5 cm^3 cubes. The moulds were filled a quarter full with curds and left for 20 min to drain and then two thirds filled and left for a further 60 min, during which time material was removed to determine the initial count. Samples, 10 g, were diluted 1:10 in 0.01 % peptone w/v and 0.85 % NaCl and further diluted, 1:100 and 100 µL was surface plated onto dried Oxford agar (OA) plates. Finally the moulds

were completely filled. The cheeses were left overnight (15.5 h) in the laminar flow cabinet to allow the whey to drain. The following morning the cheeses were turned and left for 2 h before they were brined for 75 min in 2 L of sterile of 25 % NaCl (w/v) solution in sterile stainless steel bucket. After brining the cheeses were replaced into the original moulds and left overnight (15.5 h) to drain. The next morning the cheeses were turned. After draining for a further 2 h the cheeses were sprayed with a spore suspension of 0.005 % *P. camembertii* [code] conidiospores (Wisby Laboratory, Denmark). The cheeses were placed in a ripening cabinet (perspex desiccator placed in a refrigerated incubator Vindon, Oldham), at 10 °C and 95 % relative humidity (RH), on sterile aluminium foil squares (Figure 2.8). On every 3 rd day the cheeses were turned again and on the 14 th day they were wrapped in sterile aluminium foil. Temperature and RH were logged (Grant 1000 series Squirrel, Cambridge, CB2 5QZ attached to temperature and relative humidity probes), throughout the experiment. Every week two cheeses were taken from each batch for chemical and bacterial analysis.

Figure 2.8. Cheese ripening cabinet inside a refrigerated incubator

Seven trials were completed in total, the first was to determine the titratible acidity, pH, salt, moisture and fat content of the cheeses over a 31 d period. The second trial was to examine the growth of *L. innocua* (A7) on the surface and in the centre of the cheeses. The third was a comparison of growth of *L. innocua* (A7), on the surface and in the centre of the cheeses, with and without the addition of 5.04 mM (1 g) of lauric acid L^{-1} and 0.33 mM (0.1 g) monolaurin L^{-1} of milk. Before mixing with the skimmed milk, monolaurin and lauric acid were dissolved into the cream by warming in a 40 °C waterbath for 10 min. A distinctive smell of methyl ketones was produced during ripening of these cheeses at 10 °C during this trial. Growth of *L. innocua* A7 and NCTC 11288 in the presence and absence of 0.9 mM monolaurin (25 mg L^{-1}) was undertaken during the 5 th and 6 th trials. In the 4 th and 7 th trials the effect of monolaurin on *L. innocua* A7 and NCTC 11288 were examined. The cheeses were left at ambient temperature for an extra day before incubation at 10 °C and 95 % RH.

2.10 Chemical analysis of Camembert-type cheese

2.10.1 Lactic acid concentration

The acidity of the milk was determined by direct titration at stages 1, 2, 6 and 8 (Figure 2.7) of cheese manufacture, as described in Sawyer and Kirk 1991 (BS 1741: Part 10: Section 10.1, 1989). The reagents were prepared as follows. To 110 mL of industrial methylated spirits, 1 g of phenolphthalein and 80 mL of distilled water were added. Sodium hydroxide solution 0.111 M (M/9 NaOH) was added until one drop gave a faint pink colour. This solution was diluted to 200 mL using distilled water. The reference colour solution was prepared by dissolving 1.5 g of cobalt (II) sulphate 7-hydrate in distilled water and diluting to 100 mL.

Milk was pipetted in 10 mL aliquots into 2 basins labelled as the colour control and sample. Reference colour solution (1 mL) was added to the control basin and stirred. Phenolphthalein indicator solution (1 mL) was added to the sample basin and titrated with M/9 NaOH, stirring continuously, until the colour matched the pink of the control, the volume of NaOH titrated was noted. Acidity was calculated as lactic acid (% m/v) by dividing the titration quantity (mL) of NaOH by 10.

2.10.2 Hydrogen ion concentration (pH)

The pH meter, Phillips 9640, was calibrated using the type Phillips, CE2 probe (diameter 4.5 mm and stem 80 mm, Unicam Analytical Systems, CB1 2PX) and setting the pH using standard pH 4 and pH 7 buffers (BDH, Merck chemicals, Leistershire). During manufacture at stages 1, 2, 6, and 8 (Figure 2.7), 5 mL of whey were collected from each batch of cheese and the pH measured in triplicate. During ripening of the Camembert-type cheeses (trials 5-7), 5 g of their surface and 5 g of their centre were weighed into 10 mL beakers. This was mixed with 5 mL of distilled water to form a homogeneous mixture. The pH of each sample was measured in triplicate by placing the probe into the centre of the homogenate. The results were recorded on every 7 th day during the ripening of the cheeses.

2.10.3 Salt content

The salt content of each cheese during ripening was measured electrochemically using a Corning 926 Chloride Analyser (Ciba-Corning Diagnostics Ltd, Essex, CO9 2DX). The cheese samples were prepared by adding 1 g of cheese to 100 mL of distilled water and liquidising (in a homogeniser) for 5 min. The homogenate was centrifuged for 2 min at 1000 rpm. The analyser was calibrated using 200 mg Cl^- L^{-1} standard solution and the Cl^- content of the cheese samples measured. The reading was multiplied by 100

(the dilution factor) to convert to mg $Cl^- L^{-1}$ and % NaCl calculated (equations 2.1a and b).

$$mg\ Cl^- L^{-1} \times 10 = mg\ Cl^- 100\ g^{-1} \qquad (2.1\ a)$$

$$mg\ Cl^- 100\ g^{-1} \times 1.648^* = \%\ NaCl \qquad (2.1\ b)$$

* atomic weight of NaCl = 58.44
atomic weight of Cl^{-1} = 35.45
therefore 58.44 / 35.45 = 1.648

2.10.4 Moisture determination: Dean and Stark

Dean and Stark (AOAC – 969.19 solvent and standards) methodology and distillation equipment were used. Solvent was prepared using 1 part 1-penatanol and 2 parts xylene. Fine sand was added to a 250 mL conical flask to form a 4 cm diameter circle and 10 mL of distillation solvent was pipetted onto the sand. The standard control was prepared by adding 4.5 mL of distilled water and 60 mL of solvent to the flask (this was used as the standardisation constant). The flask was closed immediately with a glass stopper and left until the cheese samples were prepared. Duplicate cheese samples were weighed accurately (11 g) into a 250 mL round bottom flasks with a 4 cm diameter circle of fine sand. After the addition of 60 mL of solvent the flasks were closed with a ground glass stopper. The flasks containing both cheese samples and control were placed onto heating mantles, (Electrothemal, London E7 9QN), on setting 7 for 50 min, and allowed to reflux. The distillation was continued until no further drops of water were seen. The condenser walls were rinsed with solvent and the distillation continued until no further water droplets were seen to condense into the receiver. The condenser was dismantled and the receiver tilted to expel some of the solvent. The flasks were left to cool and the water level recorded to the nearest 0.05 mL. The calculation for % moisture is shown in equation 2.2:

$$\% \text{ Moisture} = \frac{\text{Water level (mL) - Standardisation constant}}{\text{Cheese (g)}} \times 100 \quad (2.2)$$

2.10.5 Fat content

A 25 g sample of each cheese was macerated with a pestle and mortar and 3g of each sampled analysed for fat content by the Gerber method, Gerber Method (adapted from ISS 696 189) described in Sawyer and Kirk 1991.

2.11 Examination of *Listeria* spp. in Camembert-type cheese

During each trial two cheeses were sampled every 7 d during the ripening. Both cheeses were cut into half, the first half was used to determine the population of *Listeria* spp. on the surface and the second half was used to obtain the population of *L. innocua* in the centre of the cheese.

To prepare the surface sample, a 1 mm thick slice was cut from each surface with a sterilised scalpel and forceps. The outside of the slice contained the fungal mycelium and was discarded and a 10 g ± 0.5 g sample of the surface of the cheese was taken. To prepare a sample from the centre the upper and lower surface of the cheese was cut away (5 mm) with a sterile scalpel and the centre scooped out with a sterile spatula to give a 10 g ± 0.5 g sample. The 10 g samples were diluted 1:10 with 0.01 % peptone and 0.85 % NaCl (10 g + 90 mL diluent) in a stomacher bag and blended with a Stomacher (Laboratory Blender Stomacher 400, Seward Medical, London SE1), for 60 s. The samples were left at 0 °C in order to allow the *Listeria* spp. time to resuscitate (20 min) and then decimal dilutions were prepared in 0.01 % peptone with 0.85 % NaCl. Spread plates (100 µL) of the last 2 dilutions were prepared in triplicate for each cheese sample on dried Oxford agar and the plates were incubated at 30 °C for 48 h ±

4 h. This was repeated for both test and control batches of cheese. Only colonies that were aesculin positive were counted. Counts on TSGY agar where inaccurate due to the excessive growth of lactic acid bacteria in the initial stages of ripening.

2.12 Sensory analysis of laboratory made Camembert-type cheese

The Camembert-type cheeses were produced in the food production laboratory not a microbiological laboratory, as these cheeses would have to be safe to eat (Figure 2.7). No *L. innocua* was added to the milk and the extended production time of 25 h was used in their manufacture). To one batch of milk monolaurin (0.05 g L^{-1} of pasteurised milk) was added, this was labelled as the test cheese. The second batch was produced without monolaurin and was labelled as the laboratory control. Once manufactured the two cheeses where ripened in a cheese cabinet, in a refrigerated incubator at 10 °C and 95 % erh in the food preparation room. After 21 d the cheeses were ready for sensory analysis.

With a group of nine untrained panellists a basic sensory analysis test was conducted. This was to compare laboratory manufactured Camembert-type cheese, with and with out monolaurin to a reference sample bought from a major retailer.

Each cheese was cut into 30 slices using a cheese wire. A paper plate was divided into 3 sections, 3 samples of the cheeses were placed into each section, 2 from the same cheese and 1 from a different cheese. All slices were coded using a table of random numbers. The basic triangle difference test was used to determine whether a difference in taste could be detected, Appendix 1.

After sampling the cheese each panellist was asked to taste each of the 3 samples and to identify the different sample within each section. Each panellist was provided with a

cup of water to rinse his or her mouth between samples. They where asked to add comments on the cheeses they had tested. Results were analysed using the manual on sensory testing methods, STP 434 (American Society for Testing and Materials).

2.13 Mathematical analysis

2.13.1 Calculations from raw data

Colony forming units per mL (cfu mL^{-1}) were calculated from the counts using equation 2.3:

$$\text{cfu } mL^{-1} = \text{count} \times \frac{1}{\text{volume of inoculum}} \times \text{dilution factor} \qquad (2.3)$$

(a correction factor was used to bring the volume of the inoculum to 1 mL). This was expressed as Log_{10} using the Microsoft Excel spreadsheet package. Two experiments were completed for each strain of *listeria* spp. In each experiment there were three tests (for the first, C, O, LA and for the second O, ML, ML and LA). Results were in triplicate for each experiment. Each point on the logarithmic graph represented the average (plus/minus standard deviation) of each tests 18 plates (i.e. 2 experiments x 3 flasks x 3 plates). This was calculated by using the average and SD functions in the Microsoft Excel package. Finally the average logarithm of the bacterial count was plotted against time for ML, LA, ML and LA, O and C.

2.13.2 Analysis of bacterial curve

The experimental plot of each line curve for each *Listeria* spp. was fitted to a polynomial model created by Barayni 1993 using an Excel spreadsheet. From this a growth plot, the rate of growth, lag h (if any) and standard error were calculated.

The comparison of the slopes of the exponential phase of each curve was achieved using analysis of variance (ANOVA). The exponential phase and the beginning of the stationary phase were judged by eye to be 0 h and 120 h. Analysis of variance was calculated using the mean value of y for each linear line by Microsoft Excel data analysis. If $P > 0.05$ then the null hypothesis H_o: $a = b = c = d = e$ is accepted and there is no significant difference between slopes. Alternatively if $P < 0.05$ then H_o is rejected and there is a significant difference between the slopes. A least significant difference test was used to determine which sets of data in the columns or rows were significantly different. The calculation was as follows:

$$\text{Least significant difference} = tDF\sqrt{\frac{2 \times MS}{n}} \quad (2.4)$$

tDF = t degrees of freedom (calculated from ANOVA)

MS = Mean squares error (calculated from ANOVA)

n = number of tests - 2

The differences between the averages of each test were then found. If they were found to be greater than the calculated least significant difference then the conclusion was no difference. If the difference was found to be less than this value then there was a difference.

The growth rate was used to calculate other constants:

$$\mu \ (h^{-1}) = \text{growth rate constant } (h^{-1}) = \text{slope} \times 2.303 \quad (2.5)$$

$$t_d \ (h) = \text{doubling time (h)} = t_d = \frac{0.693}{\mu} \quad (2.6)$$

$$V \ (h^{-1}) = \text{divisions per hour} = \frac{1}{t_d} = \frac{\mu}{0.693} \quad (2.7)$$

These constants were used to describe and compare the growth of the three strains of listeria when subjected to ML and LA.

2.13.3 Log reduction

Model broth culture:

The log reduction was calculated as equation 2.8:

$N_o - N_1$ (2.8)

where: $N_o = Log_{10}$ cfu mL^{-1} of control culture at each point in time and $N_1 = Log_{10}$ cfu mL^{-1} of test culture at each point in time.

Model Camembert-type cheese:

The log reduction between the (1) surface and centre of the cheese for control and tests cheeses, (2) the surface of the control cheese and the surface of the test cheese and (3) the centre of the control and the centre of the test cheese, were calculated at each point in time as equations 2.9 – 2.11:

$SN - CN$ (2.9)

$SN_o - SN_1$ (2.10)

$CN_o - CN_1$ (2.11)

where: S = surface, C = centre, N = population Log_{10} cfu g^{-1}, 1 = test cheese and o = control cheese.

Identification of *Listeria* spp. and standardisation of incubator temperature

3.1 Identification of *Listeria* spp. using the API test strip

The results from the API computer identification of strains of *L. monocytogenes* and *L. innocua* are given in Table 3.1. Strains A3, A4, A13, A14, A15, BL/88/1b and LB were identified as *Listeria monocytogenes* (6510), strains A7 and NCTC 11288 were identified as *Listeria innocua* (7510). All strains hydrolysed aesculin to aesculetin, which formed a complex with Fe^{++} to give a black colour. All strains gave a positive reaction for α–mannoside and a yellow colour after fermentation of D-arabitol, rhamnose and α-methyl-D-glucoside due to production of acid. The listeria strains did not metabolise D-xylose, ribose, and glucose-1-phosphate.

Cells were examined using a light microscope and were measured using an eye-piece graticule. The graticule was calibrated at (x 1000) and the distance between each line in the eye-piece scale was found to be 1.0 μm. All strains were Gram positive small rods, 0.5 - 2 μm in length (Figure 3.1). Strain A7 and NCTC 11288 were positive for the DIM test, (β-napthylamine and the enzyme arlamidase reacted with the Zym B reagent to give a free amino acid and β napthylase) the other strains did not. The strains identified as *L. monocytogenes* gave a positive β-haemolysis reaction on Blood Agar whilst the strains identified as *L. innocua* did not haemolyse haemoglobin in the agar. The β-haemolytic reaction and the DIM test were used to differentiate *L. innocua.* from *L. monocytogenes.*

Table 3.1. (a) Identification of cheeseborne strains of *Listeria monocytogenes* with the API Listeria Test Strip

Test (code)	Fourme d'Ambert (A3)			Fourme d'Ambert (A4)			Brie (A13)			Brie (A14)			Brie (A15)		
	Colour[l]	Reaction	Code	Colour	Reaction	Code	Colour	Reaction	Code	Colour	Reaction	Code	Colour	Reaction	Code
DIM[a]	Pale Orange	-		Pale Orange	-		Pale Orange	-		Pale Orange	-		Pale Orange	-	
ESC[b]	Black	+	6	Black	+	6	Black	+	6	Black	+	6	Black	+	6
αMAN[x]	Yellow	+		Yellow	+		Yellow	+		Yellow	+		Yellow	+	
DARL[d]	Yellow	+		Yellow	+		Yellow	+		Yellow	+		Yellow	+	
XYL[e]	Red	-	5	Red	-	5	Red	-	5	Red	-	5	Red	-	5
RHA[f]	Yellow	+		Yellow	+		Yellow	+		Yellow	+		Yellow	+	
MDG[g]	Yellow	+		Yellow	+		Yellow	+		Yellow	+		Yellow	+	
RIB[h]	Red	-	1	Red	-	1	Red	-	1	Red	-	1	Red	-	1
G1P[i]	Red	-		Red	-		Red	-		Red	-		Red	-	
TAG[j]	Red	-	0	Red	-	0	Red	-	0	Red	-	0	Red	-	0
βHEM[k]	Clearing	+		Clearing	+		Clearing	+		Clearing	+		Clearing	+	
Catalase Test		+			+			+			+			+	
Gram stain[m]	Purple	G+CB		Purple	G+CB		Purple	G+CB		Purple	G+CB		Purple	G+CB	
Identity	*Listeria monocytogenes*			*Listeria monocytogenes*			*Listeria monocytogenes*			*Listeria monocytogenes*			*Listeria monocytogenes*		

Table 3.1. (b) Identification of cheesborne and other strains of listeria with the API Listeria Test Strip

Origin (code)	(BL/88/1b)			Lanark Blue (LB)			Brie (A7)			NCTC 11288		
	Colour[l]	Reaction	Code	Colour	Reaction	Code	Colour	Reaction	Code	Colour	Reaction	Code
DIM[a]	Pale Orange	-		Pale Orange	-		Orange	+		Orange	+	
ESC[b]	Black	+	6	Black	+	6	Black	+	7	Black	+	7
αMAN[x]	Yellow	+		Yellow	+		Yellow	+		Yellow	+	
DARL[d]	Yellow	+		Yellow	+		Yellow	+		Yellow	+	
XYL[e]	Red	-	5	Red	-	5	Red	-	5	Red	-	5
RHA[f]	Yellow	+		Yellow	+		Yellow	+		Yellow	+	
MDG[g]	Yellow	+		Yellow	+		Yellow	+		Yellow	+	
RIB[h]	Red	-	1	Red	-	1	Red	-	1	Red	-	1
G1P[i]	Red	-		Red	-		Red	-		Red	-	
TAG[j]	Red	-	0	Red	-	0	Red	-	0	Red	-	0
βHEM[k]	Clearing	+		Clearing	+		None	-		None	-	
Catalase Test		+			+			+			+	
Gram stain [m]	Purple	G+CB		Purple	G+CB		Purple	G+CB		Purple	G+CB	
Identity	*Listeria monocytogenes*			*Listeria monocytogenes*			*Listeria innocua*			*Listeria innocua*		

The strains where cultured for 24 h at 30 °C on HBA agar, before inoculation into API Listeria Test Strip (Bio-Mèrieux, France). Results were read after incubation at 37 °C for 24 h. Code 6510 = *Listeria monocytogenes*, code 7510 = *Listeria innocua.* [a] Differentiation between *L. innocua* and *L. monocytogenes* (colour recorded after the addition of Zym b reagent); [b] Aescultin; [c] α-Mannoside; [d] D-Arabitol; [e] D-Xylose; [f] Rhamnose; [g] a-Methyl-D-Glucoside; [h] Ribose; [i] Glucose-1-Phosphate; [j] Tagatose; [k] β-Haemolysis; [l] Colour of microtubes after incubation; [m] G+, Gram positive, CB, cocco-bacilli.

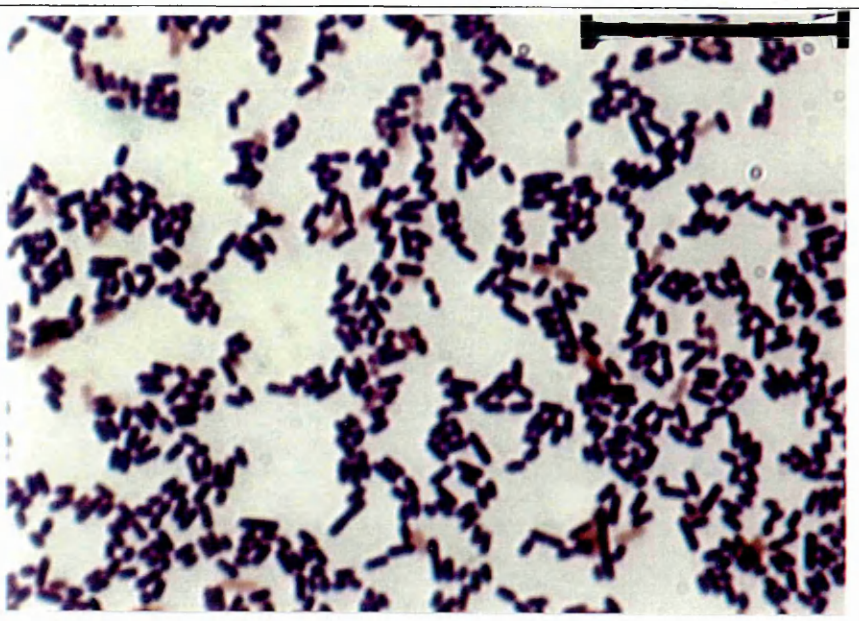


Figure 3.1. Gram stain of *Listeria innocua* (A7)

Listeria innocua was cultured in enriched TSB for 18 h at 30 °C and examined under the light microscope (at x 1000). Scale bar = 10 μm.

3.2 Examination of *Listeria monocytogenes* for motility with the light micoscope

A number of cells were examined with the light microscope to see if lauric acid and monolaurin affected their motility. The cells used were actively growing ones derived from the logarithmic phase of the growth cycle. A survey of the listeria cells was completed to determine motility. *Listeria* spp. exhibit a characteristic tumbling motility at 25 ° C. Table 3.2 shows that when lauric acid was added to the medium, the listeria cells were less motile when compared to the control. When both monolaurin and lauric acid were used in combination this effect was even greater. The addition of butter oil alone had no effect on the motility of the cells. There was also no difference between the motility rate of the control cells and those subjected to monolaurin. The non-viable cells did exhibit some movement but this was attributed to Brownian movement and still graded as zero (0). These results suggest either that the listeria cells had less energy to move when grown in the presence of lauric acid and monolaurin or that the cells did

not produce flagella. *Listeria innocua* was found to be more sensitive to the inhibitors than *L. monocytogenes*. The reduction in motility of the cells could be due to the interruption of the proton motive force, and a reduction in available ATP or the cells simply did not have the energy to produce actin for making flagella. The investigation of listeria cells with the electron microscope was designed to see if the listeria cells still grew flagella when subjected to lauric acid.

Table 3.2. Effect of monolaurin and lauric acid on motility of *Listeria* spp.

Strain	Test	Number of Samples	Rating[a]
L. monocytogenes (A3)	Non-viable cells[b]	3	0
	Control	3	5
	Oil	3	5
	Monolaurin	3	5
	Lauric acid	3	4
	Monolaurin and lauric acid	3	3
L. innocua (NCTC 11228)	Non-viable cells	3	0
	Control	3	5
	Oil	3	5
	Monolaurin	3	5
	Lauric acid	3	3
	Monolaurin and lauric acid	3	2

Cell were grown in TS broth and investigated after 96 h (logarithmic phase).
[a] control (listeria cells were boiled for 1 min); [b] cells were scored between 0 and 5, 5 = motility with tumbling movement, 0 = no tumbling movement or simple Brownian movement

3.3 Examination of *Listeria* monocytogenes for flagella with the Transmission Electron Microscope (TEM)

When *L. monocytogenes* was examined by Transmission Electron Microscopy (TEM) it was harder to find cells that had been grown in the presence of lauric acid as there were fewer *L. monocytogenes* cells present. A more concentrated suspension of listeria cells was achieved by centrifugation. The physical agitation due to centrifugation resulted in the flagella to falling off of the cells. It was decided omit the centrifugation step in

future experiments. Figures 3.2 to 3.4 show electron micrographs of *L. monocytogenes* strains. It can be seen that all the cells were round-ended rods or cocco-bacillus, with some of the cells under-going cell division by binary fission. The cell dimensions of the *L. monocytogenes* were taken from TEM photographs using the calibration bar on the electronmicrograph. This was achieved by measuring the calibration bar in mm. The cells were measured on the photomicrograph with a ruler. The cell width and length could be calculated from this in μm.

Table 3.3 gives the cell dimension of a number of strains of *L. monocytogenes* cells in the logarithmic and stationary phase of growth. It can be seen for all three strains that cells grown in the presence of lauric acid were shorter in length than those grown in the absence of lauric acid. Assuming that the cells were perfectly cylindrical, the formula $\pi r^2 h$ was used to calculate cell volume. The listeria cells grown in the presence of lauric acid were found to be smaller in volume (50 % reduction in cell volume) than those grown in the absence of lauric acid. There was no difference in cell size between the listeria cells which were measured in their stationary phase of growth compared to those cells measured in the exponential phase of growth. The listeria cells have adapted to the presence of lauric acid.

Table 3.3. Cell size of three *Listeria monocytogenes* strains

Strain	Growth Phase	Treatment	n	Length (μm)	Width (μm)	Volume (μm^3)
LB	Logarithmic	O	2	1.55 1.55	0.35 0.35	0.15 0.15
		LA	2	0.98 0.97	0.65 0.65	0.34 0.34
	Stationary	O	2	1.18 1.32	0.78 0.53	0.56 0.29
		LA	2	1.00 0.88	0.70 0.69	0.38 0.33
A3	Logarithmic	O	1	1.25	0.4	0.16
		LA	NF	NF	NF	NF
	Stationary	O	2	1.05 0.82	0.67 0.67	0.36 0.28
		LA	2	0.71 0.63	0.55 0.52	0.18 0.14
A13	Logarithmic	O	2	1.00 1.13	0.62 0.60	0.35 0.32
		LA	NF	NF	NF	NF
	Stationary	O	2	1.26 1.43	0.65 0.72	0.44 0.90
		LA	2	0.80 0.86	0.57 0.56	0.20 0.22

Cells were cultured in 50 mL TSGYB with 5 g of butter oil on the surface and incubated at 10 °C. Samples were taken at logarithmic phase of growth (72 h) and at the stationary phase of growth (144 h). These were photographed with the TEM. Two cells from each growth stage were measured. O = cells grown with butter oil (control), LA = Cells grown with oil and lauric acid (5.05 mM, with respect to aqueous phase). n = number of cells measured. LB = *L. monocytogenes* from Lanark Blue cheese, A3 = *L. monocytogenes* from Brie cheese and A13 = *L. monocytogenes* from Fourme d'Ambert cheese. NF = not found

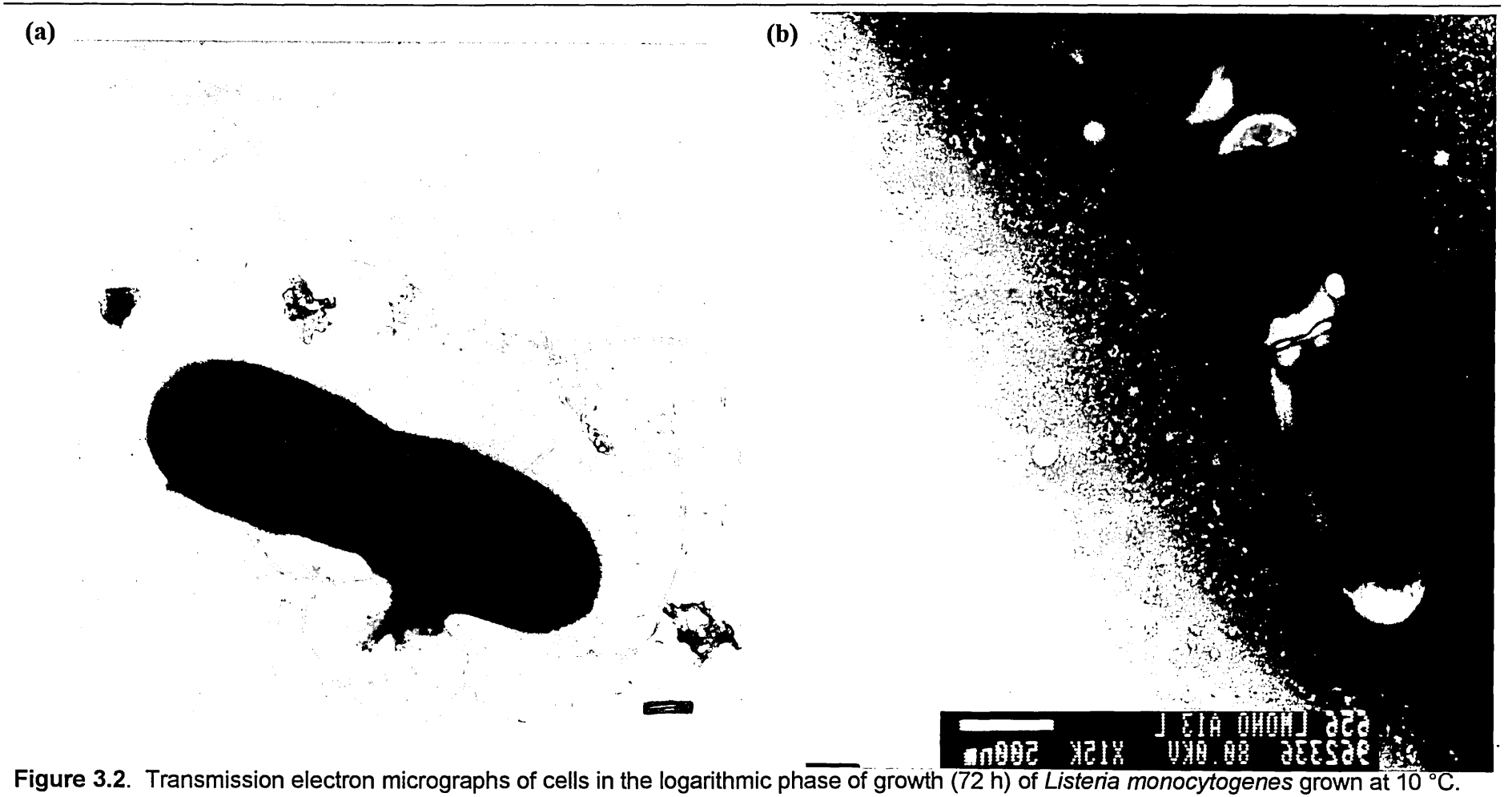


Figure 3.2. Transmission electron micrographs of cells in the logarithmic phase of growth (72 h) of *Listeria monocytogenes* grown at 10 °C.
(a) Strain A13 from Fourme d'Ambert cheese grown with butter oil, scale bar = 0.2 μm;
(b) Strain A13 grown with 5.05 mM lauric acid, scale bar = 0.5 μm.

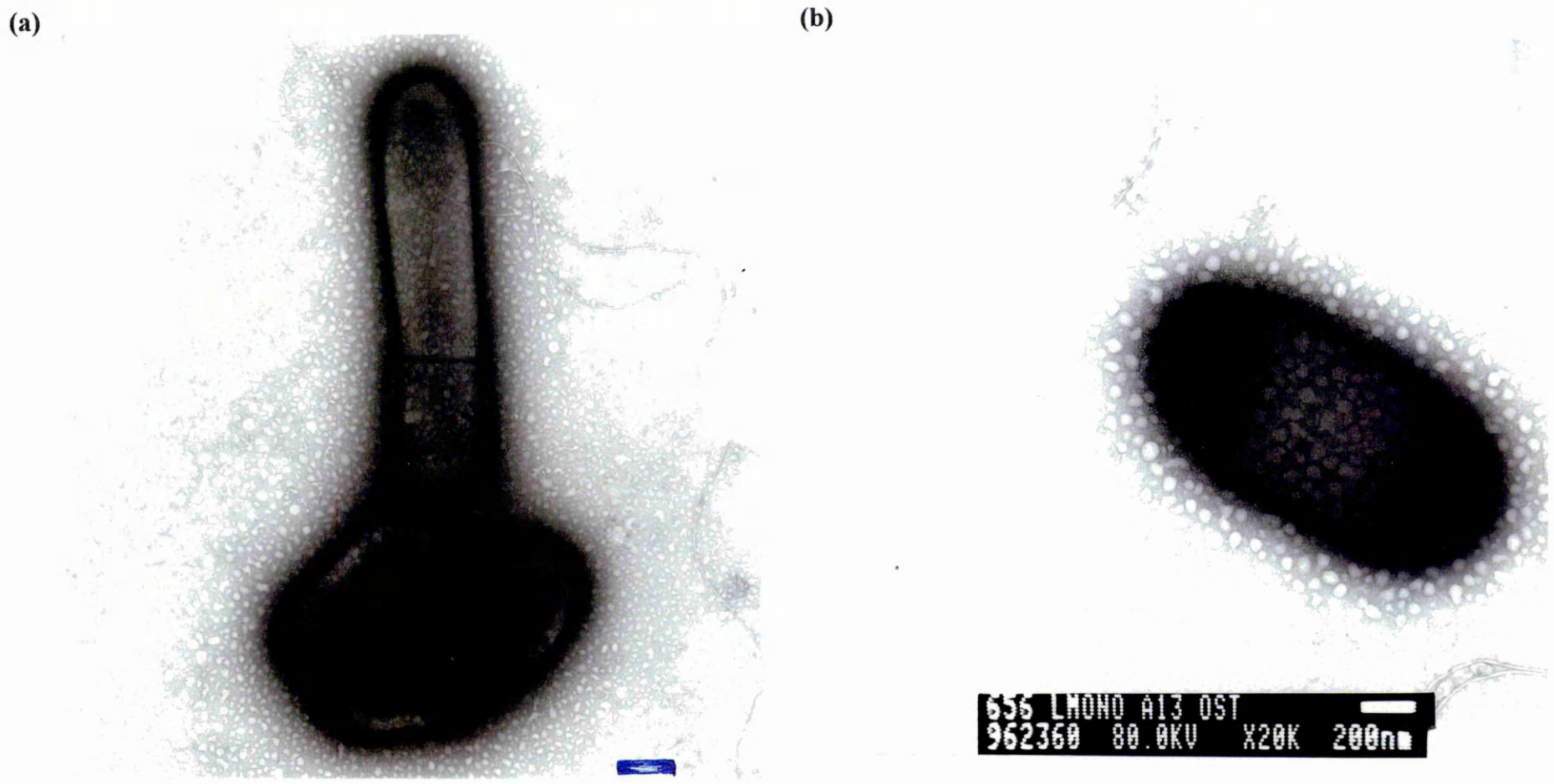


Figure 3.3. Transmission electron micrographs of cells in the stationary phase (144 h) of *Listeria monocytogenes* A13 (from Fourme d'Ambert cheese), grown at 10 °C with butter oil; (a) Scale bar = 0.2 μm; (b) Scale bar = 0.5 μm.

Figure 3.4. Transmission electron micrographs of cells in the stationary phase (144 h) of *Listeria monocytogenes* grown at 10 °C with 5.05 mM (with respect to aqueous phase) lauric acid; (a) *Listeria monocytogenes* A3 from Brie cheese, Scale bar = 0.2 μm; (b) *Listeria monocytogenes* from Fourme d'Ambert cheese, Scale bar = 0.5 μm.

3.4 Calibration of the refrigerated incubator to give an incubation temperature of 10 °C

Temperature calibration of the refrigerated incubator was very important in order to maintain constant conditions for cell growth in shake culture. Table 3.4 gives the flask temperature and air temperature of five separate experiments. The first experiment was run for 14 days where the air temperature was 10.0 ± 0.2 °C. The shaker was set at 200 rpm which gave a water loss of 0.32 % per 24 h. Flasks with a wide neck (30 mm diameter) lost more water than those with a narrow neck (20 mm diameter). Flasks with 20 mm diameter were used in subsequent experiments.

The second experiment was designed to monitor both air temperature and the temperature of the broth in the flask. The shaker speed was reduced to 75 rpm in order reduce the heat produced by the shaker motor and the rate of water loss. It was found that the flask temperature was 0.6 °C higher that that of the air. The slower shaker speed slowed the evaporation rate of water loss by 0.10 % per day. The third and fourth experiments were designed to determine the setting of the temperature dial on the incubator which would give a flask temperature of 10 °C. It was found that an air temperature of 8.5 °C gave a broth temperature of 10 °C with a setting of 75 rpm on the shaker. Water loss was also reduced to 0.21 % per day. The last experiment was designed to find if butter oil had an effect on the rate of water loss, water loss was finally reduced to 0.20 % per day.

In subsequent experiments a shaker speed of 75 rpm, an air temperature of about 8.5 °C (resulting in a broth temperature of 10 °C) and a flask neck size diameter of 20 mm was used to minimise water loss.

Table 3.4. Calibration of the refrigerated incubator

Date	Period (d)	Vindon setting	Butter oil	Air Temperature °C ± SD	Flask Temperature °C ± sd	Speed [a] (rpm)	Loss [b] ($g\ d^{-1}$)	Loss [c] ($\%\ d^{-1}$)
8.12.94	14	7.77	-	10.0 ± 0.2	NT	200	0.15	0.32
22.12.94	18	7.77	-	10.0 ± 0.3	10.6 ± 0.6	75	0.11	0.22
9.1.95	7	7.67	-	9.0 ± 0.3	10.0 ± 0.3	75	0.10	0.22
24.1.95	6	7.62	-	8.5 ± 0.2	10.0 ± 0.2	75	0.10	0.21
30.1.95	7	7.62	+	8.5 ± 0.2	10.0 ± 0.2	75	0.10	0.20

The temperature was recorded throughout the five experiments, and the temperature means ± standard deviations (sd) were calculated. Percent weight loss of broth was due to the mass transfer of the water in the broth to the refrigerated coils in the incubator. [a] Speed of shaker; [b]weight loss of broth d^{-1}; [‡] in g; [c] as a %.

The incubator was defrosted between each experiment and broth and flask temperatures were monitored during each experiment. The average temperature of the culture broth after calibration was kept at 10.0 °C ± 0.2 °C for all experiments, as described in Chapter 4.

3.5 Summary of results

1. Listeria strains A3, A4, A13, A14, A15, BL/88/1b and LB were identified as *L. monocytogenes* by the API computer identification system. All strains gave a positive reaction for the DIM test and the β-haemolysis test.
2. Listeria strains A7 and NCTC 11288 were identified as *L. innocua* by the API computer identification system. Both strains were negative for the DIM test and the β-haemolysis test.
3. Lauric acid (5.05 mM) and monolaurin (0.35 mM) reduced the motility of *L. monocytogenes* A3 and *L. innocua* NCTC 11288. Butter oil and monolaurin alone had no effect on motility.
4. There was a 50 % reduction in cell volume of listeria cells when grown in the presence of lauric acid in both stationary and exponential phases of growth.
5. Calibration of the refrigerated incubator with frequent defrosting was essential in the development of a constant incubator temperature for growth in shake culture.

Growth of *Listeria spp.* in a model bi-phasic system in broth culture

4.1 Incubation conditions and concentration of preservative

Table 4.1 gives the experimental conditions (temperature, lauric acid and monolaurin concentration) which were used to measure the growth of *Listeria* spp. in broth culture. This Table demonstrates that the average temperature of incubation was 10 °C ± 0.5 °C for most of the experiments. There were two factors which affected the incubation temperature, the first was the incubator design. Temperature variation was reduced to a minimum during each experiment by placing the shaker in exactly the same position in the incubator. This meant that the relationship between the position of the shaker, heating coils and evaporator were the same for all experiments. The second factor was the heat produced by the shaker motor. This was reduced as much as possible by insulation of the shaker with an additional two layers of foam between the top of the shaker and the tray with clips for attaching the flasks to the shaker.

All five strains of listeria were grown with butter oil (5 g), with lauric acid (5.05 mM) and monolaurin (0.35 mM) in broth culture. The concentration of the inhibitors was calculated as though they were dissolved in the aqueous phase (broth) of the system. There was a variation in the concentration of the individual inhibitors between individual flasks. This was due to the small quantities that were dispensed into butter oil and the difficulty in weighing exactly 5 g into each flask.

Table 4.1. Experimental conditions used to determine the effect of monolaurin and lauric acid on *Listeria* spp.

Strain	Experiment	n[f]	Temperature (°C ± SD)	Lauric acid (mM ± SD)[g]	Monolaurin (mM ± SD)
L. monocytogenes A3[a]	Control	2	9.9 ± 0.2	-	-
	Control with oil	4		-	-
	Lauric acid	2		4.98 ± 0.13	-
	Control with oil	4	10.0 ± 0.2	-	-
	Monolaurin	2		-	0.36 ± 0.01
	Lauric acid and monolaurin	2		5.65 ± 0.18	0.35 ± 0.01
L. monocytogenes A13[b]	Control	1	10.1 ± 0.2	-	-
	Control with oil	4		-	-
	Lauric acid	2		5.04 ± 0.00	-
	Control with oil	4	9.8 ± 0.2	-	-
	Monolaurin	2		-	0.35 ± 0.02
	Lauric acid and monolaurin	2		5.34 ± 0.03	0.38 ± 0.00
L. monocytogenes LB[c]	Control	2	10.1 ± 0.2	-	-
	Control with oil	4		-	-
	Lauric acid	2		5.01± 0.07	-
	Control with oil	4	10.0 ± 0.2	-	-
	Monolaurin	2		-	0.36 ± 0.01
	Lauric acid and monolaurin	2		5.19 ± 0.15	0.35 ± 0.01
L. innocua A7[d]	Control	2	9.9 ± 0.2	-	-
	Control with oil	4		-	-
	Lauric acid	2		5.10 ± 0.12	-
	Control with oil	4	9.8 ± 0.2	-	-
	Monolaurin	2		-	0.36 ± 0.00
	Lauric acid and monolaurin	2		4.99 ± 0.04	0.36 ± 0.00
L. innocua NCTC 11288[e]	Control	2	10.0 ± 0.3	-	-
	Control with oil	4		-	-
	Lauric acid	2		5.00 ± 0.02	-
	Control with oil	4	10.1 ± 0.3	-	-
	Monolaurin	2		-	0.36 ± 0.01
	Lauric acid and monolaurin	2		5.22 ± 0.02	0.34 ± 0.01

Strains were grown in a bi-phasic system TSGY broth (initial pH 7.00) and butter oil in shake culture at 75 rpm. Monolaurin and lauric acid were dissolved in the butter oil.

[a] *L. monocytogenes* from Fourme d'Ambert Cheese, [b] *L. monocytogenes* from Brie Cheese,
[c] *L. monocytogenes* from Lanark Blue Cheese. [d] *L. innocua* from Brie Cheese,
[e] *L. innocua* from the National Collection of Type Cutures, [f] n = number of experiments.
[g] Calculated as if the preservative was completely dissolved in the aqueous phase.

4.2 Growth of cheeseborne strains of *Listeria* spp. in a bi-phasic system in broth culture at 10 °C

The viable count $\log_{10}$ N mL^{-1} for all experiments are given in Appendix 2. The viable count Log_{10} N mL^{-1} against incubation time has been plotted for all experiments and is given in Figures 4.1 a to 4.5 a. The data was fitted using a predictive model programme written by Baranyi and Roberts (1994). The fitted growth curves are given in each Figure 4.1 to 4.5 as inserts b, c, d, e and f. This programme has been written to fit a lag phase to the data if it was present. In this work the inoculum comprised of cells already in exponential growth phase as they were incubated at 30 °C for 24 h and then 20 h. These cells were re-inoculated into the same medium although incubated at a lower temperature (10 °C). This meant that the listeria cells were already adjusted to TSGB and did not have to adjust to a new medium before continuing to grow at a constant rate. A lag phase was present for strains *L. monocytogenes* (A3) and *L. innocua* (NCTC 11288), with all the other strains no lag phase was seen (Table 4.2).

It was thought that this lag phase was due to the lower incubation temperature (10 °C). Table 4.2 gives the cell population of *Listeria* spp. at 192 h, the lag phase (if present), the slope and r^2. This Table demonstrates that 0.05 mM lauric acid reduced the cell population by 1 to 2 log orders for *L. monocytogenes* and 2 to 3 log orders for *L. innocua* depending on strain at 192 h. When monolaurin was combined with lauric acid the cell population at 192 h was reduced by up to five log orders.

Table 4.2. Effect of monolaurin and lauric acid on multiplication of *Listeria* spp. at 10 °C

Strain		Test	N_{192}[f] ($\log_{10}$ cfu mL^{-1})	Lag (h)	r^2	Slope (h^{-1})	Standard error (h^{-1})
L. monocytogenes	A3[a]	Control	9.95	20.51	0.99	0.069	0.007
		Control with oil	9.90	14.86	0.99	0.066	0.003
		Lauric acid	8.22	15.56	0.99	0.039	0.003
		Monolaurin	9.67	None	0.99	0.057	0.002
		Lauric acid and monolaurin	5.44	None	0.99	0.014	0.002
	A13[b]	Control	9.66	None	0.99	0.047	0.001
		Control with oil	9.72	None	0.99	0.046	0.002
		Lauric acid	7.51	None	0.99	0.030	0.001
		Monolaurin	9.64	None	0.99	0.057	0.002
		Lauric acid and monolaurin	6.05	None	0.99	0.018	0.002
	LB[c]	Control	9.79	None	0.99	0.059	0.001
		Control with oil	9.75	None	0.99	0.061	0.002
		Lauric acid	7.53	None	0.99	0.028	0.001
		Monolaurin	9.61	None	0.99	0.063	0.003
		Lauric acid and monolaurin	2.81	None	0.99	NG	0.001
L. innocua	A7[d]	Control	10.11	None	0.99	0.059	0.005
		Control with oil	9.93	None	0.99	0.059	0.002
		Lauric acid	6.79	None	0.99	0.020	0.002
		Monolaurin	10.02	None	0.99	0.059	0.002
		Lauric acid and monolaurin	4.82	None	0.99	0.043	0.002
	NCTC 11288[e]	Control	9.96	14.44	0.99	0.022	0.003
		Control with oil	10.04	None	0.99	0.020	0.002
		Lauric acid	7.64	23.51	0.99	0.012	0.002
		Monolaurin	10.04	None	0.98	0.019	0.004
		Lauric acid and monolaurin	5.47	74.56	0.98	0.070	0.002

Strains were grown in a bi-phasic system in TSGY broth (initial pH 7.0) and butter oil in shake culture at 75 rpm. Monolaurin and lauric acid were dissolved in the butter oil. [a] *L. monocytogenes* from Frourme d'Ambert cheese, [b] *L. monocytogenes* from Brie cheese, [c] *L. monocytogenes* from Lanark Blue cheese. [d] *L. innocua* from Brie cheese,d *L. innocua* from the national Collection of Cultures. [f] Counts taken from inoculated TSGY agar after incubation at 30 °C for 48 h. The curve fitting was calculated using a spreadsheet programme written by Baranyi 1994.

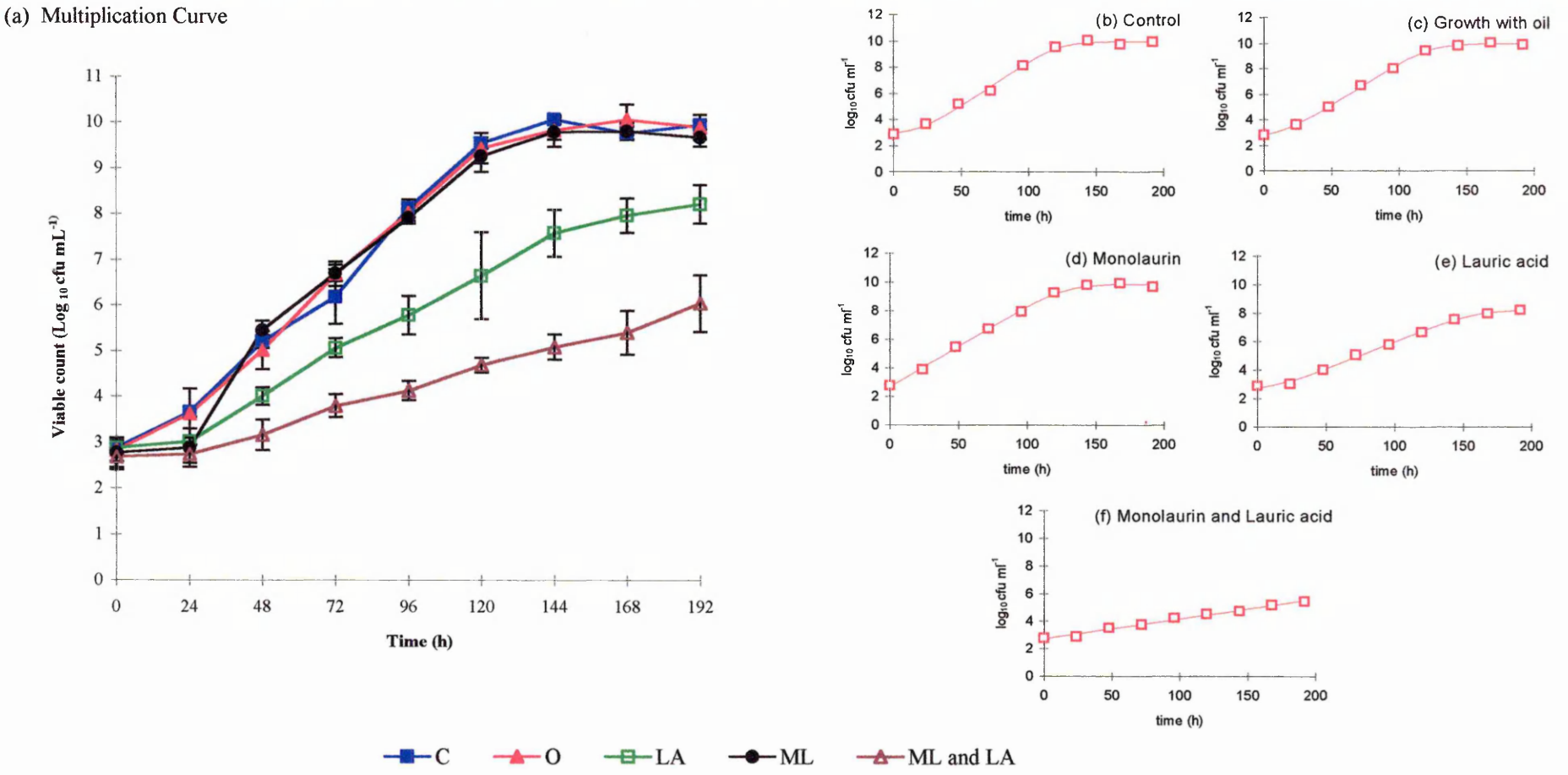


Figure 4.1. Growth of *Listeria monocytogenes* (A3) in a bi-phasic system containing butter oil, monolaurin 0.36 (mM), lauric acid (4.98 mM)and a combination of monolaurin (0.35 mM) and lauric acid (5.65 mM) at 10 °C, pH 7.0 and 75 rpm. Legend definitions are C: growth without oil; O: growth with oil; ML: growth with monolaurin; LA: growth with lauric acid; ML and LA: growth with monolaurin and lauric acid

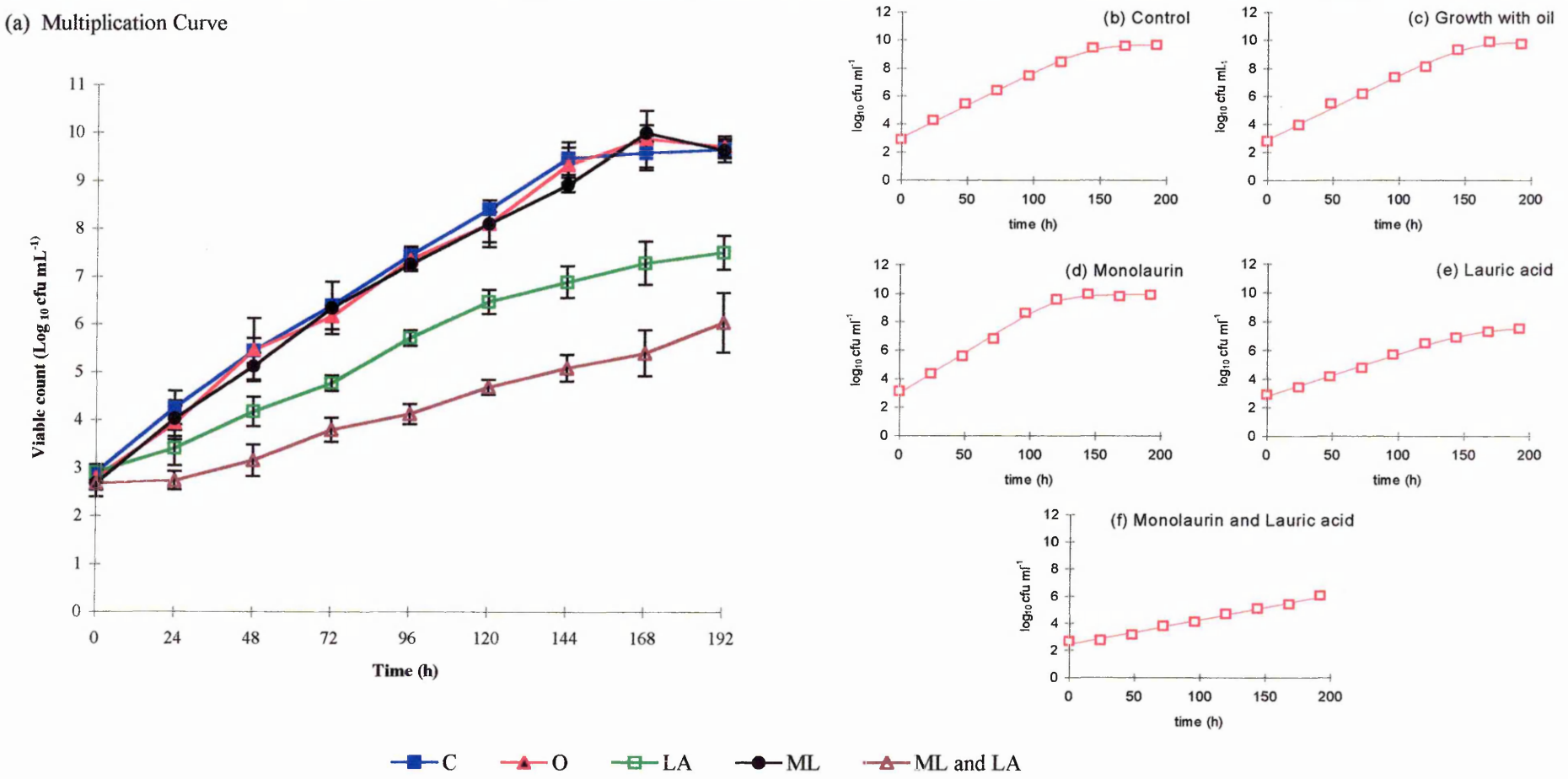


Figure 4.2. Growth of *Listeria monocytogenes* (A13) in a bi-phasic system containing butter oil, monolaurin (0.35 mM), lauric acid (5.04 mM) and a combination of monolaurin (0.38 mM)and lauric acid (5.34 mM) at 10 °C, pH 7.0 and 75 rpm. Legend definitions are C: growth without oil; O: growth with oil; ML: growth with monolaurin; LA: growth with lauric acid; ML and LA: growth with monolaurin and lauric acid

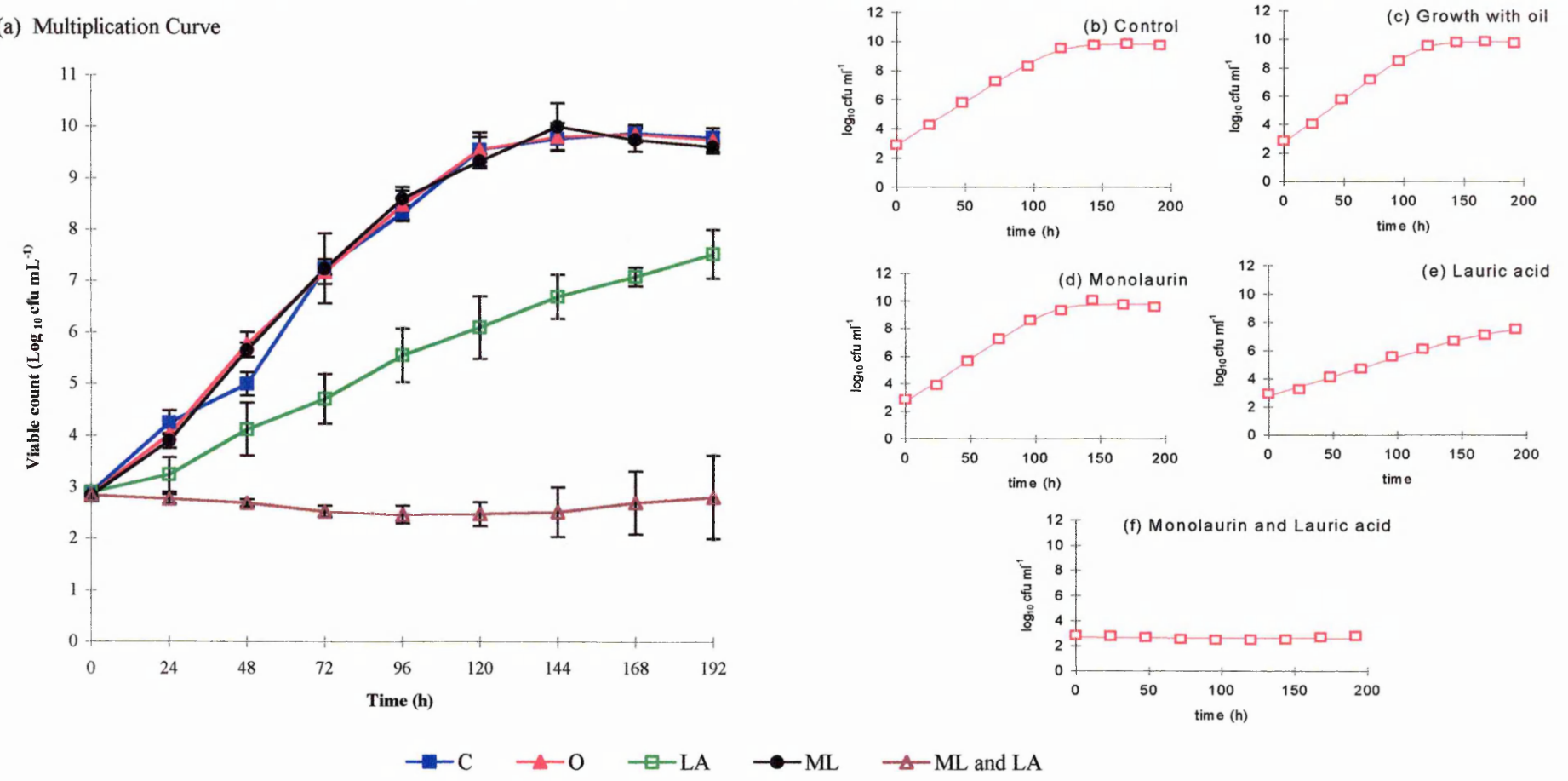


Figure 4.3. Growth of *Listeria monocytogenes* (LB) in a bi-phasic system containing butter oil, monolaurin (0.36 mM), lauric acid (5.01 mM) and a combination of monolaurin (0.35 mM) and lauric acid (5.19 mM) at 10 °C, pH 7.0 and 75 rpm. Legend definitions are C: growth without oil; O: growth with oil; ML: growth with monolaurin; LA: growth with lauric acid; ML and LA: growth with monolaurin and lauric acid

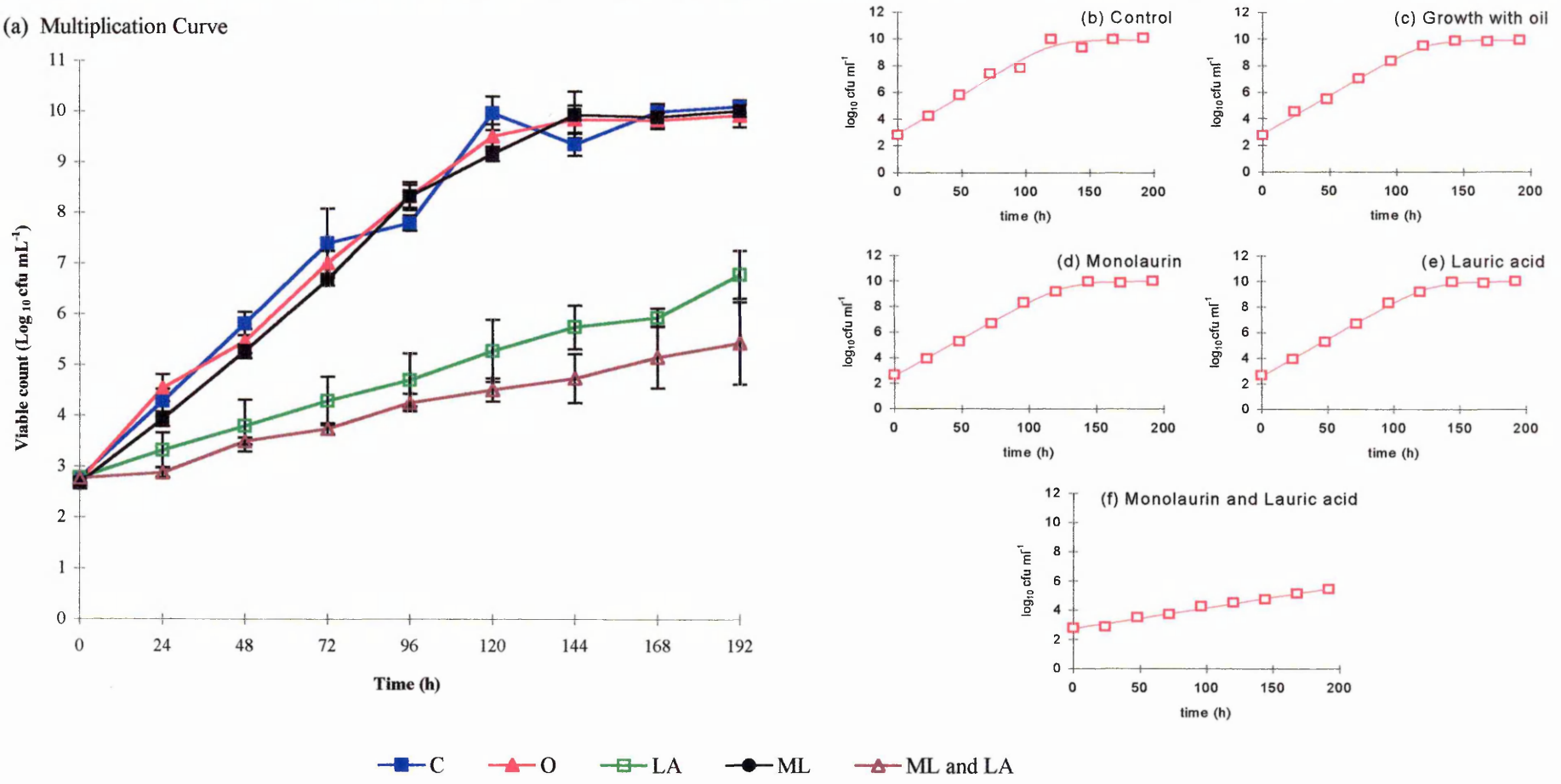


Figure 4.4. Growth of *Listeria innocua* (A7) in a bi-phasic system containing butter oil, monolaurin (0.36 mM), lauric acid (5.10 mM) and a combination of monolaurin (0.36 mM) and lauric acid (4.99 mM) at 10 °C, pH 7.0 and 75 rpm. Legend definitions are C: growth without oil; O: growth with oil; ML: growth with monolaurin; LA: growth with lauric acid; ML and LA: growth with monolaurin and lauric acid

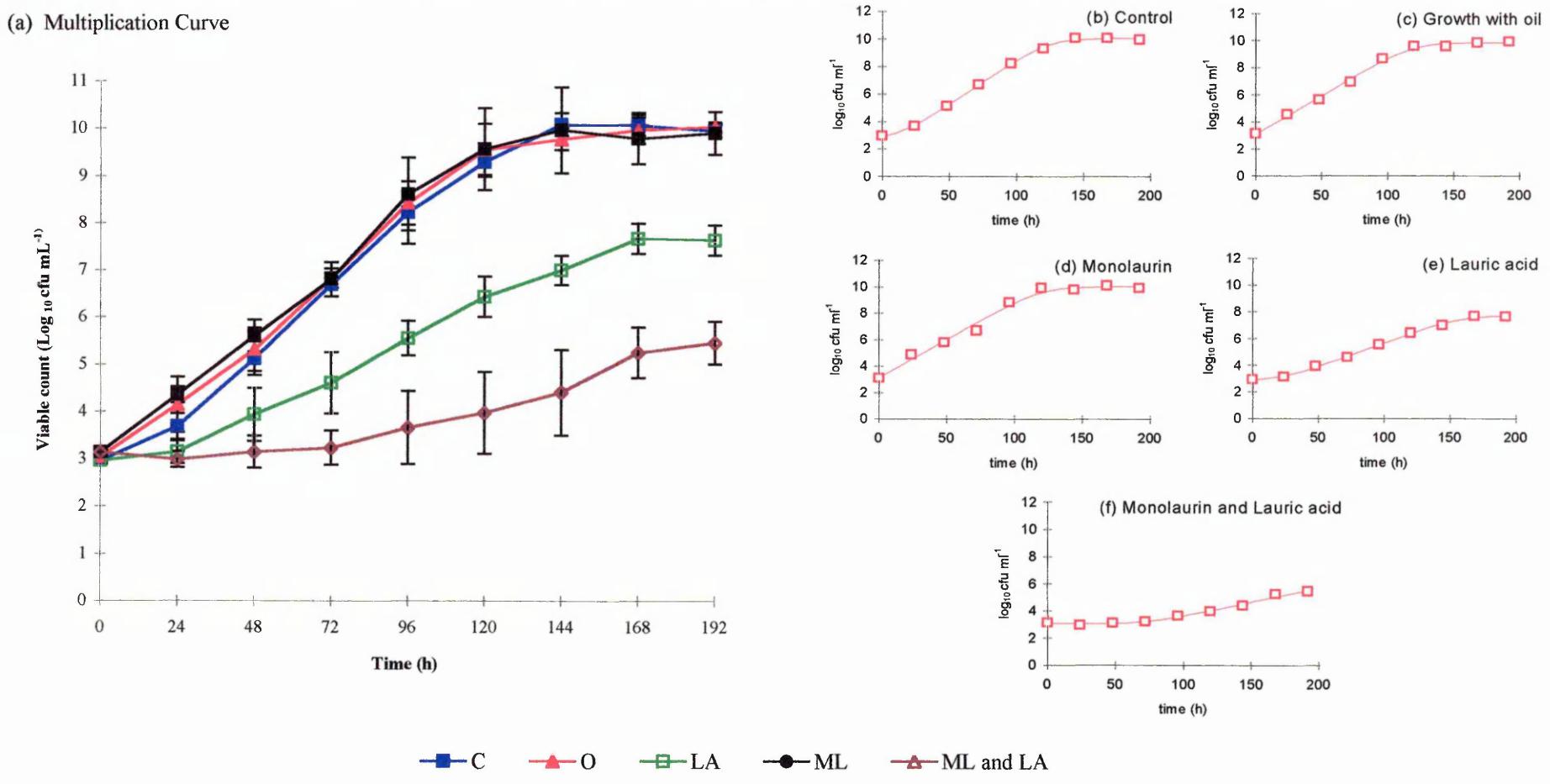


Figure 4.5. Growth of *Listeria innocua* (NCTC 11288) in a bi-phasic system containing butter oil, monolaurin (0.36 mM), lauric acid (5.00 mM) and a combination of monolaurin (0.34 mM) and lauric acid (5.22 mM) at 10 °C, pH 7.0 and 75 rpm. Legend definitions are C: growth without oil; O: growth with oil; ML: growth with monolaurin; LA: growth with lauric acid; ML and LA: growth with monolaurin and lauric acid

4.3 Effect of butter oil on the growth of *Listeria* spp.

Growth of the listeria strains with and without butter oil was similar. This is shown in the ANOVA test given in Table 4.4 ($P > 0.05$). When the population of listerias was compared and the log reduction (as described in chapter 2) was plotted the data was almost a straight line on the x axis. This demonstrates that most strains of listerias were slightly affected by the presence of 5 g of butter oil. *Listeria monocytogenes* (A13) was the only strain which gave reduced growth with the presence of butter oil and this occurred between 100 and 120 h. The four other strains showed slight acceleration of growth between 100 and 120 h.

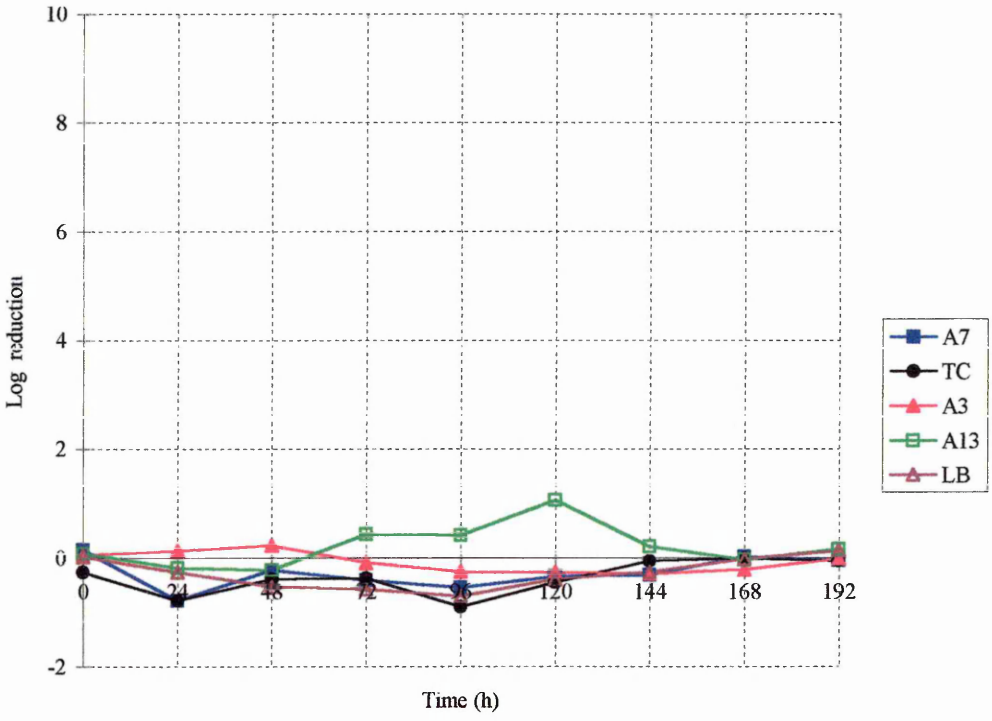


Figure 4.6. Effect of butter oil on multiplication of *Listeria* spp.
Log reduction = $LogN_0$ / Log N_1. A7 = *L. innocua* from Brie cheese, TC = *L. innocua* NCTC 11288 from the National Collection of type cultures, A3 = *L. monocytogenes* from Brie cheese, A13 = *L. monocytogenes* from Fourme d'Ambert cheese and LB = *L. monocytogenes* from Lanark Blue cheese.

4.4 Effect of lauric acid on the growth of *Listeria* spp.

There was a reduction in the cell populations for all five listeria stains with addition of lauric acid to the butter oil followed by incubation at 10 °C when compared to both the controls. The statistical analyses is given in Tables 4.4. This Table shows that there was no significant difference ($P > 0.05$) between each growth curve for all five strains of listeria. There was however a significant difference between all the results for growth with lauric acid and growth of the controls ($P < 0.05$) Table 4.4.

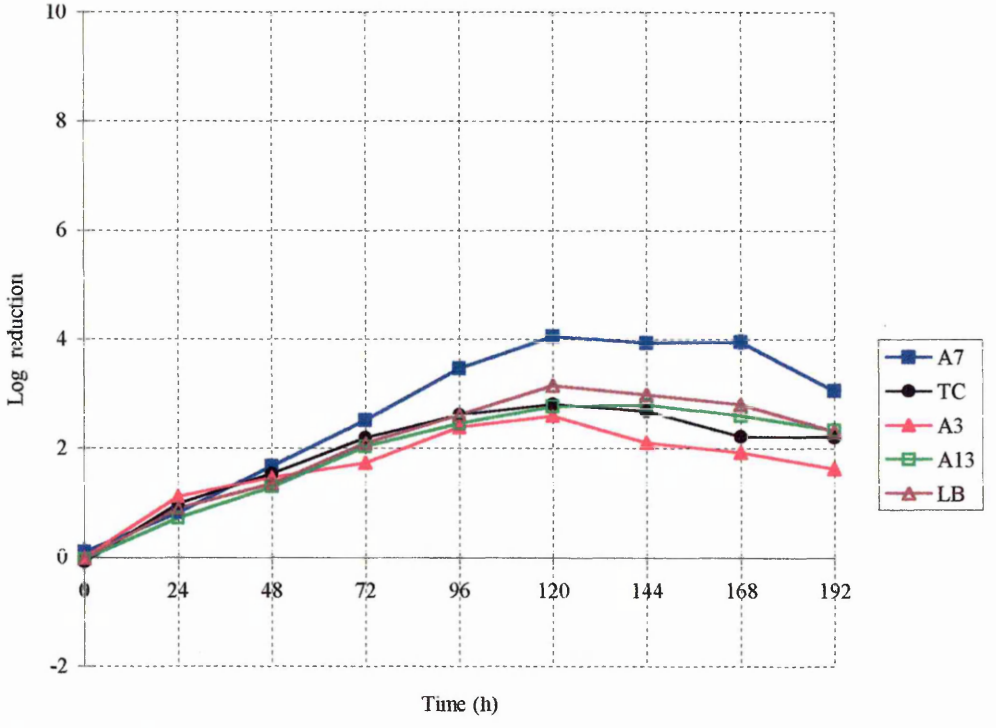


Figure 4.7. Effect of lauric acid on the multiplication of *Listeria* spp.
Log reduction = $LogN_0$ / Log N_1. A7 = *L. innocua* from Brie cheese, TC = *L. innocua* NCTC 11288 from the National Collection of type cultures, A3 = *L. monocytogenes* from Brie cheese, A13 = *L. monocytogenes* from Fourme d'Ambert cheese and LB = *L. monocytogenes* from Lanark Blue cheese.

Figure 4.7 demonstrates the effect of adding lauric acid to butter oil on the growth of all strains of listeria at 10 °C. From this Figure it can be seen that the log reduction increases until 160 h when it begins to fall. *Listeria innocua* (A7) was the most sensitive strain where a 4 log reduction was seen at 120 h and *L. monocytogenes* (A3) was the most resistant strain where a 2 log reduction was seen after 120 h. Although

statistical analysis demonstrated that there was no strain difference at 192 h. It is obvious from Figure 4.7 that the growth rate up to 192 h was different between the strains.

4.5 Effect of monolaurin on the growth of *Listeria* spp.

The statistical analysis given in Table 4.5 shows that there was no significant difference ($P > 0.05$) between multiplication of *Listeria* spp. in the control with oil compared to monolaurin at 192 h for the five listeria strains. Statisical analysis (Table 4.4) shows that there was no significant difference between the five strains of listeria ($P > 0.05$) at 192 h.

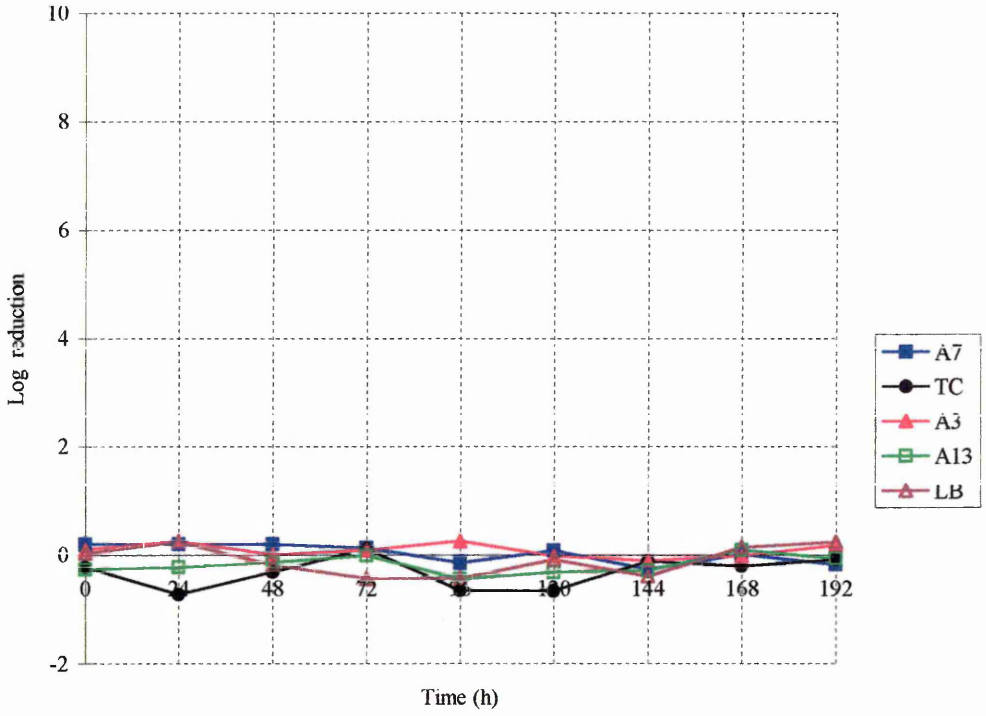


Figure 4.8. Effect of monolaurin on multiplication of *Listeria* spp.
Log reduction = $LogN_0$ / Log N_1. A7 = *L. innocua* from Brie cheese, TC = *L. innocua* NCTC 11288 from the National Collection of type cultures, A3 = *L. monocytogenes* from Brie cheese, A13 = *L. monocytogenes* from Fourme d'Ambert cheese and LB = *L. monocytogenes* from Lanark Blue cheese.

Figure 4.8 illustrates the log difference between growth of *Listeria* spp. with and without monolaurin. Monolaurin only had a slight effect on the growth of these listeria strains in a model bi-phasic system as all of the log reduction lines gather around zero on the x axis. There was no statistical difference between the way all the five listeria strains at 192 h reacted to 0.35 mM monolaurin. Figure 4.8 demonstrates that there were slight differences in the growth rates of the five strains.

4.6 Effect of monolaurin and lauric acid on the growth of *Listeria* spp.

The inhibitory effect of lauric acid on *Listeria* spp. was increased by the addition of monolaurin. Monolaurin has an augmentative effect on lauric acid. There was a significant decrease in all cell growth parameters with the addition of 0.35 mM monolaurin to the lauric acid in the butter oil. Statistical analysis (Table 4.5) showed that there was a significant difference between the strains of *Listeria* spp. when monolaurin and lauric acid were added together to the butter oil ($P < 0.05$). The results for each strain were similar, although *L. monocytogenes* (LB) showed no growth with monolaurin and lauric acid at 192 h. Further statistical analysis showed that there was a significant difference ($P < 0.05$) for all five strains between results obtained with oil (control) and with monolaurin and lauric acid, Table 4.4. There was also a significant difference ($P < 0.05$) between growth curves found for lauric acid alone and when combined with monolaurin, Table 4.4.

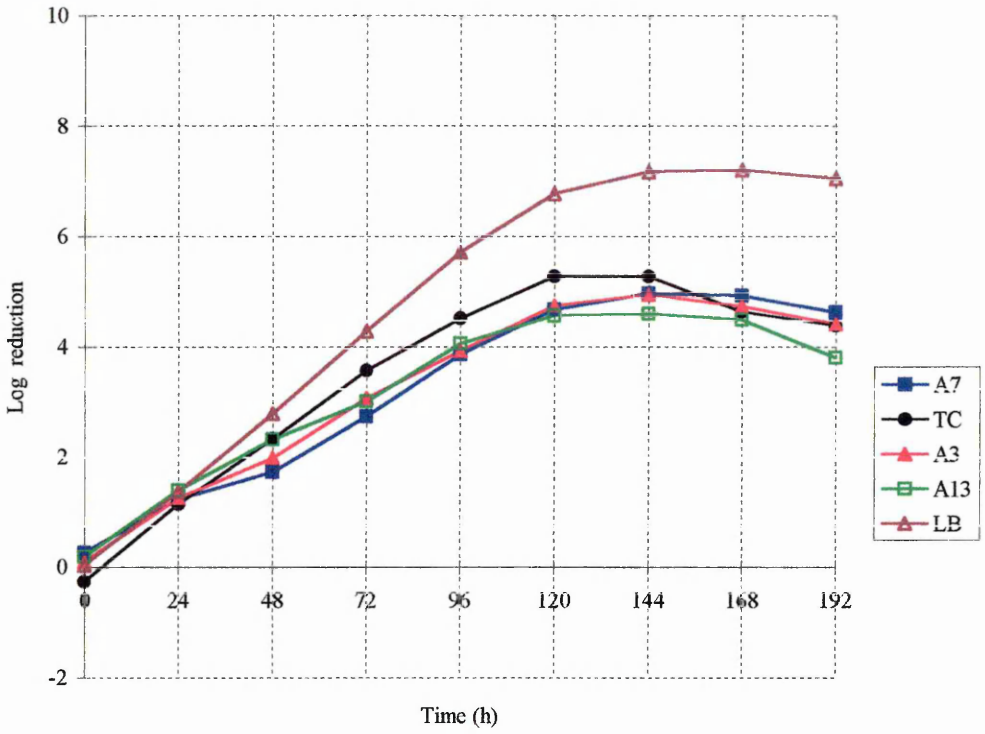


Figure 4.9. Effect of lauric acid and monolaurin on the multiplication of *Listeria* spp. Log reduction = $LogN_o$ / Log N_1. A7 = *L. innocua* from Brie cheese, TC = *L. innocua* NCTC 11288 from the National Collection of type cultures, A3 = *L. monocytogenes* from Brie cheese, A13 = *L. monocytogenes* from Fourme d'Ambert cheese and LB = *L. monocytogenes* from Lanark Blue cheese.

The effect of both monolaurin and lauric acid can be demonstrated in Figure 4.9. listeria strains *L. innocua* (A7) and (NCTC 11288) and *L. monocytogenes* (A3) and (A13) showed similar trends in log difference. The two inhibitors exhibited approximately 4 to 5 log differences after 120 h and 192 h of growth. *Listeria* monocytogenes (LB) was the most sensitive strain; there was a 7 log difference during this time period

Table 4.3. Effect of monolaurin and lauric acid on the growth of *Listeria monocytogenes*

Strain		Experiment		Slope[d] (h^{-1})	μ (h^{-1})	ν (h)	t_d (h)
L. monocytogenes	A3[a]	Control	mean	0.06	0.15	0.21	4.79
			± SD	0.01	0.02	0.02	0.53
		Control with oil	mean	0.06	0.15	0.21	4.78
			± SD	0.01	0.02	0.03	0.52
		Lauric acid	mean	0.04	0.09	0.13	8.23
			± SD	0.01	0.02	0.03	1.74
		Monolaurin	mean	0.06	0.13	0.19	5.32
			± SD	0.00	0.01	0.01	0.26
		Lauric acid and monolaurin	mean	0.02	0.04	0.05	20.34
			± SD	0.00	0.01	0.01	3.76
	A13[b]	Control	mean	0.05	0.11	0.16	6.41
			± SD	0.00	0.01	0.01	0.34
		Control with oil	mean	0.04	0.10	0.15	6.89
			± SD	0.01	0.01	0.02	0.95
		Lauric acid	mean	0.03	0.07	0.10	10.11
			± SD	0.00	0.01	0.01	0.68
		Monolaurin	mean	0.03	0.11	0.15	6.58
			± SD	0.00	0.01	0.01	0.33
		Lauric acid and monolaurin	mean	0.02	0.05	0.07	15.48
			± SD	0.00	0.01	0.01	2.72
	LB[c]	Control	mean	0.06	0.14	0.20	5.02
			± SD	0.01	0.02	0.03	0.44
		Control with oil	mean	0.06	0.15	0.21	4.82
			± SD	0.01	0.01	0.02	0.39
		Lauric acid	mean	0.02	0.05	0.09	14.35
			± SD	0.00	0.01	0.01	1.64
		Monolaurin	mean	0.07	0.15	0.22	4.67
			± SD	0.01	0.02	0.02	0.42
		Lauric acid and monolaurin	Mean	NG	NG	NG	NG
			± SD				

Strains were grown in a bi-phasic system TSGY broth (initial pH 7.00) and butter oil in shake culture at 75 rpm and 10 °C. Monolaurin and lauric acid were dissolved in the butter oil.
[a] *L. monocytogenes* from Fourme d'Ambert Cheese, [b] *L. monocytogenes* from Brie Cheese,
[c] *L. monocytogenes* from Lanark Blue Cheese. Counts were taken from inoculated TSGY agar after incubation at 30 °C for 48 h. [d] The slope was calculated using a curve fitting programme written by Baranyi 1994).

Table 4.3. (Continued) Effect of monolaurin and lauric acid on the growth of *Listeria innocua*

Strain		Experiment		Slope[c] (h^{-1})	μ (h^{-1})	ν (h)	t_d (h)
L. innocua	A7[a]	Control	mean	0.06	0.15	0.21	4.73
			± SD	0.00	0.01	0.01	0.26
		Control with oil	mean	0.06	0.13	0.18	5.78
			± SD	0.01	0.03	0.04	1.48
		Lauric acid	mean	0.02	0.05	0.07	14.35
			± SD	0.00	0.01	0.01	1.64
		Monolaurin	mean	0.06	0.14	0.20	5.10
			± SD	0.00	0.01	0.01	0.24
		Lauric acid and monolaurin	mean	0.02	0.03	0.05	22.22
			± SD	0.00	0.01	0.01	4.58
	TC [b]	Control	mean	0.06	0.15	0.21	4.73
			± SD	0.00	0.01	0.01	0.23
		Control with oil	mean	0.05	0.12	0.17	4.58
			± SD	0.02	0.05	0.07	1.74
		Lauric acid	mean	0.04	0.08	0.12	8.77
			± SD	0.01	0.02	0.03	2.12
		Monolaurin	mean	0.06	0.13	0.19	5.24
			± SD	0.00	0.00	0.01	0.17
		Lauric acid and monolaurin	mean	0.03	0.07	0.09	11.53
			± SD	0.01	0.02	0.03	3.27

Strains were grown in a bi-phasic system TSGY broth (initial pH 7.00) and butter oil in shake culture at 75 rpm and 10 °C. Monolaurin and lauric acid were dissolved in the butter oil.
[a] *L. innocua* from Brie Cheese, [b] *L. innocua* from the National collection of type cultures.
Counts were taken from inoculated TSGY agar after incubation at 30 °C for 48 h. [c] The slope was calculated using a curve fitting programme written by Baranyi 1994).

Table 4.4. Statistical Analysis of mean cell populations[a] of each growth curve by Analysis of Variance (ANOVA)

(a) Sum (Σ), average ($\overline{X}$) and variance (ν) of mean cell populations

Strain	Test					Rows		
	Control	Oil	Monolaurin	Lauric acid	Monolaurin and lauric acid	Σ	$\overline{X}$	ν
L. innocua (A7)	6.33	6.26	6.01	4.03	3.71	26.35	5.27	1.66
L. innocua (NCTC 11288)	6.00	6.43	6.53	4.44	3.36	26.76	5.35	1.94
L. monocytogenes (A3)	5.94	5.94	6.00	5.57	3.61	26.06	5.21	1.17
L. monocytogenes (A13)	5.81	5.64	6.53	5.58	3.53	25.92	5.18	1.27
L. monocytogenes (LB)	6.35	6.31	6.26	4.44	2.63	25.99	5.20	2.72
Columns Σ	30.43	30.59	31.15	22.06	16.84			
Columns $\overline{X}$	6.09	6.12	6.23	4.41	3.37			
Columns ν	0.06	0.10	0.05	0.05	0.19			

Table 4.4. (b) ANOVA analysis[b]

Source of variance	Sum of Squares	Degrees of Freedom	Mean squares	P-value	H_o[c]
Strains	0.10	4	0.02	0.92	Accept
Tests	33.31	4	8.32	2.72×10^{-10}	Reject
Error	1.70	16	0.11		
Total	35.11	24			

$$\text{Least significant difference} = t_{16} DF \sqrt{\frac{2 \times MS}{n}} \qquad (4.1)$$

DF = degrees of freedom
t = t distribution at 16 degrees of freedom = 2.12
MS = Mean squares
n = DF for tests

$$2.12 \sqrt{\frac{2 \times 0.11}{4}} = 0.49$$

Table 4.4. (c) Difference between mean cell populations [d]

	Control	Oil	Monolaurin	Lauric acid	Monolaurin and lauric acid
Control	0				
Oil	0.03	0			
Monolaurin	0.14	0.11	0		
Lauric acid	**1.67**	**1.71**	**1.82**	0	
Monolaurin and lauric acid	**2.72**	**2.75**	**2.86**	**1.05**	0

Values are in Log_{10} cfu L^{-1}. [a] Mean of each value of each curve was calculated using a Microsoft Excel worksheet. [b] Analysis of Variance calculated using Microsoft Excel data analysis package. [c] H_o for strains – A7 = NCTC 11288 = A3 = A13 = LB; for tests - Control = Oil = monolaurin = lauric acid = monolaurin and lauric acid. H_o accepted if P-value > 0.05 and rejected if P-value < 0.05. [d] H_o rejected for tests, there is a significant difference between the tests. The least significant difference was used to determine which tests differed. Those in bold show a significant difference.

4.7 Effect of growth of *Listeria* spp. at 10 °C on pH in broth culture

Table 4.5 gives the initial and final pH of all the growth experiments in the broth culture medium at 10 °C. In experiments where there had been very little growth there was little change in the pH. This can be seen in all experiments where lauric acid alone (5.05 mM) or lauric acid in combination with monolaurin was used.

Table 4.5. Effect of multiplication of Listeria spp. at 10 °C, on the final pH

Strain	Experiment		pH			
		n[k]	Initial	± SD	192 h	± SD
L. monocytogenes (A3)[a]	C	2	7.00	0.00	4.41	0.09
	O	4	7.00	0.00	4.60	0.12
	LA	2	7.00	0.00	5.59	0.84
	O	4	7.00	0.01	4.72	0.19
	ML	2	7.00	0.01	4.75	0.12
	ML+ LA	2	7.00	0.01	6.83	0.05
L. monocytogenes (A13)[b]	C	2	6.99	0.02	4.86	0.04
	O	4	6.99	0.02	4.81	0.03
	LA	2	6.99	0.02	6.86	0.07
	O	4	7.01	0.00	4.71	0.10
	ML	2	7.01	0.00	5.11	0.36
	ML+ LA	2	7.01	0.00	6.86	0.06
L. monocytogenes (LB)[c]	C	2	7.00	0.01	4.48	0.01
	O	4	7.00	0.01	4.68	0.16
	LA	2	7.00	0.01	6.71	0.15
	O	4	7.02	0.01	4.61	0.07
	ML	2	7.02	0.01	4.54	0.10
	ML+ LA	2	7.02	0.01	6.81	0.05
L. innocua (A7)[d]	C[f]	2	7.00	0.00	4.16	0.04
	O[g]	4	7.00	0.00	4.24	0.06
	LA[h]	2	7.00	0.00	6.47	0.08
	O	4	7.00	0.00	4.59	0.06
	ML[i]	2	7.00	0.00	4.73	0.02
	ML+ LA[j]	2	7.00	0.00	6.89	0.03
L. innocua (NCTC 11288)[e]	C	2	6.99	0.06	4.49	0.07
	O	4	6.99	0.06	4.63	0.09
	LA	2	6.99	0.06	6.61	0.27
	O	4	7.06	0.01	4.47	0.66
	ML	2	7.06	0.01	4.59	0.12
	ML+ LA	2	7.06	0.01	7.00	0.05

Monolaurin and LA were dissolved in the butter oil. The pH was recorded before and after each experiment.

[a] A3 = *L. monocytogenes* from Fourme d'Ambert Cheese, [b] A13 = *L. monocytogenes* from Brie.

[c] LB = *L. monocytogenes* from Lanark blue cheese, [d] A7 = *L. innocua* from Brie Cheese ,

[e] NCTC = *L. innocua* type culture 11288, obtained from the National collection of type culture,

[3] flasks and 18 plates, [f] control of growth with no butter oil, [g] control of growth with butteroil, [h] growth with lauric acid, [i] growth with monolaurin, [j] growth with monolaurin and lauric acid.

[k] number of experimental runs,

In all cases the pHs of the culture media were reduced from 7 to below 5 (for the controls with and without oil), after 192 h incubation at 10 °C. When monolaurin was added to the medium the pH of the system was reduced after 192 h to below 5.2. When lauric acid was added alone only a small reduction in the pH of the broth was observed. When *L. innocua* (A7) and *L. monocytogenes* (A13) were subjected to LA the pH after 192 h was above 6.4, and was 5.6 for *L. monocytogenes* (A3). In all cases when monolaurin and lauric acid were used together the pHs of the broths were greater than 6.4 after 192 h incubation confirming that very little growth had taken place. It was not possible to calculate the H^+ concentration produced during metabolism as the medium was buffered with KH_2PO_4.

4.8 Summary of Results

1. Butter oil had only a slight influence over the growth rate of *Listeria* spp in broth culture.
2. Monolaurin by itself did not reduce the final population of *Listeria* spp. in the model bi-phasic system in broth culture.
3. Lauric acid reduced the final population of *L. monocytogenes* by 1 to 2 log orders and *L. innocua* by 2 to 3 log orders after 192 h at 10 °C.
4. The listeriostatic effect of lauric acid was increased with the addition of monolaurin. This effect can be described as an augmented one. The final populations of *Listeria* spp. were reduced by about 5 log orders compared to the controls without lauric acid and monolaurin.
5. The pH of the medium was reduced to about 4.5 in the controls and with monolaurin alone after 192 h growth at 10 °C.
6. There was relatively little change in the pH of the culture medium when growth was inhibited.

Growth of *Listeria innocua* in a model cheese system at 10 °C and 95 % erh

The listeria strains tested in broth culture at 10 ° C (Chapter 4) showed a considerable reduction in growth with the addition of monolaurin and lauric acid to butter oil (ca. 5 log orders). It would seem worthwhile to test the effect of a combination of monolaurin and lauric acid in a food system such as soft-ripened cheese. Growth of *L. monocytgoenes* in soft mould-ripened cheese has been discussed in Chapter 1 Section 1.6. It was decided to use a model soft-ripened cheese to study the growth of *L. innocua* with these antimicrobial compounds. Initially the concentrations of lauric acid (5.04 mM) and monolaurin (0.33 mM) were similar to those used in the model broth culture.

The concentration of monolaurin used in the broth culture had no effect on the growth of *Listeria* spp. in the model system, although evidence suggests that monolaurin has inhibitory at higher concentrations (Bautista *et al* 1993). The concentration of monolaurin was increased to 0.9 mM (0.25 g L^{-1}) in the model cheese system in subsequent experiments in the hope that an inhibitory effect would be seen. Bautista *et al.* (1993) suggest that 0.25 g L^{-1} monolaurin inhibited the growth of *L. monocytogenes* in cottage cheese although the difference in taste can be detected with the higher levels of monolaurin.

5.1 Manufacture of cheese

The Camembert-type cheeses were produced using a traditional method (Figure 2.5). After 14 d of ripening growth of *P. camemberti* was seen on the surface of the cheese.

The mould had a greyish-white colour and a cotton wool-like texture. On the 14th day the cheeses were wrapped in aluminium foil and left to ripen. After 21 d of ripening the surface of the cheeses were wet, this was attributed to secondary fermentation by *P. camembertii*. After 28 d of ripening, the cheeses were completely sealed by mould growth and the interior was soft and creamy in texture and had a yellowish colour, Figures 5.1 and 5.2. The cheeses with monolaurin had a more creamy, solid and were softer in texture than those without monolaurin. This was shown by direct visual comparison of the two cheeses, Figures 5.2 a and b.

(a)

Figure 5.1. (a) Laboratory made Camembert-type cheese, ripened for 21 d at 10 °C and 95 % erh.

(b)

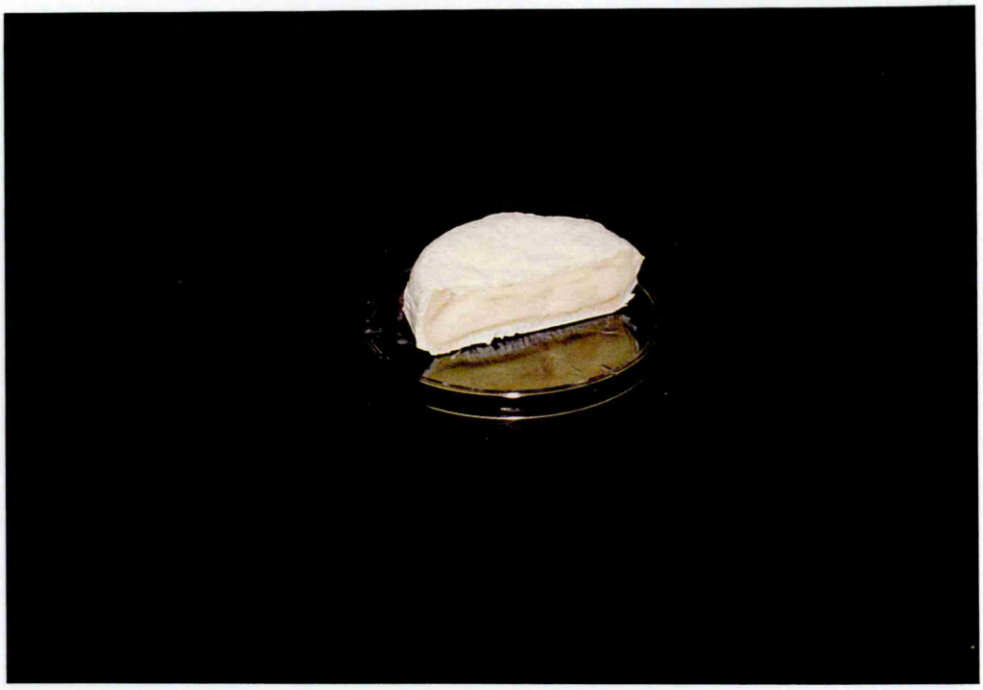


Figure 5.1. (b) Cross-section of the cheese to show the yellow creamy centre and the surface with 1 mm thick layer of the mould

(a)

Figure 5.2. (a) Camembert-type cheeses inoculated with *Listeria innocua* A7, no mould growth control cheese on the left, test cheese on the right (cheeses were ripened for 7 d at 10 °C and 95 % erh).

(b)

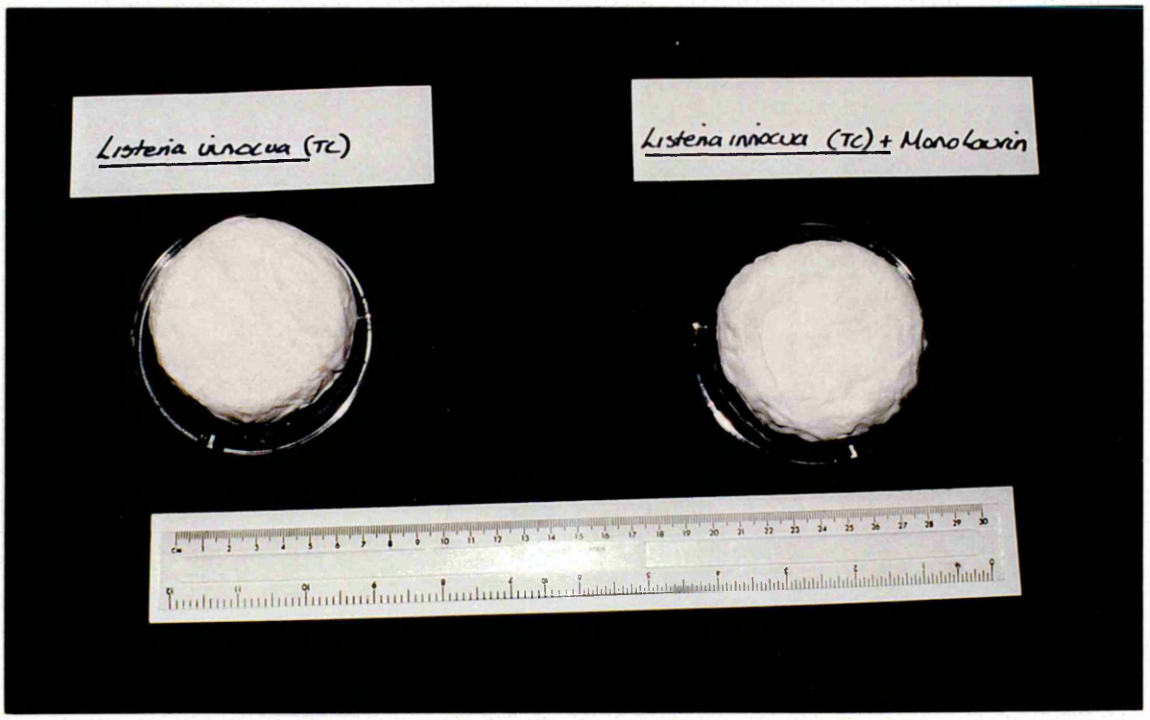


Figure 5.2. (b) as (a) but inoculated with *L. innocua* NCTC 11288, with mould growth, control cheese on the right and test cheese on the left (cheeses were ripened for 28 d at 10 °C and 95 % erh). The textures of Camembert-type cheeses were denser with the *L. innocua* than the texture of the control

5.2 Compositional analysis of Camembert-type cheese

The percent lactic acid and pH of the cheeses during the first day of production are guven in Table 5.1. This Table demonstrates that no reduction in pH of the milk and curds took place during drainage of the cheeses. The lactic acid content of the cheese curds doubled from 0.1 % to 0.2 % during drainage of the whey from the cheese curds (Table 5.1). Table 5.2 gives the chemical composition of the cheeses during ripening. The moisture content of the cheeses decreased from 62 % to 56 % in the ripening period. The fat content of the cheeses increased from 19 % to 30 % and there was a concomitant increase in pH (from pH 4.3 to 7.5). The pH of the centre of the cheeses was consistently lower than that on the surface throughout production and ripening. After the 3 d of draining the pH of the cheeses had decreased, from 6.8 to 5.03 on the surface and 4.87 in the centre. Once the mould had grown on the surface of the cheeses

cabinet ranged from 9.82 to 10.06 °C. The humidity in the ripening cabinet rose during ripening. This was caused by the transfer or moisture from the cheeses to the trays underneath the cheeses. The mean erh for all the experiments ranged from 94 % to 98 %. The concentration of lauric acid and monolaurin in the cheeses related to their original concentrations in the milk. The first batch contained the same concentrations of lauric acid (5.04 mM) and monolaurin (0.33 mM) to those used in the model bi-phasic broth culture. In the subsequent batches lauric acid was eliminated and the concentration of monolaurin increased to approximately 0.9 mM (0.25 g L^{-1} milk).

Table 5.3. Ripening conditions of the Camembert-type cheese experiments

L. innocua strain	Date	Duration (d)	Temperature mean± SD (°C)	Erh mean ± SD (%)	Concentration (mM)	
					Monolaurin	Lauric acid
A7[a]	16.10.96	28	10.06 ± 0.48	98.69 ± 1.20	0.33	5.04
A7	13.01.97	28	9.99 ± 0.27	94.83 ± 1.90	0.91	-
NCTC[b]	17.02.97	34	9.82 ± 0.21	95.16 ± 1.84	1.24	-
A7[c]	18.11.96	28	10.03 ± 0.31	97.20± 1.67	0.91	-
NCTC[d]	02.04.97	34	10.02 ± 0.26	96.50 ± 1.28	0.91	-

Strains were grown in experimental Camembert-type cheeses. Monolaurin was dissolved in double cream before homogenising with skimmed milk to obtain a final concentration of 3.6 % fat. [a] *L. innocua* from Brie cheese. [b] *L. innocua* NCTC 11288 obtained form the National collection of cultures. [c] and [d] these cheeses were left for an extra 24 h to drain at ambient temperature before ripening at 10 °C.

5.4 Multiplication of *Listeria innocua* (strains A7 and NCTC 11288) at 10 °C and 95 % erh over 28 d period of ripening

Results for $\log_{10}$ N for all the experiments were shown in Appendix 2. The data $\log_{10}$ N against time have been plotted for *L. innocua* strains A7 and NCTC 11288, Figures 5.3 to 5.5. A general trend in growth of *L. innocua* strains A7 and NCTC 11288 has been demonstrated in these graphs. There was less growth of *L. innocua* took in the centre of

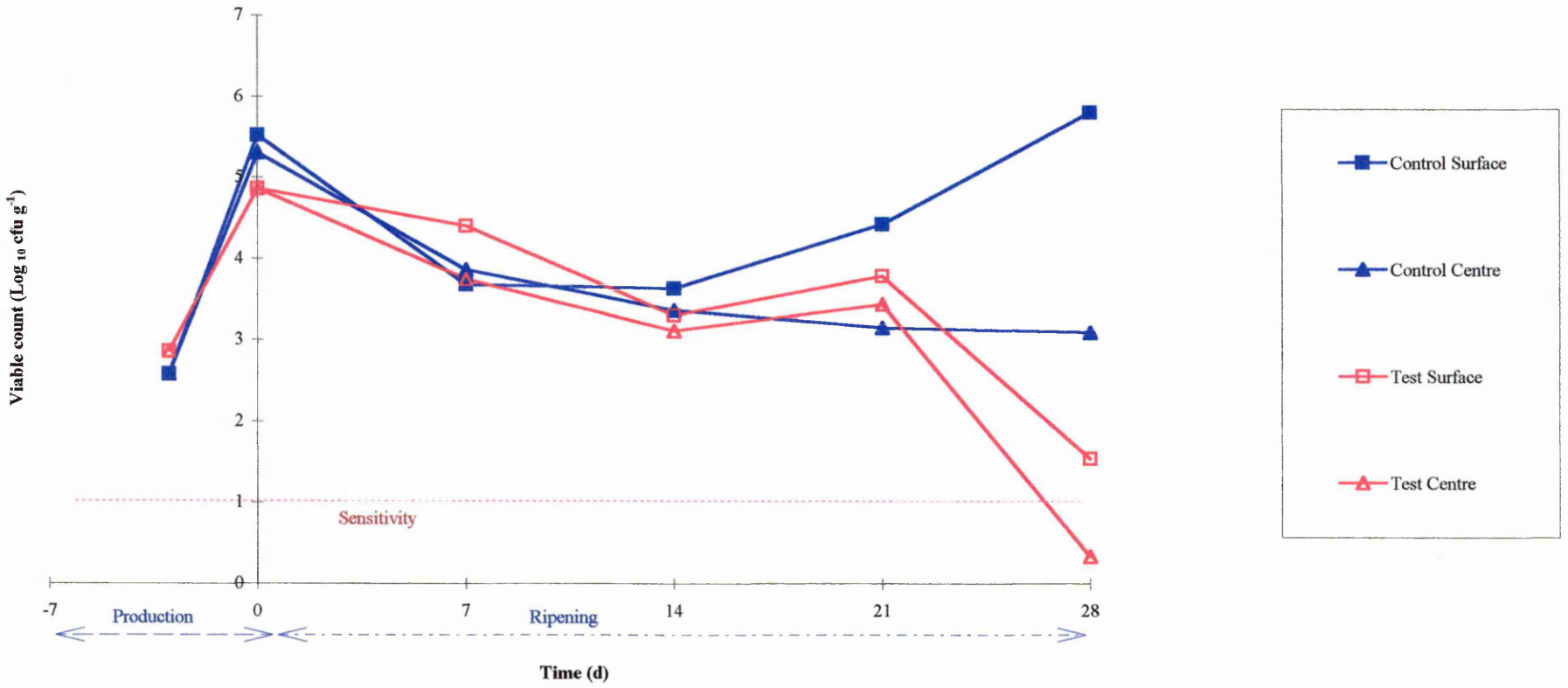


Figure 5.3. Comparison of the growth of *Listeria innocua* (A7) with and with out ML (0.3 mM) and LA (5.0 mM) on the surface and in the center of experimental Camembert-type cheese at 10 °C and 95 %erh

Test cheeses contained ML, control cheeses did not. Each point on the graph represents the mean counts from 2 cheeses that were tested in triplicate.

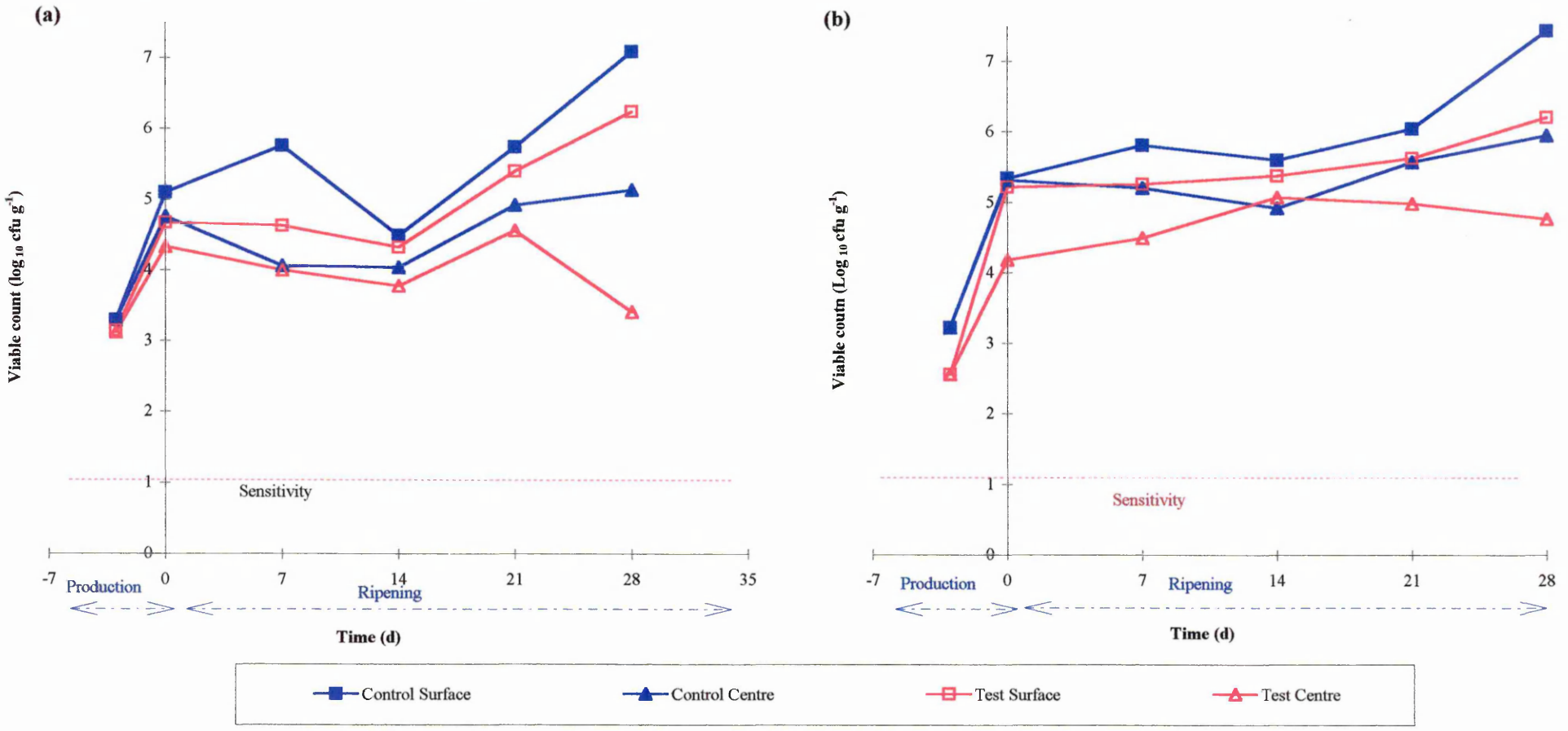


Figure 5.4. Comparison of the growth of *Listeria innocua* on the surface and in the centre of experimental Camembert-type cheese with and without monolaurin, at 10 °C and 95 % erh. (a) *L. innocua* (A7) with and with out ML (0.09 mM); (b) *L. innocua* (NCTC) 11288 with and with out ML (0.12 mM)

Test cheeses contained ML, control cheeses did not. Each point on the graph represents the mean counts from 2 cheeses that were tested in triplicate.

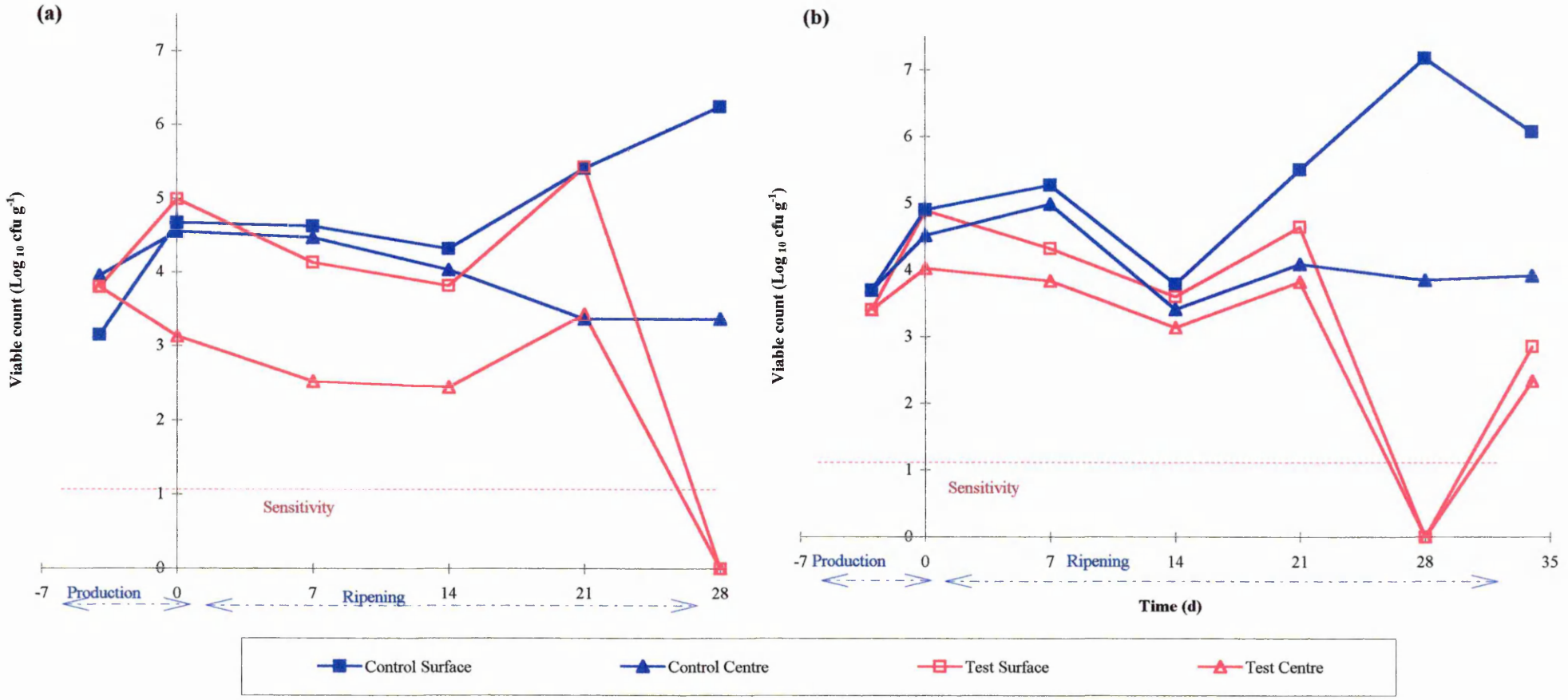


Figure 5.5. Comparison of the growth of *Listeria innocua* on the surface and in the centre of experimental Camembert-type cheese with and with out monolaurin, the production period was extended by 24 h and the cheeses ripened at 10 °C and 95 % erh. (a) *L. innocua* (A7) with and with out ML (0.09 mM); (b) *L. innocua* (NCTC) 11288 with and with out ML (0.09 mM).
Test cheeses contained ML, control cheeses did not. Each point on the graph represents the mean counts from 2 cheeses that were tested in triplicate.

the cheeses compared to the surface. There was approximately 0.5 $\log_{10}$ cfu g^{-1} difference in populations at any one time during production or ripening.

The cell populations of *Listeria* spp. both on the surface and in the centre of the cheeses increased by approximately 2 log orders during draining. After addition of the mould and ripening at 10 °C there was a reduction in the population of listerias by approximately 1.5 log orders. This was due to the production of lactic acid by the lactic acid bacteria causing the pH to drop. After a 14 d period of ripening the *Listeria* spp. began to multiply giving a final population of approximately 6 $\log_{10}$ cfu g^{-1}. This followed the increase in pH which was due to metabolism of lactic acid by the mould.

Table 5.4. pH of the surface and centre of Camembert-type cheeses during production and ripening

L. innocua Strain	Treatment	pH (d)			
		1	0	14	28
A7[a]	Control	6.82 ± 0.03	4.72 ± 0.09	5.42 ± 0.03	7.11 ± 0.20
	Monolaurin	6.73 ± 0.02	4.63 ± 0.10	5.45 ± 0.03	7.14 ± 0.15
NCTC 11288[b]	Control	6.84 ± 0.02	4.86 ± 0.08	5.50 ± 0.06	7.18 ± 0.13
	Monolaurin	6.75 ± 0.02	4.70 ± 0.02	5.54 ± 0.08	7.22 ± 7.22
A7[c]	Control	6.83 ± 0.02	5.09 ± 0.08	6.80 ± 0.22	7.04 ± 0.08
	Monolaurin	6.77 ± 0.02	5.09 ± 0.10	6.94 ± 0.13	7.21 ± 0.07
NCTC 11288[d]	Control	6.83 ± 0.02	5.20 ± 0.02	7.06 ± 0.04	7.23 ± 0.04
	Monolaurin	6.75 ± 0.03	5.25 ± 0.07	7.11 ± 0.03	7.31 ± 0.07
A7[d]	Control	6.82 ± 0.03	4.64 ± 0.03	5.21 ± 0.12	6.23 ± 0.24
	Monolaurin	6.73 ± 0.02	4.60 ± 0.05	5.16 ± 0.05	6.17 ± 0.09
NCTC 11288	Control	6.84 ± 0.02	4.71 ± 0.01	4.79 ± 0.08	6.76 ± 0.11
	Monolaurin	6.75 ± 0.02	4.63 ± 0.02	4.81± 0.06	6.84 ± 0.10
A7	Control	6.83 ± 0.02	4.91 ± 0.02	5.02 ± 0.02	6.71 ± 0.10
	Monolaurin	6.77 ± 0.02	4.95 ± 0.02	4.89 ± 0.15	6.89 ± 0.12
NCTC 11288	Control	6.83 ± 0.02	4.96 ± 0.03	5.54 ± 0.03	6.89 ± 0.12
	Monolaurin	6.75 ± 0.03	5.05 ± 0.03	6.12 ± 0.15	7.07 ± 0.05

The pH was the average ± SD of 3 cheese samples. Draining 1 d: initial pH of curds at the start of draining (ambient temperate); 0 d: start of ripening at 10 °C; 14 d: on the 14 d of ripening at 10 °C; 28 d: the 28 d of ripening at 10 °C. [a] *L. innocua* (A7) from Brie cheese. [b] *L. innocua* NCTC 11288 obtained form the National collection of type cultures, [c] and [d] these cheeses were left for an extra 24 h at ambient temperature to drain before incubation at 10 °C.

The pH of the cheeses both in the centre and on the surface follows the same trend as the counts, (Table 5.4). The pH in the centre of all the cheeses was lower (0.5 log orders) than that of the surface. The pH of the cheese drained for an extra 24 h was higher than those drained for a shorter time at ambient temperature.

5.5 Effect of monolaurin alone and in combination with lauric acid on *Listeria innocua* (strains A7 and NCTC 11288) in experimental Camembert-type cheese

The combination of monolaurin (0.03 mM) and lauric acid (0.50 mM) reduced the final cell population of *L. innocua* both on the surface and in the centre of the cheeses, Figure 5.1 and Table 5.5. The cheeses were ripened for 28 d at 10 °C and 95 % erh.

Table 5.5. Effect of monolaurin and lauric acid on the multiplication of two strains of *L. innocua* on the surface and in the centre of Camembert-type cheese

L. innocua strain	Treatment	N_0 (log_{10} cfu g L^{-1})	Surface counts (log_{10} cfu g^{-1})		Centre counts (log_{10} cfu g^{-1})	
			N_1	N_{28}	N_1	N_{28}
A7[a]	Control	2.58	5.53	5.81	5.53	3.09
	Monolaurin and lauric acid	2.86	5.31	1.54	4.87	0.33
A7	Control	3.30	5.10	7.20	4.76	5.14
	Monolaurin	3.15	4.68	6.25	4.34	3.41
NCTC [b]	Control	3.23	5.34	7.44	5.32	5.95
	Monolaurin	2.56	5.22	6.21	4.18	4.77
A7[c]	Control	3.15	4.68	6.25	4.56	3.38
	Monolaurin	3.81	4.99	<1.00	3.13	<1.00
NCTC[d]	Control	3.70	4.91	7.18	4.61	3.85
	Monolaurin	3.41	4.90	<1.00	4.03	<1.00

[a] *L. innocua* from Brie cheese. [b] *L. innocua* NCTC 11288 obtained form the National collection of type cultures. [c] and [d] these cheeses were left for an extra 24 h at ambient temperature before incubation at 10 °C. N_0: Initial count of *Listeria innocua* in the milk; N_1: Count of *Listeria innocua* on the first day of ripening; N_{28}: count of *Listeria innocua* on the 28 d of ripening.

Figure 5.6 gives the log reduction between cheeses treated with monolaurin and lauric acid and those which remained untreated. The greatest difference was seen on the surface of the cheeses (approximately 4 log orders). The least difference was seen between the surface and centre of the treated cheeses (1 log order).

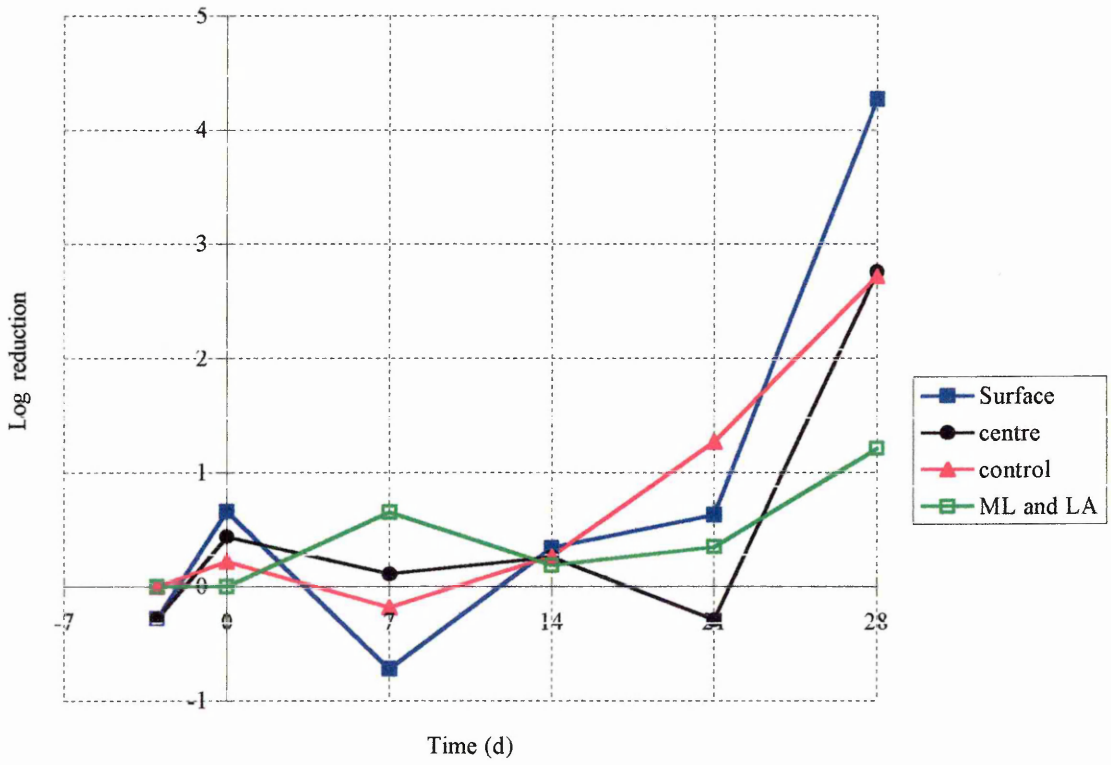
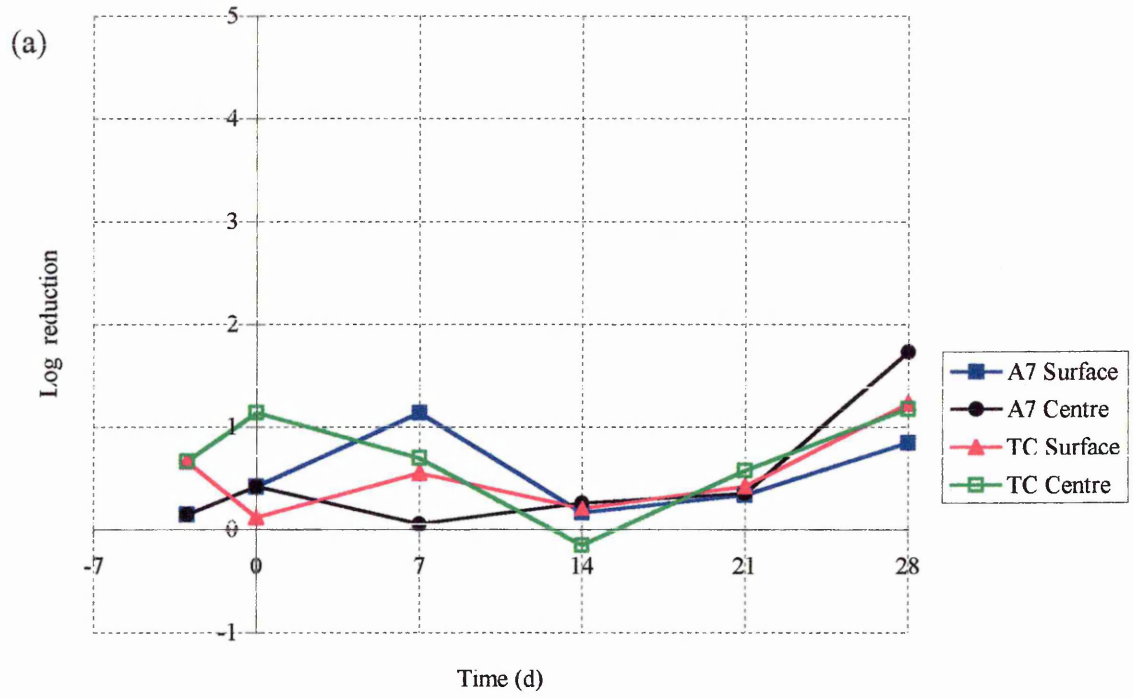


Figure 5.6. Effect of monolaurin and lauric acid on *L. innocua* (A7) in Camembert-type cheese.
Log reduction calculated as log N_0 / Log N_1. A7 = *L. innocua* from Brie cheese, TC = *L. innocua* NCTC 11288 from the National Collection of Type Cultures, A3 = *L. monocytogenes* from Brie cheese, A13 = *L. monocytogenes* from Fourme d'Ambert cheese and LB = *L. monocytogenes* from Lanark Blue cheese.

When the ripening cabinet was opened on day14 of ripening a distinct smell of methyl ketones was detected. This was attributed to metabolism of the lauric acid by *P. camembertii* to produce 2-undecanone. It was decided to eliminate lauric acid from the production of the cheeses and to use monolaurin alone but at a higher concentration.



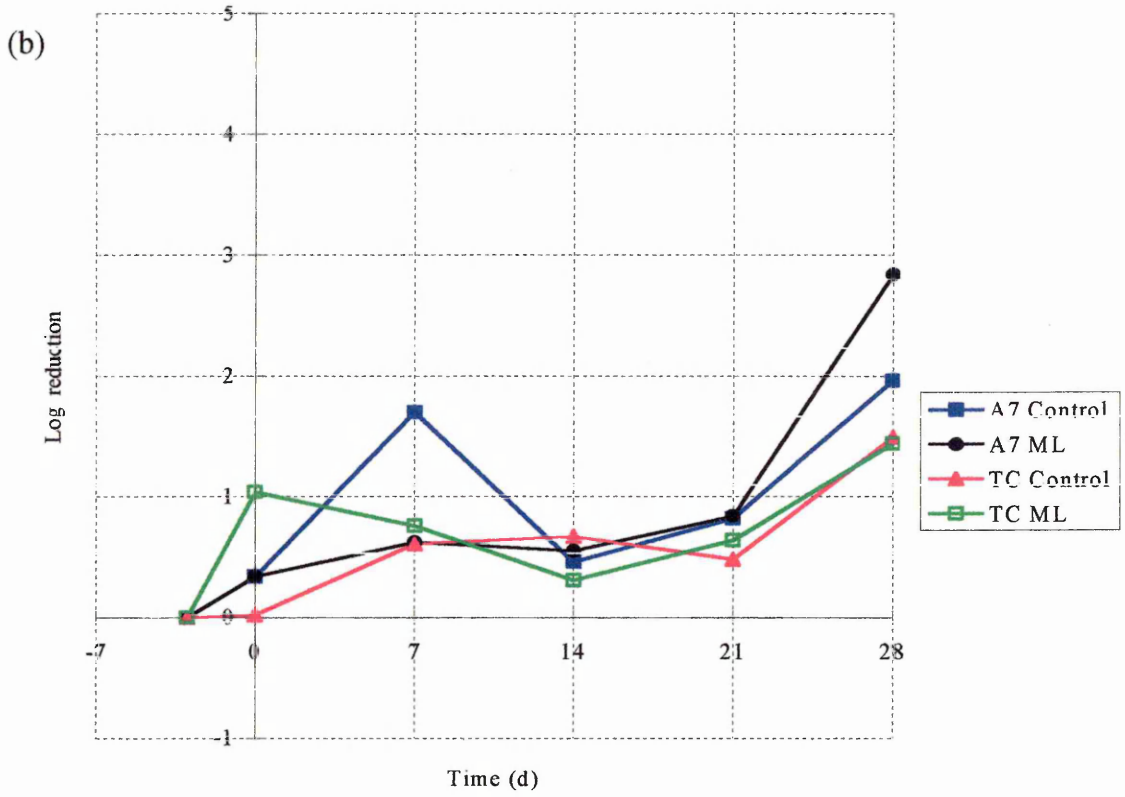


Figure 5.7. (a) Effect of monolaurin on *L. innocua* on the surface and in the centre of Camembert-type cheese, (b) log reduction between counts of *L. innocua* on the surface and in the centre of Camembert-type cheese.

Log reduction calculated as log N_o / Log N_1. A7 = *L. innocua* from Brie cheese, TC = *L. innocua* NCTC 11288 from the National Collection of Type Cultures, A3 = *L. monocytogenes* from Brie cheese, A13 = *L. monocytogenes* from Fourme d'Ambert cheese and LB = *L. monocytogenes* from Lanark Blue cheese.

Monolaurin alone (0.91 mM for strain A7 and 1.24 mM for strain NCTC) was added to the cheeses. This gave a reduction in the population of *L. innocua* throughout the ripening period. Inhibition of *L. innocua* A7 and NCTC 11288 occurred in the presence of monolaurin but to a lesser extent when compared cheese made with lauric acid and monolaurin.

Figure 5.7 (a) gives the log reduction of the population of *L. innocua* (A7) and (NCTC 11288) between the surface and centre of the cheeses. This Figure shows that the log difference for both strains spanned from zero to two log orders. Figure 5.7 (b) gives the log reduction between cheeses treated with monolaurin and those not treated with monolaurin. This Figure demonstrates a 1 to 3 log reduction between the population of *L. innocua* treated with monolaurin and which remained untreated. This trend was seen both on the surface and in the centre of the cheeses. Monolaurin reduced the growth of *L. innocua* both on the surface and in the centre of Camembert-type cheese. The combination of monolaurin and lauric acid was more effective than when monolaurin was added alone.

5.6 Multiplication of *Listeria innocua* (A7 and NCTC 11288) with monolaurin and temperature abuse during the production and ripening of Camembert-type cheese

The Camembert-type cheeses where unavoidably left draining for an extra 24 h at ambient temperature, before addition of *P. camembertii.* These cheeses had been inoculated with *L. innocua* and monolaurin had been added before draining. It was decided that it would be worthwhile leaving the cheeses to ripen and monitor the growth of *L. innocua* as normal.

In the presence of monolaurin and with the additional time (24 h) spent at ambient temperature, both populations of *L. innocua* had reduced to < 1 $\log_{10}$ cfu g^{-1}. This was seen both on the surface and in the centre of the cheeses. The population of *L. innocua* NCTC 11288 was reduced by 6.18 log orders and 2.8 log orders for surface and centre respectively (Table 5.5). Figure 5.3 b shows that after 28 d strain NCTC 11288 recovered and began to multiply. These results demonstrate that the effect was listeriostatic rather than listeriocidal.

Figure 5.8 (a) gives the plot of the log reduction between the growth of *L. innocua* with monolaurin and those cheeses not containing monolaurin after they were left for an extra 24 h. This Figure shows 2 plots for both strains, these are the difference between the surface of the control and treated cheeses and the second between the centre of the control and treated cheeses. It can be seen that after 21 d there was a sudden increase in log reduction, this illustrates a drop in the population of *L. innocua* when treated with monolaurin.

Figure 5.8 (b) gives the log reduction between the surface and centre of the Camembert-type cheeses. There are two plots for each strain, the first compares surface and centre populations of the control cheese. The second compares the surface and centre populations of the cheese treated with monolaurin. The log difference of *L. innocua* in both control cheeses increased suddenly after 14 d to approximately 3 log orders. The log difference of *L. innocua* in cheeses with monolaurin is consistent at approximately 1.5 log orders until 14 d and then is reduced to zero after 28 d. This indicates that in cheese treated with monolaurin and left for an additional 24 h during draining, the surface population of *L. innocua* was reduced to a similar level as that in the centre of the cheese.

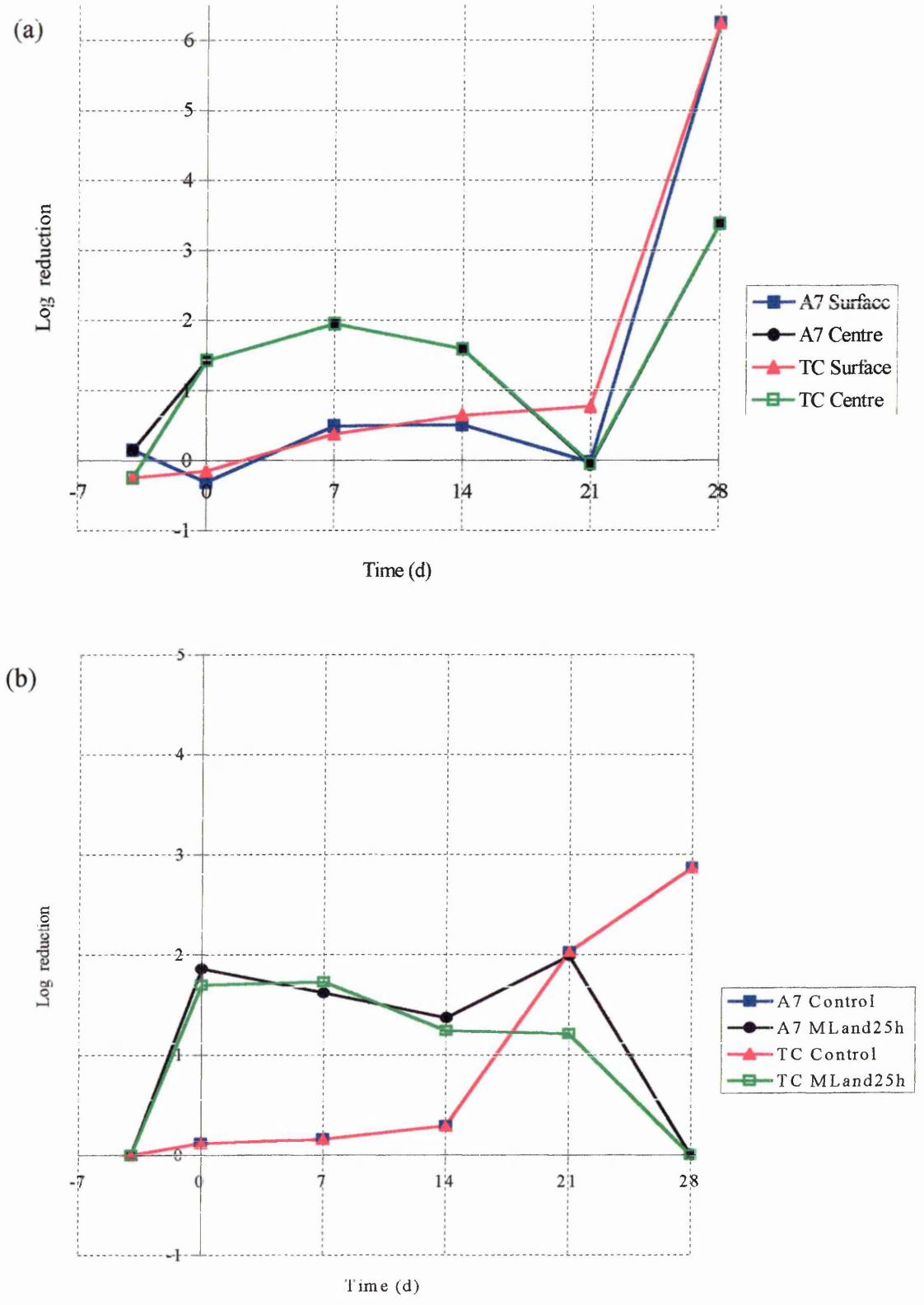


Figure 5.8. (a) Effect of monolaurin and an extra 24 h of draining at ambient temperature on *L. innocua* in Camembert-type cheese, (b) log reduction between counts of *L. innocua* on the surface and in the centre of Camembert-type cheese.

Log reduction calculated as log N_0 / Log N_1. A7 = *L. innocua* from Brie cheese, TC = *L. innocua* NCTC 11288 from the National Collection of Type Cultures, A3 = *L. monocytogenes* from Brie cheese, A13 = *L. monocytogenes* from Fourme d'Ambert cheese and LB = *L. monocytogenes* from Lanark Blue cheese.

extra 24 h of draining at ambient temperature there was a 2 log order reduction seen throughout ripening compared to the control. After 28 d there was no reduction in counts, this was because all were recorded to be below 1 $\log_{10}$ cfu g^{-1}. This indicated that by extending the draining time at ambient temperature and adding monolaurin the listerias were unable to survive in the Camembert-type cheese.

5.7 Sensory analysis of laboratory made Camembert-type cheese with and without monolaurin

The panellists were able to detect a difference between all three cheeses, $p < 0.05$. No preference to either of the cheeses was recorded. Table 5.6 shows the number of correct identifications made by panellists during sensory analysis if Camembert-type cheese using triangle difference test methodology. A significant difference ($P < 0.05$) was found between samples A (the laboratory control) and B (the laboratory test), a total of 6 correct identifications were made by the 9 panellists. A significant difference at the 1 % level ($P < 0.01$) was found between samples A and C (retailers sample), 7 correct identifications were made by the panellists. The panellists made 6 correct identifications of sample C, showing a significant difference ($P < 0.05$) between samples B and C.

Comments used to describe this difference were for sample A: Strong aftertaste, soft, smooth, mature, harsh compared to C, runny, bitter and a farm yard smell. For sample B: again strong aftertaste, bitter, soapy, mature, not as nice as sample A, smoother,

Table 5.6. Sensory analysis of three Camembert type cheeses using the triangle difference test

DATA SET	Sample comparison	Different sample	No. of Panellists	No. of correct identifications [d]	Significance % [e]
ONE	A [a] and B [b]	B	9	6	5
TWO	A and C [c]	A	9	7	1
THREE	B and C	C	9	6	5

Camembert type cheeses were produced as per methodology Figure 2.7, with extra time (24 h) at ambient temperature. Cheeses were ripened for 28 d at 10 °C and 96 % erh. [a] Laboratory made Camembert type cheese (lab. control); [b] Laboratory made Camembert cheese with 0.91 mM monolaurin; [c] Camembert-type cheese control from a major retailer; [d] A correct identification was detecting the from different sample the 3 given; [e] Significance calculated from table 5.7 below.

Table 5.7. Minimum number of correct identifications required for a significant difference at various levels in the triangle test

Number of Judgements	Level of Significance		
	5%	1%	0.10%
9	6	7	8

Adapted from ASTM special Technical Publication 434, 1968.
Chance probability is 33.3 %, and the hypothesis is one-tailed.

more solid and creamier than sample A. For sample C: again strong aftertaste, soapy, creamy, bland, not as acidic, milder and a farm yard taste.

The samples produced in the laboratory were described as soapy, bitter, strong tasting and more mature than the sample brought from a major retailer. This could have been

due to the rennet used in production of the cheese causing proteolysis of the casein to give a bitter peptide.

5.8 Summary of Results

1. The texture of the cheeses containing monolaurin (0.3 mM) was more uniform and softer than those not containing monolaurin.
2. Compositional analysis of the Camembert-type cheese gave a final water content of 55.9 %, fat content of 29.5 %, salt content of 3 % and pH of 7.3.
3. There was less growth of *L. innocua* in the centre of the cheeses compared to the surface.
4. Monolaurin had an augmented effect on lauric acid, reducing the final population of *L. innocua* (A7). A strong smell of methyl ketones was detected during cheese ripening.
5. Monolaurin alone reduced the final populations of *L. innocua* (A7) and (NCTC 11288), but this was a greater effect when combined with lauric acid.
6. Extending the draining time at ambient temperature by 24 h with the addition of monolaurin reduced *L. innocua* (A7) and (NCTC 11288) to < 1 log10 cfu g^{-1} in the model Camembert-type cheeses.
7. Sensory analysis proved that there was a difference in taste between the Camembert-type cheeses containing monolaurin and without monolaurin. There was a difference between the laboratory control cheese and the cheese brought from a major retailer. A bitter, strong, more mature after taste was detected in the cheeses containing monolaurin which was not detected in the retail controls after-taste. However, the laboratory made controls tasted bitter, perhaps due to proteolysis of the casein leading to the production of a bitter peptide.

Discussion

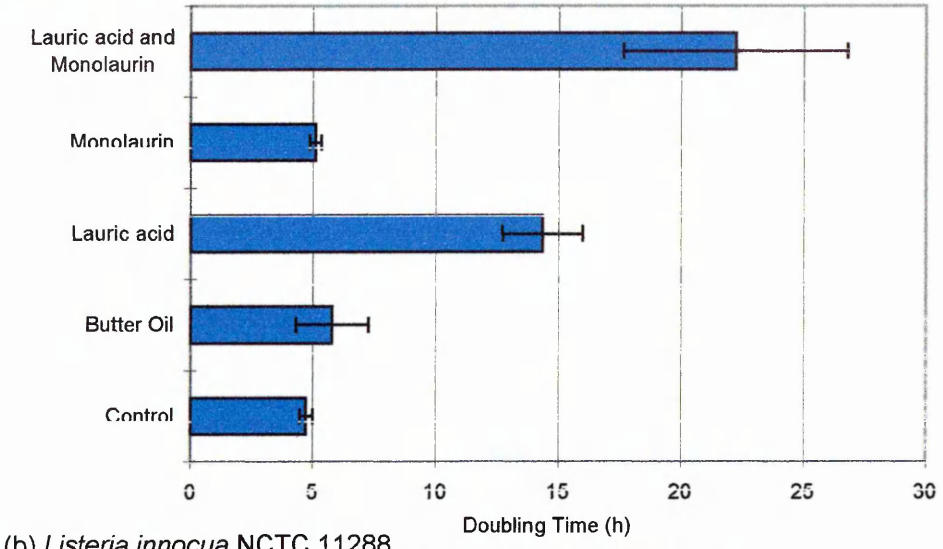
6.1 Effect of monolaurin and lauric acid in bi-phasic systems at 10 °C

Fatty acids and monoacylglycerides are known to have antimicrobial activity in model systems. In the present study, lauric acid had antilisterial activity in an aqueous broth when added to butter oil as a carrier at 10 °C. The antilisterial effect of lauric acid alone, monolaurin alone and monolaurin in combination with lauric acid on the three strains of *L. monocytogenes* and the two strains of *L. innocua* is given in Figure 6.1. This Figure demonstrated that the doubling time during the exponential growth phase increased with the addition of lauric acid from approximately 5 h to between 8 – 14 h, depending on the strain. *Listeria innocua* (A7) was the most sensitive strain to 5.0 mM lauric acid with a doubling time of 14.35 h and *L. monocytogenes* (A3) was the most resistant one with a doubling time of 8.23 h.

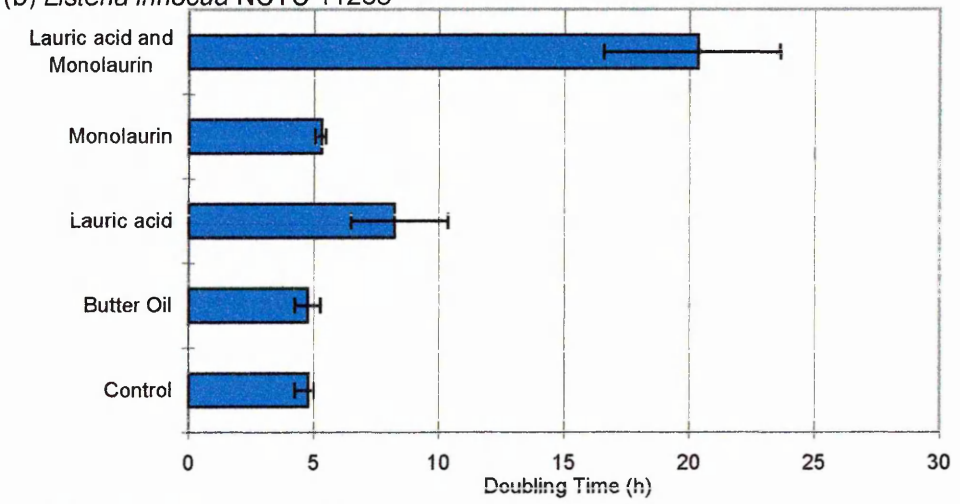
Monolaurin by itself had relatively little effect. With some strains there was slight inhibition, whilst with other ones there was a slight decrease in the doubling time. The effect of monolaurin combined with lauric acid increased the doubling time significantly. The most sensitive strain was *L. monocytogenes* (LB) which had been isolated from Lanark blue (showed no growth), whilst the most resistant strain was *L. innocua* NCTC 11288 (11.53 h).

There were differences in the doubling time in the controls with butter oil, where *L. monocytogenes* (A13) had the highest doubling time (6.89 h) and *L. innocua* (NCTC 11288) had the shortest doubling time (4.58 h).

(a) *Listeria innocua* A7



(b) *Listeria innocua* NCTC 11288



(c) *Listeria monocytogenes* A3

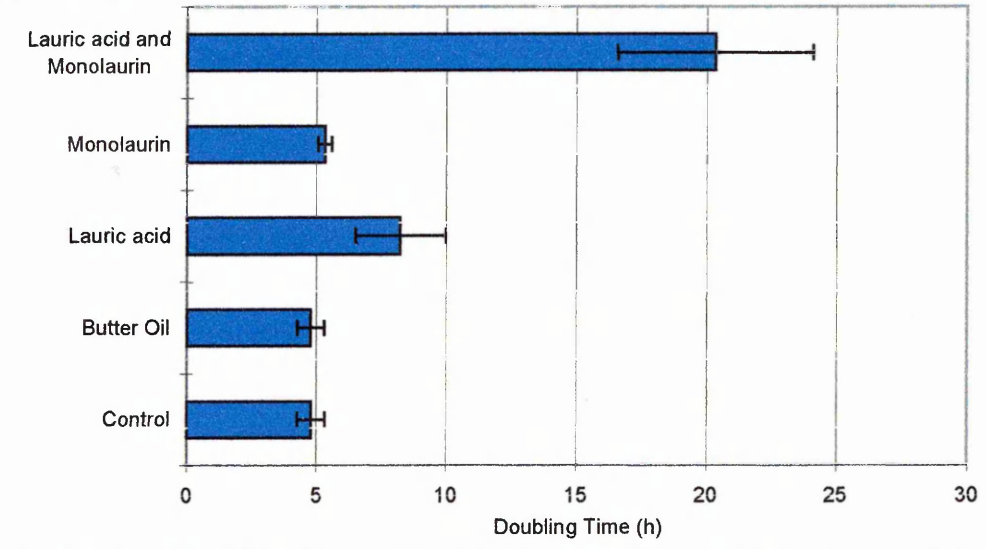


Figure 6.1. Doubling times of five strains of listeria exposed to butter oil, lauric acid and monolaurin in TSGYB at 10 °C with an initial pH of 7.
(a) from Brie Cheese, (b) from the National Collection of type cultures and (c) from Brie cheese

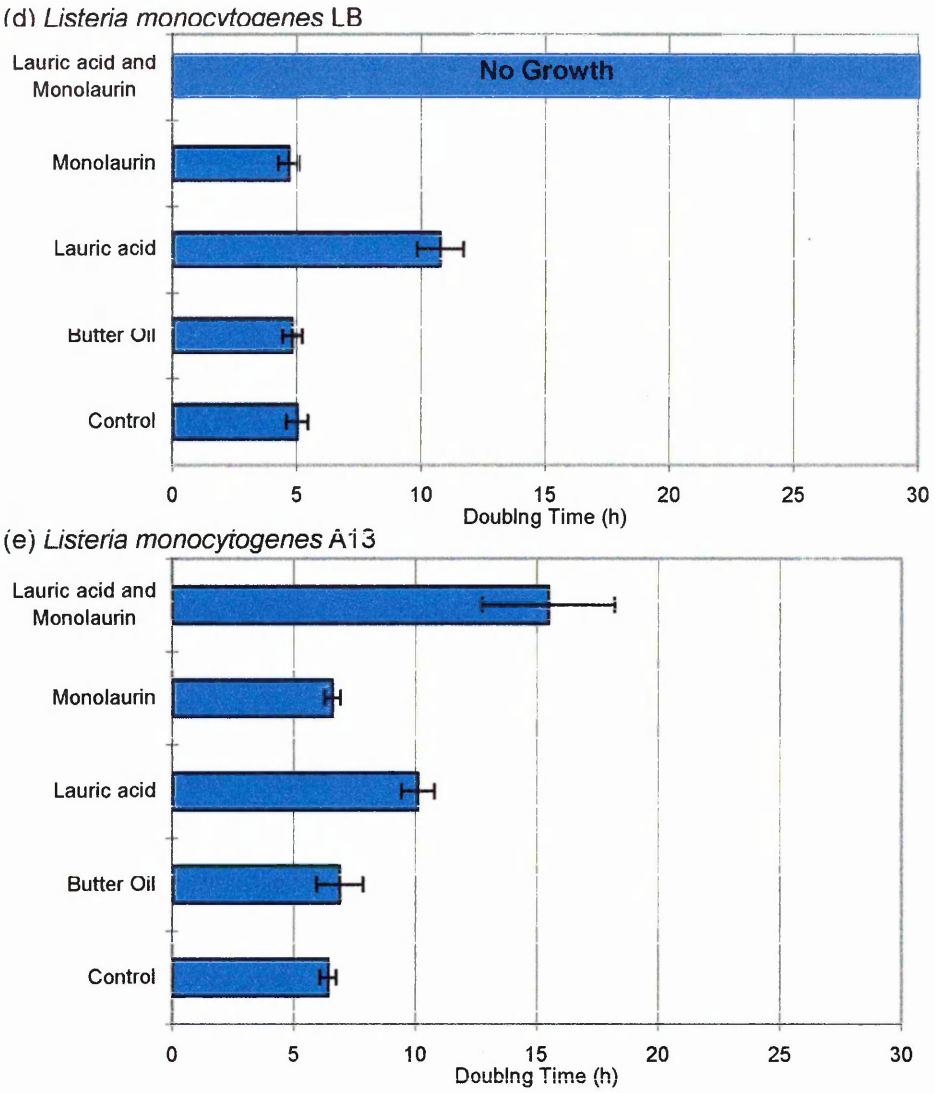


Figure 6.1. (continued) Doubling times of five strains of listeria exposed to butter oil, lauric acid and monolaurin in TSGYB at 10 °C with an initial pH of 7. (d) from Fourme d'Ambert cheese and (e) from Lanark blue cheese

Results are the mean doubling time ± SD. The data was derived from the results given in Chapter 4.0. n = 18 for each treatment and n = 36 for the butter oil control.

Table 6.1. Effect of monolaurin, lauric acid and production time on *L. innocua*

Test	*L. innocua* strain	Log reduction (Log_{10} cfu mL^{-1})	
		Surface	Centre
Lauric acid and Monolaurin	A7 (Brie)	4.27	2.76
Monolaurin	A7	0.85	1.23
Monolaurin	NCTC 11288	1.73	1.18
Monolaurin and 24 h	A7	6.25	6.25
Monolaurin and 24 h	NCTC 11288	3.38	3.38

Results are expressed as log reduction. Defined as log N_o/Log N_1. See Chapter 2 Section 13.3

Unlike the experiments in a model bi-phasic broth system, monolaurin by itself had considerable antilisterial activity in a soft-ripened cheese (Table 6.1). The log reduction on the surface of the cheese with was 0.8 – 1.7 depending on the strain, at the end of 28 d of ripening. When lauric acid was added to the model cheese, it was converted to a volatile ketone (2-undecanone), resulting in the loss of lauric acid. The loss of lauric acid was due to a fungal bio-conversion of the acid to 2-undecanone (Kinderlerer 1987). Due to the bio-conversion of lauric acid to a volatile ketone, lauric acid was omitted in the other cheese experiments and the concentration of monolaurin increased from 0.33 mM to 0.9 mM. This concentration was within the limits allowed in the food preservative regulations (Butterworth 1997).

In the experiments without lauric acid and with 0.9 mM monolaurin there was a reduction in growth of listeria on the surface of the cheese after 28 d of ripening. With extended ripening, there was a significant decrease in the population of listeria (3 – 6 log order reduction depending on the strain). With extended processing at ambient temperature there was a considerable reduction in the population of *L. innocua*, presumably due to the increase in the concentration of lactic acid in the cheese. Monolaurin is soluble in fat so during the drainage of the cheese would stay bound to the curd. As the whey drained from the cheese the concentration of monolaurin with respect to the curd would increase. The apparent increase in concentration of monolaurin in the curd may account for the reduction in the population of *L. innocua* in cheese which were drained for an extra 24 h at ambient temperature.

Fat is present in many foods as an emulsion. Butterfat is a water-in-oil emulsion, whereas cream is an oil-in-water emulsion (Coupland and McClements 1997). In order

to add lauric acid and monolaurin to the experimental cheese, they were dissolved in cream. The cream was homogenised with skim milk to give a re-constituted whole milk with a fat content of 3.6 % (w/v). This re-constituted milk was used to make the Camembert-type cheese. The advantage of adding the inhibitor to the oil phase of a water-in-oil emulsion was to increase the area available for diffusion of lauric acid and monolaurin into the aqueous phase. Work by the Robins group at the Institute of Food Research, Norwich has demonstrated very clearly that in an oil-in-water emulsion the bacteria are found at the interface of the aqueous phase surrounding the oil droplets (Brocklehurst *et al.* 1995). The concentration of lauric acid and monolaurin would be highest at the interface.

It has been demonstrated that organic acids and medium chain length fatty acids inhibit spores and vegetative cells of Gram positive bacteria (Gianozzi and Zaritzky 1996, Cotton and Marshall 1997, Karabara and Eklund 1991, Oh and Marshall 1992, 1995 b and c). The Wisconsin group have studied the effect of fatty acids and found that lauric acid had greater activity against *L. monocytogenes* than the longer chain length acids, palmitic (C16:0) or myristic (C14:0) (Wang and Johnson 1992). In the Wisconsin study 1 –2 % ethanol was used as the release agent. At these concentrations, ethanol was shown to have no effect on the viability of the pathogen.

The use of butter oil as a release agent reduced the effect of monolaurin as an antilisterial agent. There was no statistical difference ($P > 0.05$) between the final populations of the five strains of listeria. The Wisconsin group found a similar trend in full (whole) fat milk compared to skim milk. It was believed that the fat in the whole milk reduced the effectiveness of monolaurin (Wang and Johnson 1992). The protective effect of butter oil was lost when monolaruin and lauric acid were combined.

In this case monolaurin had an augmentative effect and it enhanced the inhibition which was observed when lauric acid was dissolved in the butter oil.

6.2 Sensory evaluation of Camembert-type cheese

In cheese much of the flavour is due to the release of volatiles which are influenced by the food matrix (Urbach 1997). The volatiles, which give soft unripened cheeses their characteristic aroma/flavour have been attributed to the action of the lactic acid starter culture (Cogan 1995). The starter culture in this project was Chr. Hansens Redi-Set mesophillic starter type LD. Type L contains citrate positive *Leuconostoc lactis*, *L. mesenteriodes* ssp. *cremoris*. Type D contains *Lactococcus lactis* spp. *cremoris* and *L. lactis* spp. *lactis*. Diacetyl (a flavour compound) is produced by *Leuconostoc* spp. from citrate (Cogan 1995) and from *Lactococcus lactis* ssp. *lactis* biovar *diacetylactis*. An additional flavour in soft mould-ripened cheese is due to the production of mushroom alcohol (1-octen-3-ol) produced after the fungal mycelium on the outside of the cheese is cut when the cheese is bitten. The cutting action of the teeth has allowed enzyme and substrate to come together with the release of the mushroom-like odour of 1-octen-3-ol (Kinderlerer *et al.* 1993).

Sensory analysis of the cheese made with monolaurin demonstrated a bitter, soapy, mature aftertaste. Concentrations of 250 and 500 ppm monolaurin in cheese ripened at 15 °C produced a chemical aftertaste (Bautista *et al.* 1993). Emulsifiers are known to increase the intensity of flavour compounds and to make the flavour last longer (Bakker 1997). The concentration of monolaurin used in this study was approximately 250 ppm, this should be lower than the published threshold for detection of a soapy taste, but it was not.

The production of bitter peptides from the hydrolysis of casein is the major factor contributing to bitterness in mature cheese. Amino acids which exhibit a bitter taste are methionine, histidine, lysine, tryptophan, isoleucine, arginine, phenylalanine (El-Soda 1997). Some peptides formed through protein degradation exhibit bitter flavours due to certain terminal amino acids (Scott 1986). Under normal ripening conditions, the terminal amino acid is cleaved by proteolytic action of the rennet or proteolytic enzymes produced by the lactic acid bacteria and bitterness is lost. In unripe cheeses the terminal amino acid is not cleaved and the cheese tastes bitter. Some microbial rennets are known to give a bitter taste to the cheese. Under suitable conditions the rennet will coagulate the cheese curd but will have too much proteolytic action. If the coagulum is hydrolysed too quickly the yeild of cheese is reduced and the cheese may have a bitter taste (Fox and McSweeny 1997). *Penicillium camembertii* can play a major role in the degradation of hydrophobic peptides, and can bring about proteolysis (Grippon 1997). If the pH of the cheese is too high *P. camembertii* has enhanced production of proteases (Grippon 1997). Some of the cheeses produced in this study were discarded as they had a watery texture when cut. Two factors could account for this effect (1) insufficient acidification or (2) a high moisture content (Grippon 1997). The cause of bitterness in Camembert-type cheese could have been due to:

1. Proteolysis of the casein by *P. camembertii*,
2. Storage at a high relative humidity,
3. Extra proteolytic activity due to the microbial rennet.

6.3 Solubility of saturated fatty acids

Figure 6.2 gives the solubility of various saturated fatty acids in 100 g of water. This Figure demonstrates that the solubility of the fatty acid is higher at 30 °C compared to

0 °C. The length of the carbon chain also affects the solubility, the shorter chain length fatty acids are more soluble in water than the longer chain length ones (Figure 6.3). Lauric acid (C12:0) is relatively insoluble in water (0.0037 g 100 g^{-1} at 0 °C and 0.0063 g 100 g^{-1} at 30 °C). From Figure 6.3 it can be seen that the solubility of all fatty acids is increased with an increase in temperature. Some of the variation in temperature given in Chapter 4 may account for the slight difference in the growth rate and in the doubling time.

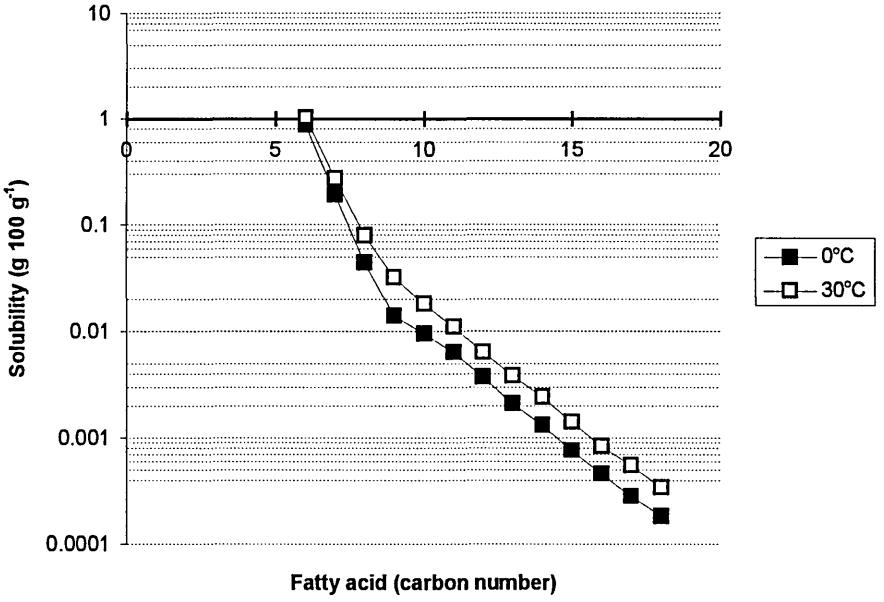


Figure 6.2. Solubility of saturated fatty acids (CRC Handbook of Biochemistry and Microbiology 1969)

6.4 Mode of inhibition of lauric acid

The mechanism of inhibition of growth of Gram positive bacteria by hydrophilic acids has been discussed by Freese *et al.* (1973) in their classic paper on food preservatives. The site of action of lipophilic acid is thought to be the cytoplasmic membrane in prokaryotic organisms. Lipophilic acids diffuse across the cellular membrane in their protonated or undissociated form (Gould *et al.* 1983). Carriers in the cell membrane linked with energy production aid in the uptake of fatty acids (Kabara and Eklund

1991). Lipophilic organic acids enter the cell in their undissociated form and once inside the cell dissociate, causing the pH of the cytoplasm to decrease (Kouassi and Shelef 1995). The cell reacts to maintain a constant internal pH by using energy to remove the protons from the cell.

The dissipation of the proton motive force results in a reduction of ATP and cellular growth (Cherrington *et al.* 1991 and Christensen and Hutkins 1992, Gould *et al.* 1983 and Kouassi and Selef 1995). Lowering of the cytoplasmic pH does not appear for all microbial inhibition by lipophilic acids (Young and Foegeding 1993). Salts of organic acids, which are fully dissociated in an aqueous solution, demonstrate anti-microbial activity (Eklund 1983 and Kouassi and Shelef 1995).

Cells of *L. monocytogenes* can maintain a pH gradient of 1.0 to 1.5 units over a medium between pH of 3.5 to 6.5 (Ita and Hutkins 1991). Other organic acids (citric, lactic, malic and tartaric acid) are thought to be inhibitory due to their ability to chelate iron, (Piccinin and Shelef 1995).

Evidence in this study suggests that lauric acid and lauric acid in combination with monolaurin have inhibited the energy metabolism of the cell. Cell growth in the presence of monolaurin and lauric acid resulted smaller cells than without the additives. Weak organic acids, such as lauric acid, are undissociated at their pHs above pKa (4.8 for lauric acid, Freese *et al.* 1973). In this system inhibitory action of lauric acid was demonstrated at pH 7. At pH 7.0 the acid will be disassociated and therefore not able to diffuse through the cell membrane. Figures 6.3 (a) and (b) demonstrate at a higher pH, the minimum inhibitory concentration (MIC) increases for *L. monocytogenes* at 20 °C. However the concentration of undissociated acid is relatively constant, particularly for caprylic acid at pH 4.99 and 5.59.

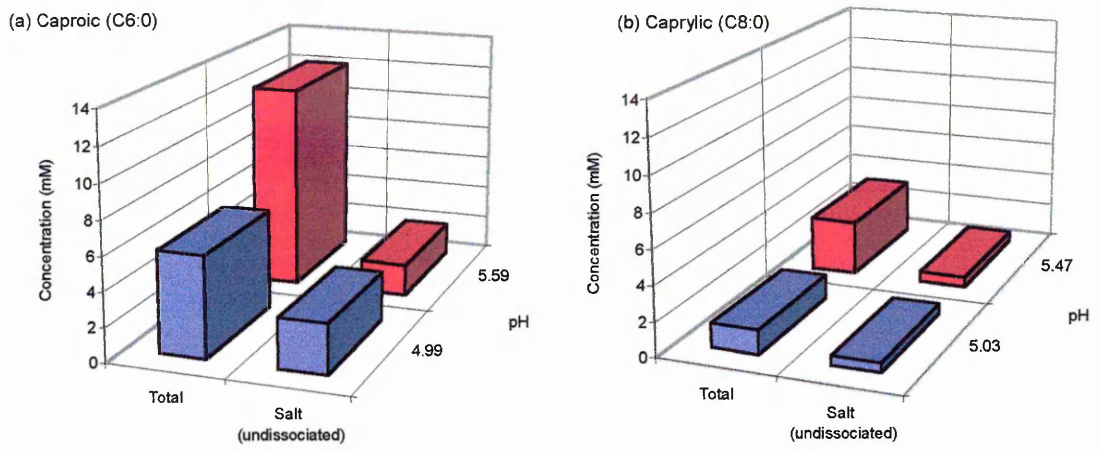


Figure 6.3. Effect of pH on the concentration of total and undissociated acid on the MIC for *L. monocytogenes* at 20 °C (data derived from Kinderlerer and Lund 1991)

Some small non-polar and fat-soluble substances (fatty acids and alcohols) can enter and exit the cell by dissolving in the lipid phase of the cell membrane. Bacterial cells are attracted to the lipid layer of the emulsion (Brocklehurst *et al.* 1995). Lauric acid was dissolved in butter oil in the shake culture, and in the cream in the Camembert-type cheese. The listeria cells would be attracted to the fat layer increasing the chances of contact of the cells with lauric acid and the absorption of the molecule into the cytoplasm of the cell. The uptake of the acid could also have been due to a concentration gradient across the cell membrane. The outside of the cell membrane would have a higher pH the acid could diffuse through the cell membrane into the lower pH of the cytoplasm.

6.5 Control of *Listeria* spp. in Camembert-type cheese

Factors which affect the growth of spoilage organisms in foods can be divided into 4 groups (Mossel and Ingram 1955):

1) Intrinsic factors; the physio-chemical properties of the food, (nutrients, pH, redox potential, a_w and antimicrobials),

2) Extrinsic factors; conditions of the storage environment, (relative humidity, temperature and gaseous atmosphere,

3) Implicit factors; properties and interactions of the micro-organisms present, (growth rate, synergism, antagonism and commensalism),

4) Processing factors; slicing, washing, packing, irradiation and pasteurisation.

The application of microbiological criteria in combination with Good Manufacturing Practise (GMP) should be adopted to achieve microbiological safety of cheese (Roberts *et al.* 1995). Cheeses such as Camembert or Brie are often made from raw milk. The risk of contamination by *Listeria* spp. from raw milk is high (Bell and Kyriakides 1997). The Dairy Products (Hygiene) Regulations gives the microbiological criteria for *L. monocytogenes* in cheese, Table 6.2.

Raw milk is pasteurised (71.7 °C for 15 s) and cooled immediately to not more than 30 - 32 °C before use in the production of cheese. Pasteurisation has the effect of reducing a pathogen population by 3 log orders (Parry and Pawsey 1992). This study shows a 6 log reduction of *L. innocua* on the surface of the Camembert-type cheese and a 3 log reduction in the centre of the cheese. This is better than a pasteurisation process.

Table 6.2. Microbiological criteria for cheese (Roberts *et al.* 1995)

Cheese other than hard cheese	Absent in 25 g, $n = 5$, $c = 0$
Hard cheese	Absent in 1 g, $n = 5$, $c = 0$

n is the number of sample units comprising the sample; c is the number of sample units where the bacterial count may between m and M; m is the threshold value for the number of bacteria; the result is considered satisfactory if the number of bacteria in all sample units does not exceed this value; M is the maximum value for the number of bacteria; the result is considered unsatisfactory if the number of bacteria in one or more samples units is equal to or greater than this value.

From the study it can be seen that an antimicrobial agent can be applied to culture medium and to Camembert-type cheese in the fat phase. Both lauric acid and

monolaurin are effective as preservatives in broth culture and in fermented foods such as cheese. Addition of monolaurin with extended drainage of 24 h reduced the growth rate of *Listeria* spp. in Camembert-type cheese. A lag phase can be calculated for some strains in the broth culture when monolaurin and / or lauric acid were added to the butter oil. In this system oxygen appeared to be important in controlling the growth of *Listeria* spp. Reduced oxygen in the centre of the cheese leads to lower populations of listeria than on the surface.

The use of lauric acid in combination with monolaurin appears to be listeriostatic rather than listeriocidal. In a food system the use of antimicrobials, particularly those which are produced naturally and that result in a reduction in numbers of a pathogen or an increase in the lag phase, would be important in increasing the shelf-life of a product. Cheeses which contain free fatty acids or monoacyglycerides would have a natural antimicrobial system which would be useful in high risk cheeses such as Brie and Camembert if the problem of unacceptable taste/flavour could be overcome.

Conclusions

1. The suggestion by Papageorgiou and Marth (1989) that free fatty acids could inhibit growth of *L. monocytogenes* in a blue mould-ripened cheese has been proved in a bi-phasic broth culture at pH 7.0 where 5.0 mM lauric acid was dissolved in butter oil. A reduction of growth of up to 5 log orders was seen in broth culture where 5.0 mM lauric acid was dissolved in butter oil and one log order in an experimental soft-ripened cheese when 0.5 mM lauric acid was dissolved in the cream.

2. Monolaurin is known to have antimicrobial activity against Gram positive bacteria when dissolved in ethanol and added to an aqueous broth. Previous workers (Wang and Johnson 1991) have demonstrated that the antilisterial activity of monolaurin is reduced in the presence of 2 % fat in milk. This observation has been confirmed in broth culture (10 % fat) where monolaurin was dissolved in the butter oil. Slight inhibition was seen for strains *L. innocua* (A7), (NCTC 11288) and *L. monocytogenes* (A3), (A13). *Listeria monocytogenes* strain LB showed slight acceleration of growth with monolaurin.

3. Monolaurin (0.35 mM) had an augmentative effect when combined with lauric acid (5.0 mM) in broth culture at pH 7.0 and 10 °C and over 5 log reduction was seen for three strains of *L. monocytogenes* and the two strains of *L. innocua*. there were strain differences, *L. innocua* was more sensitive that *L. monocytogenes* and an isolate of *L. monocytogenes* from Lanark Blue cheese was extremely sensitive.

4. In soft-ripened cheese made with *L. innocua* there were more listerias on the surface of the cheese than in the centre. It was suggested that this difference was due to the higher oxygen concentration on the outside of the cheese than the centre.

5. Monolaurin (0.35 mM) when added to the cream and incorporated into an experimental soft-ripened cheese inhibited the growth of two strains of *L. innocua*. Inhibition of growth was increased significantly when the experimental cheeses were drained for an extra day at ambient temperature before under going ripening at 10 °C.

6. Monolaurin could be detected in cheese by an untrained taste panel at a level of 250 ppm. Monolaurin appears to give a mature taste to the cheese which was liked by some panellists.

7. It is suggested that fat could be used as a release agent in foods for additives which are insoluble in water.

Further work

1. The minimum inhibitory concentrations of monolaurin and lauric acid in soft mould-ripened cheese should be determined to investigate the maximum threshold of bitter and soapy aftertastes

2. The mode of action of lauric acid at pH 7.0 should be investigated, in particular the effect of this antimicrobial as a function of oxygen tension in shake culture.

3. Oxygen tension of the cheese has an important role in control of the pathogen. Further research would determine if control of oxygen could be used in conjunction with fatty acids to control *Listeria* spp. in soft mould-ripened cheeses.

4. To improve the microbiological quality of cheese a longer period at ambient temperature during drainage in combination with the addition of monolaurin should be investigated in a range of different cheese types.

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SENSORY ANALYSIS CODED SHEET
(A SIMILAR VERSION WAS GIVEN TO ALL PANELLISTS)

Name: _____ Control Sheet

Date: _____

You have been given three sets of samples of each set two are identical and the third is different. Taste the samples in the order indicated and identify, using a cross in the relevant space, the odd or different sample.

Rinse, using palate cleanser in between samples.

Identify the odd or different sample.

DATA SET ONE

Code	Odd or different sample	Why is this sample different?
286	A	
311	B	
574	A	

Code	Odd or different sample	Why is this sample different?
897	C	
955	A	
669	C	

Code	Odd or different sample	Why is this sample different?
438	B	
743	B	
562	C	

Appendix Two

2.1. Effect of monolaurin and lauric acid in a model broth culture at 10 °C

(a) *Listeria monocytogenes* (A3)

Control

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.99	3.11	3.06	3.03	3.14	2.96	2.74	2.76	2.84	2.75	2.91	2.78	2.66	2.91	2.98	2.72	2.79	2.73
24	3.58	3.77	3.75	3.56	3.61	3.58	3.42	3.60	3.73	3.66	3.61	3.69	3.82	3.79	3.83	3.75	3.60	3.57
48	5.19	5.63	5.14	5.03	5.02	5.13	5.13	5.09	5.23	5.32	5.21	5.38	5.23	5.22	5.25	5.25	5.25	5.18
72	5.72	5.83	5.83	5.48	5.46	5.53	5.66	5.53	5.56	6.69	6.76	6.69	6.64	6.76	6.83	6.90	6.81	6.79
96	8.13	8.30	8.17	7.91	7.86	7.97	7.86	7.87	7.89	8.36	8.41	8.28	8.31	8.28	8.16	8.16	8.24	8.19
120	9.51	9.60	9.58	9.45	9.43	9.34	9.49	9.38	9.51	9.62	9.66	9.63	9.46	9.43	9.56	9.76	9.64	9.76
144	10.00	10.10	10.09	10.16	10.08	10.23	10.03	10.06	10.01	10.05	10.06	10.11	9.92	9.93	9.87	10.08	10.15	10.12
168	10.00	10.00	9.96	9.79	9.94	9.85	9.78	9.85	9.86	9.56	9.62	9.62	9.76	9.73	9.65	9.57	9.60	9.69
192	9.91	9.87	9.87	10.14	9.95	10.11	10.05	9.96	9.98	10.05	9.81	9.92	9.89	9.97	9.88	9.97	9.91	9.82

Oil

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.99	3.11	3.06	3.03	3.14	2.96	2.74	2.76	2.84	2.75	2.91	2.78	2.66	2.91	2.97	2.72	2.79	2.73
24	3.72	3.82	3.75	3.81	3.76	3.77	3.97	3.96	3.97	3.65	3.61	3.61	3.79	3.75	3.72	3.70	3.74	3.81
48	4.26	4.45	4.26	4.28	4.20	4.30	4.58	4.60	4.40	5.11	5.09	5.04	5.14	5.13	5.22	5.23	5.25	5.30
72	6.59	6.66	6.74	6.76	6.78	6.76	6.77	6.86	6.81	6.60	6.53	6.64	6.51	6.66	6.66	6.54	6.65	6.65
96	7.92	7.94	8.00	8.08	8.23	8.17	8.35	8.36	8.34	7.72	7.75	7.73	8.03	8.11	8.06	8.19	8.24	8.18
120	9.75	9.62	9.79	9.61	9.64	9.57	9.46	9.45	9.53	9.48	9.56	9.54	9.54	9.38	9.40	9.43	9.46	9.34
144	10.06	10.14	10.11	9.97	10.04	10.00	10.06	10.07	9.98	9.91	9.96	9.97	9.85	9.88	10.01	9.64	9.76	9.78
168	10.58	10.88	10.54	10.30	10.54	10.63	10.38	10.57	10.30	10.03	10.06	9.99	10.16	10.12	10.11	9.93	9.91	9.99
192	10.21	10.19	10.11	10.02	10.06	10.17	10.23	10.24	10.28	10.12	10.12	9.93	10.12	10.11	10.18	10.13	10.11	10.18

Oil continued

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.52	2.49	2.56	2.56	2.23	2.53	2.45	2.34	2.58	3.01	2.91	3.06	3.09	3.16	3.10	3.06	3.10	3.10
24	3.00	3.09	2.95	2.81	2.86	2.73	2.58	2.38	2.70	4.00	3.98	3.96	4.36	4.32	4.38	4.18	4.18	4.17
48	4.94	5.13	5.28	5.29	5.17	5.31	5.23	5.41	5.31	4.96	5.08	5.03	5.63	5.34	5.34	5.36	5.69	5.49
72	6.98	7.16	7.03	6.83	6.83	6.98	7.12	7.05	6.96	6.23	6.26	6.15	6.67	6.82	6.81	6.14	6.18	6.29
96	7.91	7.94	7.96	8.11	8.22	8.23	7.98	7.85	7.88	7.96	7.98	7.97	8.16	8.12	8.13	7.88	7.81	7.75
120	8.88	9.02	8.97	9.15	9.13	9.14	8.93	8.68	8.96	9.48	9.23	9.34	10.04	10.04	9.92	9.85	9.76	9.79
144	9.85	9.95	9.79	9.89	9.94	9.84	9.62	9.60	9.74	9.80	9.90	9.79	9.70	9.67	9.77	9.23	9.36	9.36
168	9.91	9.93	9.95	9.81	9.86	9.96	9.91	9.89	9.97	9.81	10.06	9.99	9.73	9.98	9.88	9.49	9.44	9.43
192	9.81	9.68	9.72	9.83	9.83	9.72	9.73	9.68	9.56	9.75	9.76	9.67	9.64	9.68	9.54	9.34	9.40	9.45

Monolaurin

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.52	2.49	2.56	2.56	2.23	2.53	2.45	2.34	2.58	3.01	2.91	3.06	3.09	3.16	3.10	3.06	3.10	3.10
24	3.54	3.63	3.45	3.79	3.79	3.74	3.30	3.28	3.20	4.59	4:43	4.40	4.14	4.20	4.06	4.06	4.06	4.11
48	5.26	5.30	5.40	5.56	5.58	5.71	5.76	5.75	5.72	5.22	5.23	5.26	5.38	5.42	5.26	5.38	5.48	5.69
72	6.74	6.76	6.81	6.83	6.93	6.96	6.93	6.86	6.89	6.62	6.72	6.68	6.40	6.48	6.36	6.64	6.62	6.59
96	7.82	7.72	7.85	7.94	8.05	8.02	7.72	7.83	7.70	7.85	8.09	7.95	7.94	8.06	8.03	8.13	7.88	7.91
120	9.04	8.96	8.83	9.09	9.18	9.21	8.86	8.95	8.64	9.48	9.78	9.51	9.67	9.62	9.32	9.60	9.61	9.40
144	10.09	10.23	10.18	10.06	10.03	9.96	10.02	10.04	10.08	9.54	9.51	9.30	9.53	9.61	9.26	9.76	9.45	9.56
168	9.98	9.91	9.93	9.88	9.89	9.83	9.93	9.91	9.87	10.06	10.15	10.08	9.58	9.48	9.73	10.03	9.98	10.20
192	9.84	9.88	9.89	9.93	9.96	9.80	9.68	9.72	9.57	9.59	9.45	9.60	9.34	9.40	9.46	9.65	9.68	9.56

Lauric acid

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.99	3.11	3.06	3.03	3.14	2.96	2.74	2.76	2.84	2.75	2.91	2.78	2.66	2.91	2.97	2.72	2.79	2.73
24	3.11	2.91	3.03	3.07	3.08	3.00	2.99	3.05	3.07	3.04	3.01	3.06	3.06	3.06	3.10	2.89	2.94	2.92
48	4.12	4.12	4.14	4.10	4.10	4.06	4.21	4.23	4.27	3.95	3.68	4.03	3.96	4.02	3.75	4.02	3.70	3.70
72	5.15	5.13	5.14	5.21	5.27	5.28	5.30	5.36	5.38	4.92	4.73	4.79	5.12	4.86	5.08	4.83	4.88	4.88
96	6.20	6.23	6.22	6.16	6.12	6.10	6.19	6.17	6.21	5.51	5.26	5.08	5.64	5.38	5.48	5.48	5.20	5.53
120	6.90	6.95	6.96	7.31	7.36	7.33	7.54	7.51	7.52	6.40	6.30	6.34	6.49	6.30	6.32	6.38	3.40	6.40
144	7.82	8.00	8.00	8.20	8.20	8.13	8.05	8.15	8.05	7.03	6.97	6.96	7.20	7.20	7.19	7.11	7.10	7.02
168	8.42	8.34	8.28	8.45	8.39	8.42	8.25	8.17	8.13	7.53	7.45	7.42	7.79	7.70	7.60	7.81	7.81	7.58
192	8.54	8.50	8.47	9.02	8.96	8.86	8.36	8.09	8.09	7.79	7.76	7.73	7.91	8.11	7.96	7.93	7.90	7.89

Monolaurin and Lauric acid

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.52	2.49	2.56	2.56	2.23	2.53	2.45	2.34	2.58	3.01	2.91	3.06	3.09	3.16	3.10	3.06	3.10	3.10
24	2.73	2.86	2.79	2.75	2.72	2.88	2.75	2.86	2.81	2.93	2.90	2.86	2.91	2.95	3.02	3.05	3.00	3.06
48	4.04	4.03	4.08	3.99	3.98	3.93	3.83	3.86	3.71	3.04	2.96	3.01	3.11	2.97	3.08	3.07	3.20	3.08
72	4.38	4.44	4.45	4.37	4.33	4.38	4.25	4.28	4.02	3.15	2.90	3.00	3.56	3.08	3.00	3.15	3.08	3.48
96	4.53	4.49	4.44	4.49	4.47	4.51	4.48	4.52	4.58	3.81	3.85	3.92	4.04	4.05	4.07	4.09	4.11	4.16
120	4.88	4.96	4.98	4.96	4.98	4.96	4.88	4.93	4.99	4.01	3.97	3.99	4.10	4.25	4.03	4.06	4.12	4.07
144	5.04	5.06	5.04	5.06	5.06	5.04	5.09	5.16	5.03	4.39	4.41	4.33	4.50	4.48	4.39	4.46	4.42	4.33
168	5.54	5.58	5.62	5.71	5.81	5.86	5.43	5.32	5.36	4.78	4.79	4.82	4.68	4.66	4.64	4.81	4.76	4.72
192	5.68	5.72	5.59	5.79	5.86	5.93	5.91	5.75	5.86	5.06	5.17	5.02	5.20	5.08	5.09	5.13	5.08	5.09

(b) *Listeria monocytogenes* (A13)

Control

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	3.06	3.06	3.03	3.03	3.02	3.12	3.09	3.06	3.06	2.86	2.78	2.81	2.85	2.81	2.68	2.68	2.60	2.86
24	4.77	4.49	4.54	4.61	4.62	4.34	4.60	4.65	4.67	3.90	3.85	3.92	3.87	4.03	3.84	3.98	4.01	4.01
48	5.72	5.70	5.75	5.66	5.73	5.60	5.64	5.72	5.76	5.19	5.18	5.14	5.25	5.19	5.26	5.18	5.23	5.13
72	6.42	6.42	6.43	6.62	6.62	6.60	6.42	6.46	6.30	6.43	6.38	6.21	6.35	6.36	6.27	6.32	6.34	6.28
96	7.60	7.59	7.60	7.64	7.57	7.59	7.64	7.62	7.63	7.24	7.09	7.29	7.29	7.28	7.26	7.38	7.35	7.40
120	8.38	8.37	8.38	8.45	8.44	8.45	8.47	8.46	8.45	8.37	8.47	8.45	8.35	8.40	8.43	8.38	8.34	8.34
144	9.13	9.12	9.03	9.13	9.10	9.13	9.23	9.29	9.16	9.80	9.84	9.86	9.74	9.75	9.81	9.81	9.87	9.72
168	9.42	9.47	9.35	9.24	9.27	9.25	9.30	9.32	9.33	10.06	10.15	9.94	9.68	9.79	9.82	9.72	9.81	9.73
192	9.72	9.81	9.64	9.65	9.56	9.38	9.40	9.30	9.49	9.70	9.74	9.62	9.79	9.83	9.90	9.86	9.81	9.76

Oil

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																		
0	3.06	3.06	3.03	3.03	3.02	3.12	3.09	3.06	3.06	2.86	2.78	2.81	2.85	2.81	2.68	2.68	2.60	2.86	2.78
24	3.62	3.73	3.58	3.93	3.94	3.93	3.58	3.57	3.38	3.73	3.82	3.90	3.92	4.01	3.97	4.00	3.96	3.96	3.76
48	5.13	5.60	5.76	5.86	8.79	5.79	5.60	5.76	5.78	5.40	5.39	5.38	5.46	5.44	5.43	5.45	5.52	5.52	4.89
72	6.60	6.65	6.60	6.43	6.45	6.43	6.43	6.28	6.36	6.23	6.28	6.27	6.25	6.24	6.23	6.37	6.42	6.35	5.91
96	7.76	7.75	7.72	7.79	7.83	7.85	7.66	7.58	7.62	7.26	7.15	7.18	7.07	7.21	7.19	7.25	7.33	7.30	7.15
120	8.64	8.72	8.73	8.75	8.62	8.85	8.72	8.76	8.82	8.36	8.47	8.43	8.26	8.33	8.33	8.47	8.52	8.47	7.64
144	9.60	9.76	9.57	9.43	9.45	9.36	9.46	9.54	9.56	9.70	9.75	9.81	9.68	9.54	9.72	9.83	9.84	9.86	9.77
168	9.68	9.62	6.64	9.66	9.86	9.75	9.62	9.87	9.75	9.91	10.37	10.09	10.13	10.25	9.91	10.23	10.20	10.06	9.96
192	9.83	9.79	9.61	9.54	9.77	9.80	9.91	9.85	9.79	9.78	9.90	9.89	9.99	10.07	10.03	9.65	9.70	9.51	9.60

Oil continued

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.91	2.91	2.95	2.87	2.89	2.99	3.02	3.11	2.40	2.56	2.34	2.52	2.54	2.62	2.30	2.28	2.32	3.10
24	3.72	3.61	3.81	3.76	3.63	3.76	3.69	3.45	4.56	4.56	4.15	4.51	4.42	4.42	4.58	4.48	4.60	4.17
48	4.87	4.77	5.02	5.02	4.85	4.86	4.72	4.81	5.43	5.42	5.62	5.51	5.71	5.62	5.59	5.67	5.61	5.49
72	5.95	5.85	5.70	5.72	5.93	5.72	5.75	5.94	6.03	5.95	5.93	6.09	6.06	5.97	6.16	6.43	6.18	6.29
96	7.31	7.52	7.39	7.53	7.19	7.25	7.18	7.20	7.20	7.22	7.23	7.24	7.24	7.27	7.16	7.13	7.12	7.75
120	7.72	7.73	7.75	7.62	7.85	7.72	7.76	7.82	7.43	7.48	7.54	7.65	7.63	7.69	7.56	7.58	7.53	9.79
144	8.96	8.87	8.86	8.78	8.76	8.79	8.62	8.83	9.10	9.13	9.16	9.09	9.06	9.12	9.22	9.09	9.17	9.36
168	9.81	9.85	9.81	9.96	10.01	9.70	9.75	9.89	10.08	10.16	10.13	10.16	10.22	10.20	10.22	10.10	10.13	9.43
192	9.32	9.64	9.52	9.58	9.42	9.38	9.28	9.46	9.72	9.92	9.69	10.06	9.97	10.03	9.60	9.88	9.51	9.45

Monolaurin

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.78	2.91	2.91	2.95	2.87	2.89	2.99	3.02	3.11	2.40	2.56	2.34	2.52	2.54	2.62	2.30	2.28	2.32
24	3.76	3.72	3.61	3.81	3.76	3.63	3.76	3.69	3.45	4.00	4.45	4.30	4.34	4.42	4.48	4.38	4.34	4.56
48	4.89	4.90	4.77	5.02	5.02	4.85	4.86	4.72	4.81	5.49	5.28	5.48	5.40	5.34	5.30	5.42	5.34	5.43
72	5.91	5.95	5.85	5.70	5.72	5.93	5.72	5.75	5.94	6.86	6.88	6.68	6.99	7.06	7.02	6.72	6.76	6.85
96	7.15	7.31	7.52	7.39	7.53	7.19	7.25	7.18	7.20	7.23	7.25	7.27	7.19	7.19	7.22	7.27	7.27	7.16
120	7.64	7.72	7.73	7.75	7.62	7.85	7.72	7.76	7.82	8.32	8.38	8.40	8.51	8.56	8.54	8.62	8.56	8.45
144	8.77	8.96	8.87	8.86	8.78	8.76	8.79	8.62	8.83	8.86	8.95	8.98	9.02	9.03	9.10	9.13	9.16	9.11
168	9.96	9.81	9.85	9.81	9.96	10.01	9.70	9.75	9.89	10.08	10.16	10.13	10.19	10.19	10.21	10.09	10.19	10.09
192	9.60	9.32	9.64	9.52	9.52	9.42	9.38	9.28	9.46	9.72	9.92	9.69	10.06	9.97	10.03	9.60	9.88	9.51

Lauric acid

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	3.06	3.06	3.03	3.03	3.02	3.12	3.09	3.06	3.06	2.86	2.78	2.81	2.85	2.81	2.68	2.68	2.60	2.86
24	3.81	3.82	3.71	3.71	3.81	3.79	3.74	3.72	3.75	2.88	2.92	2.86	3.12	3.11	3.12	3.16	3.25	3.20
48	4.44	4.28	4.39	4.46	4.33	4.51	4.57	4.52	4.49	3.76	3.76	3.62	3.83	4.01	3.97	4.12	4.11	4.05
72	4.84	4.76	4.84	4.65	4.69	4.76	4.63	4.76	4.71	4.89	5.06	4.91	4.50	4.54	4.52	5.03	4.90	4.86
96	5.72	5.63	5.52	5.77	5.81	5.76	5.91	5.96	5.96	5.82	5.85	5.62	5.50	5.51	5.47	5.72	5.78	5.68
120	6.62	6.73	6.72	6.68	6.75	6.76	6.72	6.66	6.62	6.36	6.43	6.43	6.11	6.12	5.98	6.25	6.30	6.31
144	6.96	7.01	7.02	7.21	7.20	7.16	7.34	7.33	7.33	6.61	6.62	6.69	6.28	6.54	6.45	6.74	6.79	6.79
168	7.62	7.72	7.76	7.64	7.76	7.72	7.62	7.88	7.68	6.99	6.76	6.78	6.76	6.72	6.73	7.08	6.92	7.13
192	7.71	7.56	7.62	7.76	7.78	7.72	8.04	8.12	8.05	7.22	7.24	7.26	7.10	7.05	7.13	7.28	7.24	7.24

Monolaurin and Lauric acid

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.78	2.91	2.91	2.95	2.87	2.89	2.99	3.02	3.11	2.40	2.56	2.34	2.52	2.54	2.62	2.30	2.28	2.32
24	2.94	3.00	2.91	2.99	2.85	2.87	2.94	2.64	2.76	2.48	2.51	2.56	2.48	2.72	2.56	2.48	2.72	2.89
48	3.40	3.82	3.34	3.62	3.32	3.58	3.30	3.28	3.32	2.88	2.96	2.96	2.91	2.82	2.84	2.82	2.81	2.81
72	3.97	3.88	4.02	3.64	3.70	3.62	3.20	3.38	3.45	3.91	3.92	3.95	4.01	4.04	4.05	3.88	3.86	3.83
96	4.55	4.39	4.45	4.35	4.16	4.09	3.89	3.86	3.70	4.09	4.10	4.11	4.13	4.13	4.14	4.06	4.02	4.05
120	4.86	5.04	4.88	4.86	4.53	4.67	4.49	4.54	4.34	4.64	4.75	4.76	4.76	4.65	4.71	4.65	4.62	4.66
144	5.67	5.68	5.59	5.16	5.30	5.15	4.95	4.95	4.98	4.85	4.89	4.90	4.92	4.95	4.91	4.90	4.94	4.93
168	6.05	6.07	6.09	5.68	5.82	5.86	5.08	5.04	5.13	5.64	5.62	5.72	5.13	4.99	5.01	4.83	4.72	4.81
192	6.99	7.10	6.93	6.60	6.61	6.91	5.32	5.36	5.46	5.75	5.70	5.72	6.00	5.96	5.88	5.48	5.59	5.59

(c) *Listeria monocytogenes* (LB)

Control

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.76	2.88	2.78	2.76	2.88	2.89	2.79	2.90	2.72	2.89	3.04	2.83	3.06	3.02	3.04	2.95	2.98	3.05
24	3.92	4.04	4.00	4.10	4.02	4.01	3.96	4.11	4.17	4.56	4.61	4.60	4.40	4.42	4.38	4.46	4.51	4.28
48	5.58	5.42	5.45	5.63	5.65	5.62	5.58	5.78	5.62	5.96	6.02	6.04	6.14	6.06	6.03	6.00	5.93	5.93
72	6.88	6.85	6.92	6.98	7.00	6.92	6.90	6.99	9.88	7.11	7.13	7.20	7.35	7.09	7.35	7.15	7.43	7.38
96	8.11	8.24	8.13	8.28	8.28	8.36	8.33	8.39	8.35	8.30	8.38	8.40	8.49	8.54	8.69	8.18	8.13	8.14
120	9.23	9.25	9.26	9.27	9.36	9.33	9.15	9.36	9.07	9.90	9.95	9.91	9.85	9.95	9.88	9.83	9.76	9.74
144	9.52	9.90	9.90	10.02	9.92	9.86	9.30	9.43	9.61	9.53	9.67	9.72	10.03	9.88	10.01	9.74	10.05	9.72
168	9.66	9.68	9.81	9.92	9.94	9.93	9.60	9.72	9.60	9.95	9.94	9.92	10.03	10.05	10.07	9.98	9.97	10.06
192	9.69	9.73	9.84	9.64	9.72	9.70	9.67	9.65	9.59	9.88	9.88	9.93	9.98	9.79	9.88	9.92	9.91	9.86

Oil

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.76	2.88	2.78	2.76	2.88	2.89	2.79	2.90	2.72	2.89	3.04	2.83	3.06	3.02	3.04	2.95	2.98	3.05
24	3.80	3.70	3.62	3.77	3.81	3.93	3.92	4.04	3.88	4.36	4.48	4.46	4.36	4.57	4.45	4.54	4.48	4.32
48	5.76	5.63	5.51	5.32	5.60	5.26	5.38	5.54	5.43	6.03	6.00	5.97	6.06	6.12	6.07	6.06	6.08	6.10
72	6.68	6.72	6.81	7.13	7.09	7.11	7.20	7.19	7.18	7.38	7.27	7.27	7.62	7.55	7.63	7.38	7.43	7.36
96	8.00	8.11	8.09	8.15	8.21	8.19	8.24	8.33	8.25	8.32	8.36	8.48	8.42	8.46	8.49	8.40	8.34	8.30
120	9.54	9.52	9.50	9.53	9.52	9.53	9.45	9.53	9.51	9.86	10.04	9.89	9.57	9.95	9.65	9.51	9.89	9.83
144	9.51	9.53	9.51	9.30	9.48	9.46	9.42	9.49	9.38	10.01	10.05	9.96	9.95	10.00	10.01	9.68	9.63	9.57
168	10.05	9.96	9.93	9.62	9.60	9.62	9.68	9.75	9.75	9.82	9.85	9.97	9.95	9.96	10.04	10.09	10.09	10.14
192	9.85	9.76	9.79	9.23	9.20	9.30	9.28	9.34	9.42	9.98	9.92	9.99	9.80	9.81	9.83	9.94	9.86	9.88

Oil continued

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.94	2.88	2.82	2.75	2.96	2.82	3.06	2.92	2.82	2.85	2.91	2.94	2.76	2.58	2.66	2.94	2.82	2.75
24	3.68	3.74	3.73	3.80	3.90	3.92	3.96	3.90	3.92	3.99	4.01	3.94	4.01	4.05	3.83	3.98	3.89	3.90
48	5.89	5.91	5.93	5.64	5.65	5.69	6.00	6.03	6.08	5.72	5.76	5.65	5.60	5.69	5.62	5.52	5.72	5.73
72	7.10	6.88	6.85	6.75	7.03	6.95	7.16	7.20	7.25	7.40	7.40	7.39	7.09	7.19	7.18	7.15	7.31	7.26
96	8.52	8.60	8.52	8.38	8.33	8.40	8.42	8.44	8.41	9.01	9.04	9.04	8.89	8.91	8.95	8.74	8.81	8.79
120	9.20	9.19	9.18	9.24	9.23	9.26	9.34	9.38	9.27	9.79	9.81	9.83	9.64	9.69	9.68	9.58	9.46	9.45
144	10.12	10.07	10.07	9.99	9.89	10.06	10.25	10.22	10.23	9.73	9.73	9.67	9.86	9.95	9.89	9.73	9.72	9.77
168	9.96	9.85	9.82	9.98	9.86	10.04	9.94	9.88	9.92	9.76	9.78	9.62	9.73	9.68	9.73	9.88	9.89	9.87
192	9.80	9.97	9.94	9.79	9.82	9.83	9.87	9.96	9.97	9.79	9.88	9.77	9.72	9.64	9.57	9.86	9.75	9.88

Monolaurin

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.94	2.88	2.82	2.75	2.96	2.82	3.06	2.92	2.82	2.85	2.91	2.94	2.76	2.58	2.66	2.94	2.82	2.75
24	3.73	3.76	3.60	3.83	3.85	3.83	4.03	4.06	4.09	4.05	4.01	4.00	3.88	4.01	3.78	3.80	3.88	3.98
48	5.66	5.62	5.68	5.77	5.74	5.76	5.79	5.88	5.91	5.60	5.60	5.72	5.43	5.46	5.58	5.69	5.60	5.38
72	7.16	7.12	7.13	7.09	7.11	7.06	7.23	7.30	7.24	7.34	7.42	7.37	7.23	7.29	7.24	7.26	7.36	7.36
96	8.39	8.39	8.38	8.51	8.66	8.58	8.34	8.38	8.47	9.09	8.97	8.95	8.74	8.75	8.67	8.51	8.64	8.45
120	9.20	9.26	9.24	9.07	9.19	9.16	9.38	9.35	9.36	9.48	9.54	9.64	9.46	9.28	9.23	9.34	9.32	9.42
144	10.39	10.57	10.45	10.63	10.60	10.58	10.53	10.52	10.46	9.76	9.74	9.63	9.54	9.66	9.46	9.65	9.63	9.67
168	9.85	9.88	9.91	10.04	9.99	9.92	9.82	9.79	9.86	9.53	9.30	9.20	9.58	9.53	9.68	9.86	9.93	9.82
192	9.61	9.56	9.63	9.60	9.58	9.63	9.77	9.80	9.83	9.48	9.36	9.48	9.63	9.62	9.48	9.72	9.60	9.57

Lauric acid

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.76	2.88	2.78	2.76	2.88	2.89	2.79	2.90	2.72	2.89	3.04	2.83	3.06	3.02	3.04	2.95	2.98	3.05
24	2.85	2.85	2.89	2.96	3.05	2.98	2.83	2.97	2.91	3.60	3.70	3.66	3.57	3.54	3.49	3.66	3.43	3.42
48	3.60	3.63	3.62	3.61	3.69	3.62	3.63	3.64	3.67	4.76	4.76	4.61	4.76	4.70	4.61	4.49	4.38	4.40
72	3.90	4.38	4.42	4.28	4.31	4.37	4.22	4.22	4.30	5.36	5.22	5.29	5.11	5.00	5.03	5.12	5.18	5.08
96	5.13	5.06	5.11	5.00	5.04	5.17	5.13	5.06	4.97	6.31	6.17	6.27	6.13	5.97	6.04	5.92	5.87	5.73
120	5.59	5.57	5.66	5.74	5.62	5.56	5.32	5.46	5.54	6.74	6.66	6.67	6.37	6.38	6.43	7.50	6.50	6.48
144	6.35	6.37	6.35	6.22	6.10	6.37	6.27	6.29	6.32	7.05	7.04	6.99	7.04	7.16	7.08	7.16	7.24	7.20
168	6.98	7.08	6.93	6.91	6.85	6.88	6.88	6.90	6.95	7.23	7.25	7.27	7.18	7.16	7.17	7.33	7.34	7.34
192	7.26	7.14	7.14	6.93	7.00	6.92	7.08	7.16	7.08	8.03	8.09	8.09	7.92	7.85	7.84	8.09	7.98	7.90

Monolaurin and Lauric acid

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.94	2.88	2.82	2.75	2.96	2.82	3.06	2.92	2.82	2.85	2.91	2.94	2.76	2.58	2.66	2.94	2.82	2.75
24	2.89	2.76	2.89	2.74	2.94	2.69	2.75	2.77	2.79	2.84	2.79	2.69	2.72	2.62	2.72	2.67	2.84	2.72
48	2.74	2.65	2.79	2.54	2.75	2.68	2.72	2.68	2.78	2.72	2.72	2.66	2.59	2.74	2.67	2.67	2.56	2.66
72	2.60	2.59	2.45	2.49	2.28	2.40	2.64	2.65	2.71	2.45	2.51	2.60	2.49	2.58	2.64	2.52	2.45	2.52
96	2.45	2.62	2.70	2.38	2.08	2.42	2.58	2.42	2.51	2.77	2.60	2.52	2.32	2.26	2.54	2.30	2.43	2.51
120	2.40	2.48	2.45	2.28	2.46	2.32	2.51	2.34	2.52	2.93	2.97	2.94	2.32	2.28	2.48	2.28	2.30	2.42
144	2.46	2.28	2.46	2.11	2.40	2.26	2.38	2.40	2.48	3.42	3.63	3.54	2.34	2.18	2.42	2.08	2.26	2.23
168	2.30	2.36	2.49	2.40	2.32	2.30	2.34	2.34	2.34	3.94	3.98	4.05	2.45	2.63	2.59	2.38	2.40	2.90
192	2.36	2.15	2.04	2.57	2.63	2.20	2.26	2.38	2.20	4.31	4.50	4.56	2.93	2.90	3.15	2.49	2.36	2.57

(d) *Listeria innocua* (A7)

Control

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.70	2.30	2.70	2.48	2.60	2.95	2.78	2.70	3.04	2.85	2.88	2.89	2.90	2.91	2.91	2.79	2.83	2.81
24	4.23	4.25	4.26							4.06	4.05	4.16	4.05	4.05	4.00	4.23	4.25	4.23
48	5.79	5.85	5.79							5.68	5.70	5.72	5.56	5.60	5.66	5.56	5.62	5.61
72	7.22	7.23	7.76							7.14	7.20	7.19	7.23	7.23	7.22	7.08	7.25	7.26
96	7.78	7.83	7.79							8.83	8.73	8.79	8.14	8.27	8.25	9.03	9.03	9.04
120	9.96	9.96	10.00							9.72	9.86	9.73	9.91	9.93	9.94	9.73	9.76	9.79
144	9.16	9.18	9.71							9.87	9.94	9.92	10.03	10.13	10.12	9.88	9.99	9.95
168	NT	NT	NT							10.00	9.88	9.92	9.88	9.83	9.79	9.97	10.04	10.07
192	10.12	10.10	10.12							9.95	9.96	9.98	10.00	10.09	10.06	9.86	9.93	9.96

Oil

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.70	2.30	2.70	2.48	2.60	2.95	2.78	2.70	3.04	2.85	2.88	2.89	2.90	2.91	2.91	2.79	2.83	2.81
24	6.27	6.28	6.27	5.97	5.98	5.98	6.00	6.00	6.00	4.19	4.14	4.15	3.98	4.00	4.07	4.04	4.02	4.02
48	5.53	5.64	5.51	5.87	5.76	6.04	5.65	5.85	5.66	5.89	5.84	5.79	5.27	5.30	5.35	5.33	5.28	5.36
72	7.14	7.10	7.05	7.19	7.23	7.26	7.13	7.19	7.15	7.08	7.08	7.11	7.19	7.20	7.21	7.19	7.21	7.20
96	8.59	8.60	8.57	8.75	8.66	8.71	8.63	8.59	8.67	8.35	8.38	8.37	8.36	8.28	8.71	8.11	8.00	8.05
120	8.90	8.97	8.98	8.89	8.90	8.88	8.88	8.86	8.87	9.99	9.93	10.01	9.88	9.85	9.85	9.95	10.00	10.06
144	9.73	9.87	9.86	9.77	9.75	9.65	9.58	9.54	9.60	9.62	9.68	9.65	9.90	9.90	9.90	9.81	9.77	9.81
168										9.91	9.87	9.97	9.95	9.89	9.94	9.91	9.85	9.81
192	10.25	10.29	10.26	10.16	10.18	10.21	10.12	10.10	10.12	10.00	9.97	10.05	9.86	9.84	9.86	9.90	9.91	9.85

Oil continued

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.53	2.61	2.65	2.59	2.60	2.58	2.53	2.53	2.63	2.75	2.77	2.89	2.73	2.77	2.80	2.63	2.79	2.83
24	3.70	3.85	3.79	3.81	3.72	3.61	3.80	3.33	3.73	4.19	4.25	4.22	4.23	4.20	4.20	4.48	4.47	4.42
48	5.18	5.21	5.15	5.27	5.29	5.28	5.26	5.26	5.21	5.36	5.43	5.28	5.38	5.52	5.40	5.52	5.53	5.57
72	6.83	6.84	6.76	7.03	6.85	6.93	6.79	6.65	6.57	6.86	6.83	6.88	6.91	6.92	6.87	6.99	6.91	6.92
96	8.06	8.23	8.11	8.21	8.19	8.14	8.11	8.09	8.27	8.22	8.26	8.21	8.24	8.25	8.18	8.26	8.27	8.27
120	9.56	9.45	9.61	9.76	9.62	9.64	9.81	9.73	9.69	9.73	9.19	9.86	9.54	9.66	9.45	9.40	9.46	9.56
144	10.13	10.06	10.02	9.90	9.90	9.74	9.79	9.68	9.60	9.97	10.03	10.05	9.85	9.89	9.91	10.12	10.23	10.22
168	9.58	9.48	9.65	9.34	9.64	9.49	9.53	9.75	9.82	9.82	9.81	9.86	10.08	10.08	10.11	10.06	10.10	10.01
192	9.57	9.61	9.64	9.48	9.68	9.63	9.62	9.83	9.96	9.99	9.95	9.95	9.86	9.90	9.81	10.02	10.09	9.94

Monolaurin

Time (h)	Viable count(count Log_{10} cfu mL^{-1})																	
0	2.61	2.65	2.59	2.60	2.58	2.53	2.53	2.63	2.75	2.77	2.89	2.73	2.77	2.80	2.63	2.79	2.83	3.10
24	3.67	3.46	3.67	3.71	3.63	3.64	3.61	3.68	4.19	4.20	4.20	4.26	4.24	4.19	4.21	4.30	4.26	4.11
48	5.02	5.01	4.99	4.93	5.01	5.01	4.98	5.03	5.60	5.54	5.54	5.57	5.59	5.52	5.56	5.59	5.48	5.69
72	6.47	6.50	6.47	6.48	6.47	6.32	6.48	6.49	6.87	7.02	7.03	6.90	6.95	6.89	6.81	6.86	6.76	6.59
96	8.45	8.50	8.16	8.30	8.27	8.19	8.23	8.28	8.28	8.26	8.32	8.34	8.40	8.36	8.48	8.43	8.42	7.91
120	9.24	9.20	8.78	8.75	8.77	9.10	9.24	9.32	9.11	9.14	9.21	9.33	9.32	9.31	9.29	9.30	9.36	9.40
144	10.20	10.19	9.94	10.01	9.96	9.60	9.75	9.88	9.83	9.84	9.76	9.95	10.01	9.94	10.08	10.00	10.02	9.56
168	9.89	9.80	9.65	9.72	9.43	9.81	9.92	9.96	10.15	10.15	10.08	9.80	9.89	9.81	10.07	10.06	10.01	10.20
192	10.03	10.04	9.72	9.83	9.75	10.10	10.06	10.10	10.15	10.14	10.08	10.06	10.10	10.12	10.10	10.07	10.05	9.56

Lauric acid

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.70	2.30	2.70	2.48	2.60	2.95	2.78	2.70	3.04	2.85	2.88	2.89	2.90	2.91	2.91	2.79	2.83	2.81
24	3.30	3.66	3.20	2.90	3.08	3.00	3.42	3.51	3.34	3.34	3.34	3.39	3.36	3.39	3.34	3.39	3.37	3.39
48	3.90	3.72	3.72	3.32	3.08	3.38	3.80	3.89	3.90	3.96	3.92	3.95	3.90	3.98	3.97	3.95	4.03	3.98
72	4.21	4.31	4.16	4.35	4.39	4.37	4.33	4.25	4.35	4.15	4.18	4.19	4.31	4.32	4.31	4.35	4.36	4.35
96	4.81	4.82	4.79	4.73	4.72	4.70	4.65	4.60	4.67	4.63	4.80	4.75	4.75	4.69	4.75	4.64	4.62	4.72
120	5.66	5.67	5.72	5.30	5.32	5.36	5.63	5.57	5.53	4.91	4.87	4.91	5.08	5.06	5.09	5.08	5.11	5.20
144	6.08	6.08	6.08	6.31	6.26	6.26	6.18	6.21	6.20	5.00	5.03	5.01	5.43	5.39	5.36	5.61	5.43	5.68
168										5.96	6.01	5.99	6.02	6.12	6.08	5.76	5.76	5.80
192	6.34	6.36	6.34	6.54	6.56	6.59	6.67	6.63	6.58	7.08	7.09	7.13	7.15	6.20	7.26	7.21	7.24	7.20

Monolaurin and Lauric acid

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	2.53	2.61	2.65	2.59	2.60	2.58	2.53	2.53	2.63	2.75	2.77	2.89	2.73	2.77	2.80	2.63	2.79	2.83
24	2.72	2.78	2.69	2.79	2.82	2.69	2.75	2.75	2.72	3.08	3.08	3.10	2.97	3.02	3.02	3.00	2.99	2.88
48	3.10	3.11	3.10	3.08	3.13	3.03	3.06	3.13	3.07	3.61	3.72	3.63	3.62	3.76	3.70	3.23	3.42	3.32
72	3.71	3.76	3.79	3.67	3.79	3.58	3.63	3.62	3.59	3.66	3.76	3.75	4.13	4.07	4.00	3.66	3.66	3.66
96	3.96	3.90	4.03	3.92	3.95	4.10	3.89	3.87	4.08	4.31	4.43	4.28	4.32	4.38	4.47	3.75	3.74	3.63
120	4.10	4.04	4.01	4.24	4.23	4.27	4.23	4.24	3.96	4.52	4.43	4.43	4.30	4.32	4.23	3.86	3.92	3.87
144	4.20	4.24	4.16	4.49	4.69	4.54	4.54	4.51	4.51	4.30	4.52	4.48	4.43	4.32	4.28	4.32	4.42	4.43
168	4.76	4.23	4.26	4.65	4.51	4.32	4.53	4.52	4.30	4.30	4.34	4.34	4.32	4.34	4.36	4.42	4.46	4.40
192	5.28	5.23	5.25	4.86	4.86	4.92	4.15	4.97	4.94	4.52	4.36	4.54	4.72	4.76	4.72	4.83	4.79	4.78

(e) *Listeria innocua* (NCTC 11288))

Control

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	3.01	3.00	2.90	2.94	2.87	3.06	2.87	2.87	3.04	3.06	2.99	2.97	2.85	3.04	2.97	2.95	2.86	2.97
24	3.93	3.97	3.86	3.96	3.93	3.92	3.97	4.00	3.90	3.45	3.49	3.43	3.54	3.53	3.46	3.56	3.28	3.20
48	5.45	5.38	5.43	5.28	5.51	5.45	5.46	5.62	5.63	4.88	4.86	4.83	4.85	4.72	4.83	4.74	4.76	4.68
72	6.91	6.88	7.08	6.73	6.87	6.88	6.94	6.87	6.88	6.75	6.76	6.62	6.56	6.40	6.28	6.26	6.30	6.54
96	8.88	8.89	8.93	8.81	8.81	8.73	8.89	8.86	8.83	7.79	7.91	7.84	7.53	7.48	7.40	7.34	7.52	7.63
120	9.45	9.48	9.49	9.51	9.58	9.59	9.65	9.69	9.60	9.18	9.13	9.21	8.95	9.06	9.09	8.92	9.02	8.87
144	10.37	10.44	10.37	10.10	10.16	10.16	10.26	10.36	10.38	9.98	10.01	10.06	9.91	9.93	9.88	9.84	9.73	9.67
168	10.26	10.36	10.36	10.29	10.16	10.22	10.26	10.16	10.19	10.00	9.95	9.83	10.16	10.13	10.08	9.76	9.70	9.62
192	9.86	9.89	9.73	10.17	10.05	10.13	10.09	10.08	10.07	9.76	9.71	9.79	10.02	10.00	10.08	9.92	9.88	9.98

Oil

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	3.01	3.00	2.90	2.94	2.87	3.06	2.87	2.87	3.04	3.06	2.99	2.97	2.85	3.04	2.97	2.95	2.86	2.97
24	3.98	4.12	4.04	4.03	4.11	4.01	4.02	4.05	4.08	3.38	3.38	3.36	3.51	3.54	3.58	3.53	3.30	3.49
48	5.26	5.31	5.21	5.36	5.45	5.30	5.40	5.42	5.38	4.73	4.61	4.65	4.76	4.74	4.81	4.53	4.73	4.58
72	6.64	6.72	6.81	6.89	6.89	7.06	6.90	6.97	6.86	6.68	6.59	6.32	6.75	6.54	6.62	6.54	6.45	6.28
96	8.54	8.58	8.64	8.67	8.68	8.74	8.81	8.68	8.54	7.72	7.76	7.72	7.60	7.76	7.58	7.62	7.76	7.73
120	9.86	9.95	9.82	9.62	9.62	9.66	9.73	9.76	9.73	9.1 82	9.2 25	9.32	8.97	9.10	9.25	8.95	9.06	9.03
144	10.09	10.08	9.91	10.02	10.11	10.09	9.82	9.92	9.90	10.08	10.10	10.10	9.98	9.91	9.86	9.77	9.83	9.89
168	10.16	10.22	10.22	10.27	10.23	10.22	9.95	9.86	9.92	10.19	10.10	10.27	10.04	10.06	9.98	9.85	10.03	10.06
192	10.09	10.12	10.15	9.87	10.00	10.00	9.98	9.96	9.94	10.36	10.32	10.38	10.29	10.27	10.30	10.21	10.24	10.17

Oil continued

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	3.16	3.15	3.09	3.07	3.10	3.11	3.11	3.09	3.01	3.22	3.15	3.24	3.18	3.23	3.16	3.06	3.16	3.15
24	4.98	4.96	4.93	5.02	5.03	5.07	5.11	5.13	5.15	4.03	4.08	4.09	3.94	3.99	4.06	4.09	4.08	4.01
48	5.88	5.90	5.62	5.94	5.94	6.00	5.94	5.98	6.06	5.62	5.54	5.52	5.31	5.27	5.26	5.11	5.28	5.27
72	6.93	6.86	6.88	6.95	6.91	6.93	6.98	6.96	6.98	7.14	7.11	7.05	6.98	6.92	6.94	7.02	6.96	6.91
96	8.79	8.84	8.83	8.88	8.80	8.66	9.06	8.96	8.92	8.72	8.68	8.69	8.34	8.32	8.41	8.40	8.50	8.44
120	10.11	10.13	10.19	10.23	10.23	10.21	10.29	10.28	10.27	9.49	9.62	9.56	8.45	8.30	8.48	9.08	9.12	9.20
144	9.28	9.40	9.38	9.52	9.49	9.46	9.56	9.65	9.43	9.66	9.81	9.66	9.64	9.72	9.75	9.76	9.78	9.79
168	9.73	9.73	9.85	10.26	10.18	10.24	10.27	10.33	10.33	9.81	9.87	9.68	9.53	9.57	9.55	9.46	9.45	9.51
192	9.93	9.88	9.91	9.95	9.98	10.02	10.11	10.08	10.06	10.06	10.10	10.03	9.65	9.76	9.79	9.85	9.93	9.80

Monolaurin

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	3.16	3.15	3.09	3.07	3.10	3.11	3.11	3.09	3.01	3.22	3.15	3.24	3.18	3.23	3.16	3.06	3.16	3.15
24	4.75	4.76	4.81	4.89	4.87	4.86	4.93	4.91	4.93	3.86	3.89	3.82	3.89	3.85	3.89	3.82	3.89	3.88
48	5.61	5.60	5.56	5.68	5.84	5.81	5.89	6.03	6.00	5.86	5.78	5.72	5.96	6.00	5.93	4.60	4.53	4.64
72	6.86	6.88	6.89	6.76	6.77	6.62	6.54	6.46	6.38	7.10	7.13	7.03	6.89	6.86	6.81	6.91	6.90	6.79
96	9.01	9.09	9.06	8.83	8.76	8.71	8.64	8.63	8.79	8.65	8.62	8.61	8.33	8.28	8.25	8.37	8.27	8.25
120	9.95	9.98	9.85	10.04	10.07	10.06	9.70	9.76	9.79	9.34	9.42	9.37	9.10	9.02	9.06	9.21	9.25	9.23
144	9.94	9.93	9.86	9.84	9.81	9.72	9.76	9.64	9.69	9.83	9.82	9.83	10.37	10.37	10.36	10.20	10.21	10.35
168	10.13	10.05	10.03	10.04	10.05	9.97	10.23	10.19	10.22	9.78	9.60	9.82	9.33	9.47	9.51	9.33	9.38	9.34
192	9.99	9.98	9.98	9.92	9.89	9.86	9.91	9.93	9.92	9.99	10.03	10.10	9.84	9.88	9.80	9.74	9.79	9.88

Lauric acid

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	3.01	3.00	2.90	2.94	2.87	3.06	2.87	2.87	3.04	3.06	2.99	2.97	2.85	3.04	2.97	2.95	2.86	2.97
24	3.46	3.40	3.48	3.25	3.16	3.10	3.43	3.48	3.43	2.88	2.99	2.94	3.01	2.91	2.94	2.90	2.98	2.91
48	4.49	4.48	4.61	4.31	4.20	4.21	4.67	4.48	4.67	3.23	3.23	3.38	3.54	3.52	3.48	3.57	3.48	3.34
72	5.23	5.32	5.37	4.89	4.87	5.01	5.46	5.38	5.51	3.99	4.03	4.06	3.86	3.93	3.96	4.02	4.07	4.08
96	5.64	5.69	5.71	5.02	5.03	5.08	6.18	6.16	6.20	5.26	5.36	5.45	5.73	5.70	5.63	5.42	5.32	5.45
120	6.83	6.80	6.90	5.81	5.88	5.81	7.11	7.10	7.08	6.38	6.27	6.17	6.48	6.29	6.33	6.29	6.33	6.11
144	7.02	7.10	7.06	6.33	6.37	6.39	7.64	7.59	7.59	7.00	6.97	6.93	6.89	7.01	7.06	7.15	7.01	6.96
168	7.89	7.92	7.89	7.24	7.18	7.20	8.09	8.24	8.25	7.67	7.68	7.59	7.64	7.63	7.51	7.45	7.54	7.55
192	7.28	7.53	7.36	7.19	7.03	7.05	7.67	7.76	7.61	7.86	7.86	7.94	7.98	7.95	7.91	7.76	7.88	7.83

Monolaurin and Lauric acid

Time (h)	Viable count (count Log_{10} cfu mL^{-1})																	
0	3.16	3.15	3.09	3.07	3.10	3.11	3.11	3.09	3.01	3.22	3.15	3.24	3.18	3.23	3.16	3.06	3.16	3.15
24	2.74	2.77	2.80	2.82	2.83	2.86	2.89	2.90	2.81	3.13	3.19	3.17	3.12	3.14	3.16	3.20	3.10	3.16
48	2.69	2.85	2.74	2.86	2.83	2.92	2.93	2.96	2.94	3.38	3.40	3.36	3.69	3.76	3.75	3.16	3.25	3.21
72	2.89	2.93	2.79	3.00	2.96	2.93	2.95	2.74	2.91	3.47	3.50	3.53	3.66	3.67	3.67	3.59	3.56	3.56
96	2.96	2.89	2.68	3.13	3.05	3.10	2.98	2.81	2.92	4.09	4.09	4.12	4.75	4.75	4.77	4.31	4.30	4.31
120	2.92	2.99	3.01	3.40	3.43	3.46	3.09	3.13	3.05	4.45	4.63	4.62	5.10	5.05	5.14	4.78	4.61	4.77
144	3.00	3.04	3.11	3.95	3.98	3.98	3.74	3.81	3.69	5.19	5.09	5.05	5.47	5.53	5.54	5.15	5.05	5.09
168	4.40	4.49	4.45	5.42	5.37	5.35	4.66	4.88	4.73	5.54	5.51	5.47	6.11	6.02	5.99	5.44	5.37	5.44
192	4.92	4.87	4.91	5.55	5.54	5.49	5.19	5.02	5.12	5.56	5.34	5.49	6.24	6.27	6.29	5.65	5.51	5.57

2.2 Effect of monolaurin, lauric acid and extending drainage time on *Listeria innocua* in Camembert-type cheese, ripened 10 °C, 95 % erh

(a) Monolaurin and lauric acid on *L. innocua* (A7)

Time (d)	Viable count (Log_{10} cfu mL^{-1}) Control				Viable count (Log_{10} cfu mL^{-1}) monolaurin and lauric acid			
	Surface	SD	Centre	SD	Surface	SD	Centre	SD
-3	2.58	0.28	2.58	0.28	2.86	0.14	2.86	0.14
0	5.53	0.1	5.31	0.07	4.87	0.37	4.87	0.36
7	3.68	0.13	3.86	0.67	4.4	0.04	3.75	0.13
14	3.64	0.26	3.37	1.4	3.3	0.17	3.11	1.28
21	4.42	0.61	3.15	0.38	3.79	0.14	3.44	0.28
28	5.81	0.7	3.09	1.6	1.54	0.21	0.33	0.82

(b) Monolaurin on *L. innocua* (A7)

Time (d)	Viable count (Log_{10} cfu mL^{-1}) Control				Viable count (Log_{10} cfu mL^{-1}) Monolaurin			
	Surface	SD	Centre	SD	Surface	SD	Centre	SD
-3	3.30	0.4	3.30	0.4	3.15	0.06	3.15	0.06
0	5.10	1.2	4.76	1.8	4.68	0.78	4.34	0.31
7	5.77	1.6	4.07	1.4	4.63	0.15	4.01	0.17
14	4.50	0.37	4.04	0.65	4.33	0.36	3.78	0.19
21	5.75	0.29	4.93	0.35	5.41	0.45	4.57	0.13
28	7.1	0.56	5.14	0.53	6.25	0.83	3.41	0.24

(c) Monolaurin on *L. innocua* (NCTC 11288)

Time (d)	Viable count (Log_{10} cfu mL^{-1}) Control				Viable count (Log_{10} cfu mL^{-1}) Monolaurin			
	Surface	SD	Centre	SD	Surface	SD	Centre	SD
-3	3.23	0.23	3.23	0.23	2.56	0.09	2.56	0.09
0	5.34	1.63	5.32	0.33	5.22	0.41	4.18	0.16
7	5.81	1.10	5.20	0.35	5.26	0.66	4.50	0.09
14	5.60	0.68	4.93	0.42	5.39	0.43	5.08	0.06
21	6.05	0.51	5.57	0.55	5.63	0.51	4.99	0.08
28	7.44	0.37	5.95	0.65	6.21	0.63	4.77	0.1

(d) Monolaurin and extended drainage time (24 h) on *L. innocua* (A7)

Time (d)	Viable count (Log_{10} cfu mL^{-1}) Control				Viable count (Log_{10} cfu mL^{-1}) Monolaurin			
	Surface	SD	Centre	SD	Surface	SD	Centre	SD
-4	3.96	0.12	3.96	0.12	3.81	0.08	3.81	0.08
0	4.68	0.08	4.56	0.13	4.99	0.31	3.13	0.58
7	4.63	0.23	4.47	0.39	4.14	0.11	2.52	1.34
14	4.33	0.38	4.04	0.25	3.82	0.75	2.45	0.48
21	5.41	0.02	3.38	0.28	5.43	0.1	3.44	0.06
28	6.25	0.03	3.38	1.66	0	0	0	0

(e) Monolaurin and extended drainage time (24 h) on *L. innocua* (NCTC 11288)

Time (d)	Viable count (Log_{10} cfu mL^{-1}) Control				Viable count (Log_{10} cfu mL^{-1}) Monolaurin and 24 h			
	Surface	SD	Centre	SD	Surface	SD	Centre	SD
-4	3.15	0.1	3.15	0.1	3.39	0.15	3.39	0.15
0	4.68	0.05	4.56	0.12	4.83	0.21	3.13	0.09
7	4.63	0.23	4.47	0.37	4.25	0.13	2.52	0.32
14	4.33	0.57	4.04	0.43	3.69	0.67	2.45	0.58
21	5.41	0.61	3.38	0.02	4.64	0.35	3.43	0.31
28	6.25	1.05	3.38	1.18	0	0	0	0

LFM - COSHH FORM

COSHH REF NO

ASSESSMENT OF HEALTH RISK ASSOCIATED WITH PROPOSED PROCEDURE

Personnel Involved

PAULINE BURGESS

Title of Experiment/Procedure ADDITION OF DODECANOIC ACID TO BUTTER OIL.

Aim: TO TEST THE EFFECT DODECANOIC ACID HAS ON LISTERIA MONOCYTOGENES AND E.COLI

Brief Description of Procedure: ACCURATELY WEIGH 2 0.05 g INTO 25 g OF STERILE BUTTER OIL

Substances used:	Hazards identified:
DODECANOIC ACID	NO SIGNIFICANT HAZARD IF USED IN LARGE QUANTITIES CAN BE AN IRRITANT.

Information sources: MANUFACTURERS CATALOGUE

Is there a less hazardous substance? NO
If so, why not use it?

Control Measures to be adopted WASH WITH WATER AND DETERGENT. WEAR GLOVES

Required checks and their frequency, on the adequacy and maintenance of control measures during the course of the experiment: NONE

Disposal procedures during and at end of experiment: N/A ALL USED

Name of Assessor	Name of Supervisor (for students only)	Head of School, or Nominee
PAULINE BURGESS	[illegible]	
Status of Assessor: POSTGRAD STUDENT		
Date 20/2/16	Date 20/2/16	Date
Signed P A BURGESS	Signed [illegible]	Signed

2

EMERGENCY PROCEDURES

If any of the substances or procedures identified overleaf is likely to pose a special hazard in an emergency, then identify below the action to be taken.

NONE

Spillage/uncontrolled release:

N/A

Fire.

N/A

If personnel are affected (fume contamination, etc) treatment to be adopted:

N/A

COMPLACENCE WITH THE ABOVE PRECAUTIONARY MEASURES WILL ENSURE HAZARD ASSOCIATED RISKS ARE MINIMISED

Anyone other than the assessor involved in this procedure should sign the statement below

I have read this document and understand it.

(Signed) ... (Date)

ASSESSMENT OF HEALTH RISK ASSOCIATED WITH PROPOSED PROCEDURE

Personnel Involved:

PAULINE BURGESS

Title of Experiment/Procedure: THE EFFECT OF NISIN AND LACTIC ACID ON LISTERIA MONOCYTOGENES AND INNOCUA

Aim: TO TEST THE ABOVE

Brief Description of Procedure: GROWTH OF LISTERIA MONOCYTOGENES AND INNOCUA IN A LIQUID BROTH MEDIA AND ON AGAR PLATES

Substances used:	Hazards identified:
LISTERIA INNOCUA	HAZARD GROUP 1
LISTERIA MONOCYTOGENES	HAZARD GROUP 2

Information sources: ADVISORY COMMITTEE ON DANGEROUS PATHOGENS. CATEGORISATION OF PATHOGENS ACCORDING TO HAZARD AND CATEGORIES OF CONTAINMENT 2ND ED

Is there a less hazardous substance? NO
If so, why not use it?

Control Measures to be adopted GENERAL ASEPTIC TECHNIQUE. SUITABLE PROTECTIVE CLOTHING. WHEN USING DO NOT EAT OR DRINK

Required checks and their frequency, on the adequacy and maintenance of control measures during the course of the experiment:

Disposal procedures during and at end of experiment: TO HAZARDOUS WASTE COLLECTION POINT. TO STERILISE

Name of Assessor:	Name of Supervisor: (for students only)	Head of School, or Nominee:
PAULINE BURGESS	J L KINGHORN	
Status of Assessor: RESEARCH STUDENT		
Date 20.2.96	Date 21/2/96	Date
Signed P A BURGESS	Signed J L Kinghorn	Signed

2

EMERGENCY PROCEDURES
If any of the substances or procedures identified overleaf is likely to pose a special hazard in an emergency, then identify below the action to be taken. N/A (ONLY SMALL QUANTITY USED)
Spillage/uncontrolled release: N/A
Fire N/A
If personnel are affected (fume, contamination, etc) treatment to be adopted: WASH WITH WATER AND DETERGENT

COMPLACENCE WITH THE ABOVE PRECAUTIONARY MEASURES WILL ENSURE HAZARD ASSOCIATED RISKS ARE MINIMISED

Anyone other than the assessor involved in this procedure should sign the statement below

I have read this document and understand it.

(Signed) ..... (Date) .....

LFM - COSHH FORM

COSHH REF NO . . . . .

ASSESSMENT OF HEALTH RISK ASSOCIATED WITH PROPOSED PROCEDURE

Personnel Involved:

PAULINE BURGESS

Title of Experiment/Procedure: THE EFFECT OF MONOLAURIN ON LISTERIA MONOCYTOGENES AND L. INNOCUA

Aim:

TO TEST THE ABOVE

Brief Description of Procedure: ACCURATELY WEIGH 0.02g OF 1 MONOLAUROYL-rac-GLYCEROL INTO BUTTER OIL

Substances used:	Hazards identified:
1 MONOLAUROYL-rac-GLYCEROL	NO SIGNIFICANT HAZARD (IF USED IN LARGE QUANTITIES IS AN IRRITANT)

Information sources:

MANUFACTURER'S CATALOGUE

Is there a less hazardous substance? NO
If so, why not use it?

Control Measures to be adopted: WEAR GLOVES
WASH WITH WATER AND DETERGENT

Required checks and their frequency, on the adequacy and maintenance of control measures during the course of the experiment: NONE

Disposal procedures during and at end of experiment: N/A ALL USED

Name of Assessor:	Name of Supervisor: (for students only)	Head of School, or Nominee:
PAULINE BURGESS	J. L. KINDERLERER	
Status of Assessor:		
RESEARCH STUDENT		
Date 20.2.96	Date 21/2/96	Date
Signed P A BURGESS	Signed J. L. Kinderlerer	Signed

2

EMERGENCY PROCEDURES

If any of the substances or procedures identified overleaf is likely to pose a special hazard in an emergency, then identify below the action to be taken

WASH WITH SOAP AND WATER. CLEAN CONTAMINATED OBJECTS WITH LYSOL. DISPOSE OF CONTAMINATED MATERIAL BY AUTOCLAVE

Spillage/uncontrolled release: COVER WITH BIOLOGICAL ABSORBING POWDER FOR 30 MINS, BRUSH UP AND AUTOCLAVE

Fire

N/A

If personnel are affected (fume, contamination, etc) treatment to be adopted

SEEK MEDICAL ADVICE

COMPLACENCE WITH THE ABOVE PRECAUTIONARY MEASURES WILL ENSURE HAZARD ASSOCIATED RISKS ARE MINIMISED

Anyone other than the assessor involved in this procedure should sign the statement below

I have read this document and understand it.

(Signed) ..... (Date)

APPENDIX 4

Effect of lauric acid and monolaurin on growth of cheeseborne strains of *Listeria monocytogenes* and *Listeria innocua*

Introduction

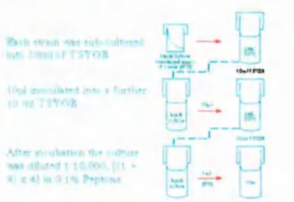
Listeria monocytogenes is recognised as a foodborne pathogen in diary products, particularly those made with unpasteurised milk[1, 7]. *Listeria monocytogenes* can multiply in soft cheeses ripened with *Penicillium camemberti*, such as Brie and Camembert [2, 6], but may be controlled in blue veined cheeses ripened with *P. roquefortii*, such as Fourme d'Ambert and Stilton [3]. It is suggested that medium chain fatty acids produced by fungal metabolism of milk fat can inhibit growth of listerias, especially in blue mould ripened cheeses, where the final pH is lower than that found in surface ripened cheese (> 7.0) [4].

Aims

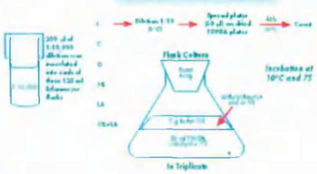
1. Was to develop a model system similar to mould ripened cheese where inhibitor was dissolved in the non-aqueous phase, (butter oil) and could diffuse into the aqueous phase (TSYG) broth.
2. Was to evaluate the effect of lauric acid (C12:0) and monolaurin dissolved in the non-aqueous phase (butter oil) on growth of cells in the logarithmic phase of growth of four cheeseborne strains of listeria at an initial pH of 7.0 and at 10 °C.

Method

1. Preparation of the Inoculum

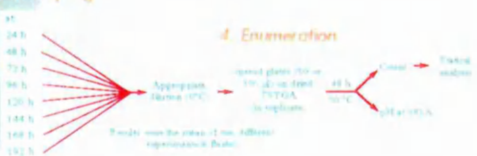


2. Inoculation



3. Sampling

4. Enumeration



Results

Photographs of cheeses to show origin of *Listeria* spp. date of isolation and concentration

Brie de Pays

Listeria innocua (A7)
Date isolated: 7/92¹
<10 cfu/g
Listeria monocytogenes (A13)
Date isolated: 12/92
<200 cfu/g

Fourme d'Ambert

Listeria monocytogenes (A3)
Date isolated: 6/92¹
<10 cfu/g

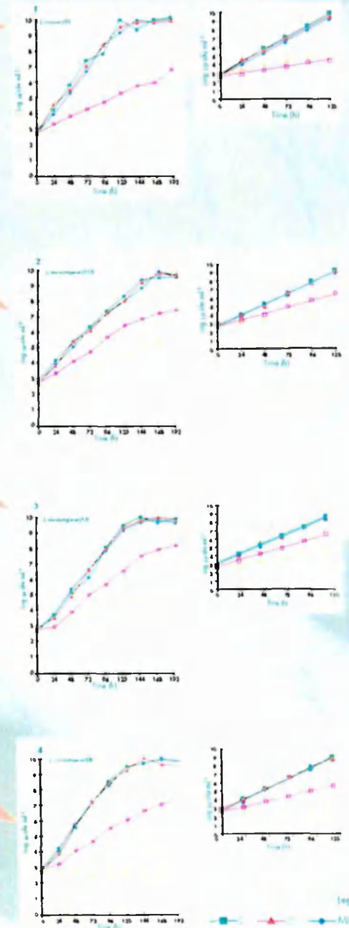
Lanark Blue

Listeria monocytogenes (LB)
Date isolated: 12/95
3.5 x 103 cfu/g

Fig 1, 2, 3and 4 to show growth of *Listeria* spp. in a bi-phasic system containing butter oil, monolaurin, lauric acid and the combination of ML and LA, at 10 °C, pH 7 and 75 rpm

Regression Analysis

Doubling time (t_d) and pH at 192 h



	t_d (h)	pH
C	5.28	4.16
O	5.37	4.41
LA	15.05	6.47
ML	5.37	4.73
ML+LA	18.81	6.89

	t_d (h)	pH
C	6.69	4.85
O	6.69	4.85
LA	10.08	6.86
ML	6.69	5.11
ML+LA	17.10	6.86

	t_d (h)	pH
C	5.28	4.41
O	5.28	4.66
LA	8.85	5.59
ML	5.47	4.75
ML+LA	18.81	6.83

	t_d (h)	pH
C	5.28	4.49
O	5.28	4.72
LA	10.72	6.26
ML	5.28	4.76
ML+LA	ND*	6.86

Conclusions

1. Addition of lauric acid to butter oil affected growth of *Listeria monocytogenes* and *Listeria innocua* in the aqueous phase, at an initial pH of 7.0 and at 10 °C.
2. Lauric acid (5 mM) decreased the rate of growth of all the strains of listeria (up to 3 log orders).
3. Monolaurin (0.35 mM) by itself had no effect on the growth of all the *Listeria* spp..
4. Monolaurin (0.35 mM) acted synergistically with lauric acid. A further decrease of 1 log order was seen for three strains and one strain was completely inhibited.
5. This study demonstrates that preservatives that are insoluble or sparingly soluble in the aqueous phase can act when dissolved in a fat phase. The addition of a sulfactant (monolaurin) increased the inhibition, presumably by increasing the solubility of lauric acid in the aqueous phase.

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[6] Back J.P., Langford S.A., Kroll R.G. (1993) *Journal of Dairy Research* 48, 421-429
[7] Kinderlerer J.L. and Lund B.M. (1992) *Letters in Applied Microbiology* 14, 271-274

List of Abbreviations

TSYGB	Tryptone Soya Broth (Unipath CM129) with added yeast extract (3g/l), Unipath and glucose (7.5 g/l) Merck Chemicals,
TSYGA	As above with 1 % (w/v) Agar Bacteriological No. 1, Unipath L11,
0.1 % Peptone	Unipath Neutralised Bacteriological Peptone L34 [5],
I	Initial count (flasks discarded after sampling),
C	Control (no oil),
O	Control (oil),
ML	Growth with 0.35 mM monolaurin,
LA	Growth with 5 mM lauric acid,
ML and LA	Growth with 0.35 mM monolaurin and 5 mM lauric acid.
	(the concentration of the inhibitors were calculated as if they had dissolved comp[letely] in the aqueous phase)

Acknowledgments

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