

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

**PERFORMANCE OF THREE COMMERCIAL LIPASES IN A  
MODEL ENZYME MODIFIED CHEESE SYSTEM**

*A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF MASTER OF TECHNOLOGY IN FOOD TECHNOLOGY*

*AT MASSEY UNIVERSITY*

*JAGATH THUSITHA KUMARA GUNARATNE*

*1999*

## ABSTRACT

---

The effects of Amano 'R' (from *Penicillium roqueforti*), Palatase (from *Mucor miehei*) and kid lipase (from kid goat) activity on hydrolysis of triglycerides in a constant enzyme modified cheese (EMC) base have been investigated. The effects of incubation time, temperature, enzyme concentration, pH, water activity ( $a_w$ ) and salt-in-moisture content on enzyme activity were studied. Under the same conditions (0.15% enzyme, 30°C, 24 h), Palatase and Amano 'R' showed a greater extent of hydrolysis (total free fatty acids) than Kid lipase. The total free fatty acids (FFAs) released by Palatase, Amano 'R' and Kid lipase were 224, 188 and 20.5 mmol FFA. kg EMC.<sup>-1</sup>, respectively. The optimum temperature for hydrolysis by Amano 'R', Palatase and Kid lipase was around 30°C, 55°C and 45°C respectively. Amano 'R' was very heat sensitive, compared to the other two enzymes. Hydrolysis increased with increasing initial pH. The optimum pH's determined for Amano 'R', Palatase and Kid lipase were 7.5, 8.0 and 5.5 respectively. Enzyme activity decreased slightly as water activity decreased and salt-in-moisture content increased, for all enzymes. The incubation time and enzyme concentration showed the expected trend.

At all process conditions, a high percentage (about 55%) of the fatty acids released by kid lipase was butyric acid. Both Palatase and Amano 'R' were relatively non selective and released large amounts of all fatty acids. Compared to Palatase, Amano 'R' selectively released a higher percentage of butyric acid (about 15% compared to 10%). Generally, the rate of release of butyric acid was greater at lower incubation temperatures for all enzymes. Also, the percentage of butyric acid release decreased with increasing initial pH for Palatase lipase.

## ACKNOWLEDGMENTS

---

I gratefully acknowledge my chief supervisor, Mr Rod Bennett, and co-supervisor Dr Mike Taylor, for their excellent supervision, understanding, and warm encouragement throughout my study, especially for their enthusiasm to help, constructive criticism, and endless patience in discussion and correction of my English. I also convey my special thanks to Mr Rod Bennett, for arrangement of this project and the research collaboration with the New Zealand Dairy Research Institute and especially for being helpful supervisor, during my post graduate study at Massey University.

I wish to thank the New Zealand Dairy Research Institute for sponsorship of my research project. I also express my grateful acknowledgment to my supervisor at New Zealand Dairy Research Institute, Dr Ross Holland, for his excellent supervision, encouragement, enormous knowledge given to me and correction of my English. I gratefully convey my heartiest thanks to Dr Ross Holland, especially appreciating his endless patience and support which he had given to me for successful completion of this project. I also wish to express my sincere gratitude to Dr. Allan Main (Cheese Food Portfolio Manager at NZDRI), Dr P. K Samal, Dr. Vaughan Crow, Dr. Tim Coolbear, Dr. Euan Cant, Julie Brown, Nicky White, Craig Dodds, Karl Gradon, Robbie Buwalda, Tracey Dodds, and all other staff at New Zealand Dairy Research Institute for their great help and friendship which has made my study in New Zealand pleasant and memorable.

I also express my thanks to Associate Professor Harjinder Singh, for nominating my chief supervisor Mr Rod Bennet and his great help during the experimental work. My gratitude and appreciation are extended to all other Staff, postgraduate students in Institute of Food, Nutrition and Human Health, College of Sciences at Massey University for their help and friendship.

Finally, and most important, my special thanks go to my dearest wife and her parents, my late father, mother and all other family members for their encouragement and constant moral support during this study.

## TABLE OF CONTENTS

---

ABSTRACT .....	i
ACKNOWLEDGMENTS .....	ii
TABLE OF CONTENTS .....	iv
CHAPTER 1: INTRODUCTION .....	1
CHAPTER 2: LITERATURE REVIEW .....	4
2.1 What is Enzyme-modified cheese .....	4
2.2 Role of lipases .....	5
2.2.1 Lipolysis and flavour development .....	6
2.2.2 The use of lipases for flavour production of different varieties of cheese.....	8
2.3 Characteristics of lipases.....	11
2.3.1 Factors affecting lipase activity .....	11
2.3.2 Specificity of lipolytic enzymes .....	13
GENERAL DISCUSSION.....	19
CHAPTER 3: MATERIALS AND METHODS.....	20
3.1 Materials.....	20
3.1.1 Cheddar ‘P’ .....	20
3.1.2 Emulsifying salts .....	20

3.1.3 Proteolytic enzymes.....	20
3.1.4 Lipase enzymes.....	20
3.2 Compositional Analysis.....	22
3.2.1 Moisture content.....	22
3.2.2 Salt content.....	22
3.2.3 Fat content.....	22
3.2.4 Total nitrogen.....	23
3.2.5 Non protein nitrogen.....	23
3.3 Microbial analysis.....	23
3.3.1 Total aerobic plate count.....	23
3.3.2 Coliform.....	24
3.3.3 Sulphite reducing clostridia.....	24
3.3.4 Coagulase-positive <i>Staphylococcus aureus</i> .....	24
3.3.5 Thermophiles.....	24
3.4 Determination of proteolysis.....	25
3.5 Determination of fatty acid composition of triglycerides.....	26
3.6 Determination of free fatty acids.....	26
3.7 Determination of volatile compounds.....	28
3.8 Preparation of Enzyme Modified Cheese base.....	28
3.9 Treatment to inhibit microbial growth during enzyme incubation.....	29
3.10 Lipolysis of Enzyme Modified Cheese base.....	31
3.10.1 Incubation time and temperature.....	31
3.10.2 Enzyme concentration.....	32

3.10.3 Initial pH.....	33
3.10.4 Water activity.....	34
3.10.5 Salt levels.....	35
CHAPTER 4: AMANO ‘R’ LIPASE ACTIVITY ON ENZYME-MODIFIED CHEESE .....	36
4.1 Incubation time .....	36
4.2 Incubation temperature .....	41
4.3 Enzyme concentration.....	45
4.4 Initial pH .....	49
4.5 Water activity ( $a_w$ ).....	53
4.6 Salt-in-moisture content.....	55
SUMMARY .....	58
CHAPTER 5: PALATASE ENZYME ACTIVITY ON ENZYME-MODIFIED CHEESE .....	60
5.1 Incubation time .....	60
5.2 Incubation temperature .....	65
5.3 Enzyme concentration.....	69
5.4 Initial pH .....	73
5.5 Water activity ( $a_w$ ).....	77
5.6 Salt-in-moisture content.....	80
SUMMARY .....	82



CHAPTER 6: KID LIPASE ACTIVITY ON ENZYME-MODIFIED CHEESE.....	85
6.1 Incubation time .....	85
6.2 Incubation Temperature .....	90
6.3 Enzyme concentration.....	94
6.4 Initial pH .....	98
6.5 Water activity ( $a_w$ ).....	102
6.6 Salt-in-moisture content.....	105
SUMMARY .....	108
CHAPTER 7: PROPERTIES OF AMANO 'R', PALATASE AND KID LIPASES .....	110
7.1 Extent of hydrolysis .....	110
7.2 Effect of key processing variables on extent of hydrolysis.....	113
7.2.1 Incubation time .....	113
7.2.2 Temperature .....	116
7.2.3 Initial pH.....	119
7.2.4 Enzyme concentration .....	122
CONCLUSIONS .....	125
REFERENCES.....	126

APPENDICES.....	134
Appendix 1 : Characteristics of enzyme modified cheese base .....	135
Appendix 2: Experimental data - lipolysis by Amano 'R' lipase .....	140
Appendix 3: Experimental data - lipolysis by Palatase lipase .....	143
Appendix 4: Experimental data -lipolysis by Kid lipase .....	146

## LIST OF FIGURES

---

Figure 2.1: Formation of flavour compounds from milk lipids .....	6
(Seitz, 1990, p. 3683)	
Figure 3.1: Effect of sucrose content on water activity of EMC base at pH 6.5 (Calibration graph) .....	34
Figure 3.2: Effect of sorbitol content on water activity of EMC base at pH 6.5 (Calibration graph) .....	35
Figure 4.1: The effect of incubation time on the amount of butyric acid and total free fatty acids released by hydrolysis ( <i>Experimental conditions:</i> <i>1% Amano 'R', 30 °C</i> ).....	38
Figure 4.2: The effect of incubation time on the percentages of short-chain fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis ( <i>Experimental conditions: 1% Amano 'R', 30 °C</i> ) .....	40
Figure 4.3: The effect of incubation time on the percentages of long-chain fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis ( <i>Experimental conditions: 1% Amano 'R', 30 °C</i> ) .....	40
Figure 4.4: The effect of incubation temperature on the amounts of butyric acid and total fatty acids released by hydrolysis ( <i>Experimental conditions: 1% Amano 'R', 24h</i> ).....	42

- Figure 4.5: The effect of incubation temperature on the percentages of C4:0, C6:0 C16:0 and C18:1 FAs (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 1% Amano 'R', 24 h*) .....44
- Figure 4.6: The relationship between the percentage of butyric acid that was released by hydrolysis (expressed as a percentage of the total FFA) and the total FFA concentration (*Experimental conditions: 1% Amano 'R', 24 h, 0 to 72 h*) .....45
- Figure 4.7: The effect of Amano 'R' concentration on the amounts of butyric acid and total fatty acids released by hydrolysis (*Experimental conditions: 30 °C, 24 h*).....46
- Figure 4.8: The effect of Amano 'R' concentrations on the percentages of C4:0, C6:0 C16:0 and C18:1 FAs (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 30 °C, 24 h*) .....48
- Figure 4.9: The effect of pH on the amounts of butyric acid and total fatty acids released by hydrolysis (*Experimental conditions 1% Amano 'R', 30 °C, 24h*) .....50
- Figure 4.10: The effect of pH on the percentages of C4:0, C6:0 C16:0 and C18:1 fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 1% Amano 'R', 30 °C, 24 h*) .....52
- Figure 4.11: The effect of water activity ( $a_w$ ) on the amounts of butyric acid and total fatty acids released by hydrolysis (*Experimental conditions: 1% Amano 'R', pH 6.5, 30 °C, 24h*) .....54
- Figure 4.12: The effect of salt-in-moisture content on the amounts of butyric acid and total fatty acids released by hydrolysis (*Experimental conditions: 1% Amano 'R', pH 6.5, 30 °C, 24 h*) .....56

- Figure 5.1: The effect of incubation time on the amounts of butyric acid and total fatty acids released by hydrolysis (*Experimental conditions: 0.15% Palatase, 45 °C*) .....62
- Figure 5.2: The effect of incubation time on the percentages of short-chain fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 0.15% Palatase, 45 °C*) .....64
- Figure 5.3: The effect of incubation time on the percentages of long-chain fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 0.15% Palatase, 45 °C*) .....64
- Figure 5.4: The effect of incubation temperature on the amounts of total fatty acids released by hydrolysis at 24 h and 48 h incubation periods (*Experimental conditions: 0.15% Palatase*) .....66
- Figure 5.5: The effect of incubation temperature on the percentages of C4:0, C6:0, C16:0 and C18:1 fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 0.15% Palatase, 24 h*) .....68
- Figure 5.6: The relationship between the percentage of butyric acid that was released by hydrolysis (expressed as a percentage of the total FFA) and the total FFA (*Experimental conditions: 0.15% Palatase, 0 to 72 h incubation period*) .....69
- Figure 5.7: The effect of Palatase concentration on the amounts of butyric acid and total fatty acids released by hydrolysis (*Experimental conditions: 45 °C, 24 h*) .....70

- Figure 5.8: The effect of Palatase concentration on the percentages of C4:0, C6:0, C16:0 and C18:1 fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 45 °C 24 h*).....72
- Figure 5.9: The effect of pH on the amounts of butyric acid and total free fatty acids released by hydrolysis (*Experimental conditions: 0.15% Palatase, 45 °C, 24 h*) .....74
- Figure 5.10: The effect of pH on the percentages of C4:0, C6:0, C16:0 and C18:1 fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 0.15% Palatase, 45 °C, 24 h*) .....77
- Figure 5.11: The effect of water activity ( $a_w$ ) on the amounts of butyric acid and total fatty acids released by hydrolysis (*Experimental conditions: 0.15% Palatase, pH 6.5, 45 °C, 24 h*) .....78
- Figure 5.12: The effect of salt-in-moisture content on the amounts of butyric acid and total fatty acids released by hydrolysis (*Experimental conditions: 0.15% Palatase, pH 6.5, 45 °C, 24 h*) .....81
- Figure 6.1: The effect of incubation time on the amounts of butyric acid and total fatty acids released by hydrolysis (*Experimental conditions: 1% Kid lipase, 40 °C*) .....87
- Figure 6.2: The effect of incubation time on the percentages of short-chain fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 1% Kid lipase, 45 °C*).....89
- Figure 6.3: The effect of incubation time on the percentages of long-chain fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 1% Kid lipase, 45 °C*) .....89

- Figure 6.4: The effect of incubation temperature on the amounts of butyric acid and total fatty acids released by hydrolysis (*Experimental conditions: 1% Kid lipase, 48 h*).....91
- Figure 6.5: The effect of incubation temperature on the percentages of C4:0, C6:0, C16:0 and C18:1 fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 1% Kid lipase, 48 h*) .....93
- Figure 6.6: The relationship of percentage of butyric acid (expressed as percentages of the total FFA) and total FFA that were released by hydrolysis at various incubation temperatures(*Experimental conditions: 1% Kid lipase, 0 to 72 h*).....94
- Figure 6.7: The effect of Kid lipase concentration on the amounts of butyric acid and total fatty acids released by hydrolysis (*Experimental conditions: 40 °C, 48 h*).....95
- Figure 6.8: The effect of enzyme concentration on the percentages of C4:0, C6:0, C16:0 and C18:1 fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 1% Kid lipase, 40 °C, 48 h*).....97
- Figure 6.9: The effect of initial pH on the amounts of total fatty acids and butyric acid released by hydrolysis (*Experimental conditions: 1% Kid lipase, 40 °C, 48 h*).....99
- Figure 6.10: The effect of initial pH on the percentages of C4:0, C6:0, C16:0 and C18:1 fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 1% Kid lipase, 40 °C, 48 h*).....102
- Figure 6.11: The effect of water activity ( $a_w$ ) on the amounts of total fatty acids and butyric acid released by hydrolysis (*Experimental conditions: 1% Kid lipase, pH 6.5, 40 °C, 48 h*).....103

- Figure 6.12: The effect of water activity on the percentages of C4:0, C6:0, C16:0 and C18:1 fatty acids (expressed as percentages of the total FFA) that were released by hydrolysis (*Experimental conditions: 1% Kid lipase, pH 6.5, 40 °C, 48 h*).....105
- Figure 6.13: The effect of salt-in-moisture content on the amounts of total fatty acids and butyric acid released by hydrolysis (*Experimental conditions: 1% Kid lipase, pH 6.5, 40°C, 48 h*) .....106
- Figure 7.1: FFA percentages (expressed as a percentages of the total FFAs) resulted from Amano 'R', Palatase and Kid lipase (*Experimental conditions: 0.15%-enzyme, 30 °C, 24 h*) .....112
- Figure 7.2: The effect of incubation time on the total amount of fatty acids released by hydrolysis: (A) Amano 'R' - 1%, 30 °C (B) Palatase - 0.15%, 45 °C (C) Kid lipase - 1%, 40 °C .....114
- Figure 7.3: Effect of time on the percentage of Butyric acid (expressed as percentages of the total FFA) that were released by hydrolysis (A) Amano 'R' - 1%, 30 °C (B) Palatase - 0.15%, 45 °C (C) Kid lipase - 1%, 40 °C.....115
- Figure 7.4: The effect of incubation temperature on the total amounts of fatty acids released by hydrolysis (A) Amano 'R' - 1%, 24 h (B) Palatase - 0.15%, 24 h (C) Kid - 1%, 48 h .....117



- Figure 7.5: The relationship between the percentage of butyric acid that was released by hydrolysis (expressed as a percentage of the total FFA) and the total free fatty acid (A) 1% Amano 'R', 0 - 72 h (B) 0.15% Palatase, 0 - 72 h (C) 1% Kid, 0 - 48 h ..... 118
- Figure 7.6: The effect of pH on the amounts of total fatty acids released by hydrolysis (A) Amano 'R'- 1%, 30°C, 24 h (B) Palatase - 0.15%, 45°C, 24 h (C) Kid lipase - 1%, 40°C, 48 h ..... 120
- Figure 7.7: The effect of initial pH on the percentage of butyric acid (expressed as a percentage of the total FFA) that was released by hydrolysis (A) Amano 'R'- 1%, 30°C, 24 h (B) Palatase - 0.15% , 45°C, 24 h (C) Kid lipase - 1%, 40°C, 48 h..... 121
- Figure 7.8: The effect of enzyme concentration on the total amount of fatty acids released by hydrolysis (A) Amano'R'-30°C, 24 h (B) Palatase - 45°C, 24 h (C) Kid lipase 40°C, 48 h..... 123
- Figure 7.9: The effect of enzyme concentration on the percentage of Butyric acid (expressed as a percentage of the total FFA) that was released by hydrolysis (A) Amano'R'- 30°C, 24 h (B) Palatase -45°C, 24 h (C) Kid lipase -40°C, 48 h ..... 124

## LIST OF TABLES

---

Table 2.1: Ratio of enzyme activity on monoglyceride and diglyceride substrates compared to activity on tributyrin .....	14
Table 2.2: Selective liberation of individual free fatty acids from milk fat by eight different lipase preparations .....	17
Table 2.3: Activities of various enzymes in triacetin solution (5%) and emulsion (15%) at pH 6.2 .....	18
Table 3.1: Microbial plate count results by after various treatments which were carried out to inhibit microbial growth during enzyme incubation .....	30
Table 3.2: The different incubation times, temperatures and enzyme concentrations used for the experiments .....	32
Table 3.3: The different enzyme concentrations, incubation times and temperatures used for the experiments .....	32
Table 3.4: The different incubation times, temperatures and enzyme concentrations used for the experiments .....	33
Table 4.1: The effect of incubation time on the amounts of individual fatty acids released by Amano 'R' lipase.....	37
Table 4.2: The effect of incubation time on the percentages of individual fatty acids released by Amano 'R' lipase.....	39
Table 4.3: The effect of incubation temperature on the amounts of individual fatty acids released by Amano 'R' lipase.....	41

Table 4.4: The effect of incubation temperature on the percentages of individual fatty acids released by Amano 'R' lipase .....	43
Table 4.5: The effect of Amano 'R' concentration on the amounts of individual fatty acids released by hydrolysis .....	46
Table 4.6: The effect of Amano'R' concentration on the percentages of individual fatty acids released by hydrolysis .....	47
Table 4.7: The effect of pH on the amounts of individual fatty acids released by Amano 'R' lipase.....	49
Table 4.8: The effect of pH on the percentages of individual fatty acids released by Amano 'R' lipase.....	51
Table 4.9: The effect of water activity ( $a_w$ ) on the amounts of individual fatty acids released by Amano 'R' lipase.....	53
Table 4.10: The effect of water activity ( $a_w$ ) on the percentages of individual fatty acids released by Amano 'R' lipase .....	55
Table 4.11: The effect of salt-in-moisture content on the amounts of individual fatty acids released by Amano 'R' lipase .....	56
Table 4.12: The effect of salt-in-moisture content on the percentages of individual fatty acids released by Amano 'R' lipase .....	57
Table 5.1: The effect of incubation time on the amounts of individual fatty acids released by Palatase lipase.....	61
Table 5.2: The effect of incubation time on the percentages of individual fatty acids released by Palatase lipase.....	63

Table 5.3: The effect of incubation temperature on the amounts of individual fatty acids released by Palatase lipase .....	65
Table 5.4: The effect of incubation temperature on the percentages of individual fatty acids released by Palatase lipase .....	67
Table 5.5: The effect of Palatase concentration on the amounts of individual fatty acids released by hydrolysis .....	70
Table 5.6: The effect of Palatase concentration on the percentages of individual fatty acids released by hydrolysis .....	71
Table 5.7: The effect of pH on the amounts of individual fatty acids released by Palatase lipase.....	73
Table 5.8: The effect of pH on the percentages of individual fatty acids released by Palatase lipase.....	76
Table 5.9: The effect of water activity ( $a_w$ ) on the amounts of individual fatty acids released by Palatase lipase.....	78
Table 5.10: The effect of water activity ( $a_w$ ) on the percentages of individual fatty acids released by Palatase lipase .....	79
Table 5.11: The effect of salt-in-moisture content on the amounts of individual fatty acids released by Palatase lipase .....	80
Table 5.12: The effect of salt-in-moisture content on the percentages of individual fatty acids released by Palatase lipase .....	82
Table 6.1: The effect of incubation time on the amounts of individual fatty acids released by kid lipase.....	86

Table 6.2: The effect of incubation time on the percentages of individual fatty acids released by kid lipase.....	88
Table 6.3: The effect of incubation temperature on the amounts of individual fatty acids released by kid lipase .....	90
Table 6.4: The effect of incubation temperature on the percentages of individual fatty acids released by kid lipase .....	92
Table: 6.5: The effect of Kid lipase concentration on the amounts of individual fatty acids released by hydrolysis .....	95
Table 6.6: The effect of Kid lipase concentration on the percentages of individual fatty acids released by hydrolysis .....	96
Table: 6.7: The effect of initial pH on the amounts of individual fatty acids released by kid lipase.....	98
Table 6.8: The effect of initial pH on the percentages of individual fatty acids released by kid lipase .....	101
Table: 6.9: The effect of water activity ( $a_w$ ) on the amounts of individual fatty acids released by kid lipase.....	103
Table 6.10: The effect of water activity ( $a_w$ ) on the percentages of individual fatty acids released by kid lipase.....	104
Table: 6.11: The effect of salt in moisture content on the amounts of individual fatty acids released by kid lipase .....	106
Table 6.12: The effect of salt-in-moisture on the percentages of individual fatty acids released by kid lipase.....	107

Table 7.1: The amounts of individual fatty acids released by Amano 'R', Palatase and Kid lipase.....	111
Table 7.2: The percentages of short-chain fatty acids released by Amano 'R', Palatase and Kid lipase.....	112

## CHAPTER 1

---

### INTRODUCTION

The use of cheese flavour in various food products is commonly accepted. As a consequence, the cheese industry faces an increasing demand for cheese with high flavour intensity from the prepared food industries. Many prepared food products require sources of cheese such as Cheddar, Swiss, Blue, Romano and others which impart typical flavour characteristics. The main alternatives to the use of natural cheese flavour are high-intensity cheese flavour concentrates, such as enzyme modified cheese (EMCs).

A number of compounds have been identified as being characteristic flavour components of certain natural cheese varieties. These flavours can be produced by procedures such as use of specific enzyme systems. To obtain pure compounds a detailed knowledge of the reaction systems is vital (Grueb and Gatfield, 1989) and is probably cost-prohibitive. Therefore, enhancement of the major flavour pathways that occur in natural cheese presently provides the most economic route to the production of intense cheese flavours.

Free fatty acids in cheese can contribute directly to flavour, but threshold values of the individual fatty acids in cheese are not known and are difficult to estimate. Further breakdown of fatty acids and reactions with other components of the maturing cheese are likely to occur, and may contribute to the formation of additional flavour components (Siezen and Van den Berg, 1994). An increase in the amount of free fatty acids in cheese is possible

---

---

by inducing lipolysis in the milk or dairy system. This technique is also used during the manufacture of enzyme modified cheese (EMC).

The degree and contribution of lipolysis to cheese flavour varies considerably between cheese varieties (Fox, 1993, Kilcawley, *et al.*, 1998). It is also concluded that for some EMC types, the flavour profile or intensity are proportional to the degree of lipolysis and release of low molecular weight free fatty acids (FFAs), as with Romano or Provolone type EMCs.

The effect of lipase on cheese flavour formation has been evaluated by a number of researchers (Dziezak, 1986). It is reported that various lipases could be selected to give the intensity of flavour required in the final product. A wide range of lipases are commercially available from a number of sources, mainly animal and microbial. The correct choice of lipase is extremely important since the FFA and flavour profiles generated vary significantly with the type of lipase used (Kilara, 1985). The flavour compounds, and their relative concentrations may also vary, depending on the conditions used to manufacture the product (Moskowitz and Noelck, 1987).

The objective of this study is to develop and collect information on the technology of controlled lipolysis in EMC production using commercially available food grade lipases. The plan is to characterise Amano 'R', Palatase and Kid lipase effects on a constant cheese substrate (EMC base) in terms of the amounts of fatty acid (FA) released by hydrolysis and the percentages of FAs released, expressed as a percentage of the total of that particular FA bound initially in the milk triglyceride, and as a percentage of the total free fatty acids (FFAs).

---



---

Total free fatty acids, expressed as mmoles per kg of EMC base, indicates the concentration of free fatty acids accumulating in the model EMC system. Total free fatty acid concentration is an indicator of EMC flavour intensity. The percentage of fatty acid released expressed as a percentage of the total amount of that particular fatty acid originally esterified in the milk fat triglyceride, indicates the “extent” of the lipase reaction (degree of hydrolysis). The percentage of fatty acid released, expressed as a percentage of the total free fatty acids released, indicates the specificity of a particular lipase. Lipase specificity impacts on the flavour profile of an EMC.

The enzyme activities will be investigated at different processing variables, namely the incubation time, temperature, enzyme concentration, pH, water activity and salt-in-moisture to find out their impact on hydrolysis of triglycerides in EMC base. Where reaction conditions are described as being optimal, this refers to the conditions required to achieve maximum enzyme activity.

The EMC base to be used is an immature processed cheese, which has undergone proteolysis but not lipolysis. A better understanding of the influence of process variables on the lipase activity and the resultant free fatty acid (FFA) profiles will provide useful information on EMC manufacture and suggest ways to produce new varieties of enzyme modified cheese.

---