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A Comparison of Lipids in Blue and White Mould-Ripened Cheeses

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ABSTRACT

The free saturated medium chain fatty acids (FMCFA's) were isolated and analysed by gas chromatography from two blue veined cheeses (Bleu d'Auvergne and Fourme d'Ambert) and two soft-ripened cheeses (Brie and Vacherin Mont d'Or) at the point of purchase. Identification of the fatty acids was carried out using a gas chromatograph coupled to a mass spectrometer (GC/MS). The cheeses were sampled in the region of conidia spores (blue veins in the blue cheeses and surface in the soft-ripened cheeses) and the region of no obvious spores (white region in the blue cheeses and centre in the soft-ripened cheeses).

In the blue veined cheeses, higher concentrations of (FMCFA's) were detected in the region of conidia spores, particularly tetradecanoic and dodecanoic acids. The concentrations of the FMCFA's in the white region of the blue cheese were similar to those in the Brie cheese. There was little difference in the concentration of the free medium chain fatty acids between the centre and surface of Brie and Vacherin Mont d'Or. Only hexanoic, octanoic and decanoic acids were detected in the Brie cheese, with octanoic acid present in the highest concentration ($\approx$ 0.4 - 0.7 mg / g cheese). In Vacherin Mont d'Or cheese, all fatty acids were detected (C6:0 - C14:0), with tetradecanoic and octanoic acids present in the highest concentrations. These results suggest that fungal metabolism is different in different regions of the cheese. The conidia in the blue cheese appear to be more metabolically active than those on the surface of the surface-ripened cheeses.

A blue veined cheese (Bleu d'Auvergne) and a surface ripened cheese (Brie) were stored under simulated ripening conditions at 12°C. The free fatty acids C6:0 - C14:0 were extracted directly from the cheeses and identified by GC/MS. The lipid fraction of the cheese that contained both free and acylated medium (MCFA's) and long chain fatty acids (LCFA's), was extracted and then esterified. Identification of saturated, mono-unsaturated and methyl branched fatty acids in this fraction was carried out by GC/MS.

The concentration of the FMCFA's in the blue region of the Bleu d'Auvergne cheese increased during storage, particularly the longer chain tetradecanoic and dodecanoic acids. In the Brie cheese and the white region of the blue cheese, the free fatty acid concentration remained constant during storage.

Differences were evident in the lipid extract containing both free and acylated fatty acids of both the Bleu d'Auvergne and Brie cheeses. In the blue region of the Bleu d'Auvergne cheese, the MCFA concentration decreased probably due to fungal metabolism of the fat, whilst the concentration of the LCFA's increased during storage. In the white region of the cheese, both the medium and long chain fatty acid concentrations increased during storage probably due to water loss from the Bleu d'Auvergne cheese. At the surface and centre of the Brie cheese, no change in the MCFA concentration of the acylglycerol fraction was seen during storage. The LCFA's decreased in concentration in both regions, again, probably due to fungal metabolism of the fat.

The pH ranged from 6.1 - 7.2 in the blue cheese and from 5.7 - 7.8 in the Brie cheese. The fat content in the blue region of the blue cheese decreased during storage, but increased in the white region, probably due to water loss from the cheese. In the Brie cheese, the fat content was higher at the surface than the centre, and decreased with time in both regions.

COURSE OF RELATED STUDIES

Courses Attended

Attended a Microbiology Part I Course and gained an average of 75 % in the coursework and examination (Feb - June 1993).

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Publications

The poster 'A study of Factors in French brie Cheeses which could affect the growth of *Listeria monocytogenes*' was displayed at the IFST Conference in York University (6 th July 1993), the 9th International Biodegradation Symposium, Leeds University (9 th Sept. 1993) and the SDT Annual Conference, Cambridge (20 th April 1993).

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Thanks,

Helen

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CHAPTER 1

INTRODUCTION

Cheese is the generic name for a group of fermented milk based food products produced in at least 500 varieties throughout the world Fox (1987).

Cheese is an example of a fermented food which is made from milk. Most cheese today is produced from cow's milk, although milk from sheep and goats is used as well. Milk consists of protein, fat, carbohydrate (lactose), vitamins and minerals. The composition of milk varies from season to season as well as with the breed and state of lactation of the cow (Scott 1986). The average composition of milk from a number of species is given in Table 1.1.

Table 1.1 Composition of milk (g/100 g)

Species		Water	Fat	Casein	Whey protein	Lactose
Cow	*Bos taurus*	87.6	3.7	2.8	0.6	4.8
Goat	*Capra hircus*	86.8	4.5	2.5	0.4	4.1
Sheep	*Ovis aries*	80.7	7.4	4.6	0.9	4.8
Human	*Homo sapiens*	87.6	3.9	0.4	0.6	7.0

Jenness and Sloane (1970)

Milk is a highly perishable liquid commodity with a relatively short shelf-life. Cheese is a semi-perishable semi-solid commodity with a considerably longer shelf-life. The increase in shelf-life in cheese compared to milk is due to three factors. 1) removal of water 2) addition of salt 3) a reduction in the pH from 6.4 - 6.6 in milk to 5 - 5.5 for most cheeses (Pearson 1976).

1.1 CHEESE

Cheese making has been established since the beginning of civilisation. Cheese is referred to in the Bible, in Job 10, 10 (1520 BC), Samuel **1** 17, 18 and **2** 17, 29 (1017 BC) (Scott 1986). It is thought that cheese was originally produced by nomadic tribes in the Middle East from cow's, goat's or sheep's milk. Perhaps these people stored milk in the stomach of a calf or sheep when travelling from place to place. When they took the milk out of the stomach they discovered that it had curdled. They realised that the curd could be eaten but better still it kept for longer than the original milk. It was this process which led to the intentional production of cheese from milk. Nothing whatsoever was known about the microbiology and biochemistry of this process. The original process by which milk was converted into cheese must have been accidental. These nomads would have introduced the art of cheese making to local populations as they travelled (Ogilvy 1976). Consequently the accidentally discovered process, cheese-making, of yesterday has given rise to the technology of the process today and the extensive range of cheeses which are available.

1.1.1 Classification of Cheese

Cheeses are broadly classified as whey, processed, soft and hard cheeses. The Cheese Regulations of 1970 (applying to England and Wales) give the following definitions for these cheeses.

Whey cheese means the product obtained by coagulation or concentration of whey with or without the addition of milk and milk fat.

Processed cheese means cheese which has been subjected to a process of melting and mixing with or without the addition of emulsifying salt.

Soft cheese means cheese which is readily deformed by moderate pressure, but does not include whey cheese, processed cheese or cheese spread, and

any reference to soft cheese including a reference to cream cheese or curd cheese.

Hard cheese means cheese other than soft cheese, whey cheese, processed cheese or cheese spread.

These regulations do not give a definition of mould-ripened cheeses because they fall into one of the above categories (soft cheese for white and soft and semi-soft for blue mould-ripened cheeses).

The composition of a range of cheeses, along with their classification is given in Table 1.2. The extra classes of cheese type have been added according to fat and water content, giving a more precise definition of cheese type, which is not included in the cheese regulations.

Table 1.2 Minimum Fat, Maximum Water and Protein Composition in Cheese

Cheese Type	Classification	Fat (g/100 g)	Maximum water (%)	Protein (g/100 g)
Parmesan	very hard	29.7	28	35.1
Cheddar	hard	33.5	37	26.0
Edam	semi-hard	22.9	43.7	24.4
Blue	semi-soft, internal mould-ripened	29.2	40.5	23.0
Camembert	soft, surface mould-ripened	23.2	47.5	22.8
Cottage	acid coagulated (soft)	4.0	78.8	13.6

Paul and Southgate (1978).

1.1.2 Fermentation of Milk

The majority of cheeses undergo one bacterial fermentation where lactose in milk is converted to lactic acid by lactic acid bacteria. This results in a pH drop from 6.4 - 6.6 in milk to 4.0 - 4.5 (Kirk and Sawyer 1991). Mould-ripened cheeses are unusual as two fermentations are involved. The initial fermentation involves the conversion of lactose by a homofermentative mesophilic lactic acid starter (*Lactococcus lactis* sub sp. *lactis* and sub sp. *cremoris*) to lactic acid. In blue cheeses the heterofermentative organisms *L. lactis* sub sp. *diacetylactis* and *Leuconostoc cremoris* may be included in the starter as well, as carbon

dioxide is produced which leads to a more open texture in the cheese, thus allowing the mould to grow. The second fermentation unlike the first is a solid-state fungal one. Two fungal species are used, the blue Penicillium mould *Penicillium roquefortii* for blue mould-ripened cheeses and the white Penicillium mould *P. camembertii* for Brie and Camembert type cheeses.

In this introduction two aspects of fat degradation, one of the breakdown processes due to the enzymes of the Penicillium mould, will be considered (Section 1.2). Firstly as a cause of flavour in mould ripened cheese and secondly as a possible source of fungal metabolites which could have antibacterial activity against potential pathogens. The interaction of fungal metabolites and their role in the safety of mould ripened cheese will be considered.

1.2 THE FAT AND FATTY ACID COMPOSITION OF MILK

The main constituents of milk fat are neutral lipids which are present in the form of globules. Triacylglycerols account for 97 - 98 %, and fatty acids account for 0.1 - 0.44 % of the total lipids (Patten and Jensen 1976). The remainder consists of phospholipids, di- and monoacylglycerols and unsaponifiable material. Triacylglycerols (triglycerides) are esters of glycerol and fatty acids. Triacylglycerols are trihydroxy aliphatic alcohols, where each hydroxyl group is esterified to a fatty acid (Jensen and Clark 1988).

Fatty acids are aliphatic carboxylic acids. Naturally occurring fatty acids usually have an even number of carbon atoms, due to the mode of biosynthesis, however odd-chain length fatty acids can be synthesised, particularly in plants. Fatty acids can be subdivided in a number of different ways. The first division depends on **chain length** (where chain length will refer to the carbon chain and

the carboxylic acid group). Fatty acids can be divided into short chain fatty acids (SCFA's) with a chain length of less than six carbon atoms, medium chain fatty acids (MCFA's), those with a chain length between six and twelve carbon atoms, and long chain fatty acids (LCFA's), those with a chain length greater than twelve carbon atoms.

The second division depends on the **degree of unsaturation**. Fatty acids can contain no (saturated), one, two or three double bonds. The commonest unsaturated fatty acid is the monounsaturated oleic acid (9 - octadecenoic acid) where the double bond appears at the ninth ($\Delta 9$) carbon atom along the chain from the methyl group. Natural unsaturated acids have the cis configuration at the double bond, which leads to a 'kink' in the molecule for each double bond (Christie 1982, Gurr and Harwood 1991[1]). For polyunsaturated fatty acids the double bonds usually appears at the sixth carbon atom first, then the ninth and then the twelfth carbon atom ($\Delta 6$, $\Delta 9$, $\Delta 12$).

The third division depends on whether the fatty acid is a **straight chain** or **branched chain** one. Branched chain fatty acids (BCFA's) occur widely in nature but tend to be present only as minor components, except in bacteria (Christie 1982). Ha and Lindsay (1993) found that *Penicillium roquefortii* lipases produced high amounts of branched chain fatty acids, predominantly hexadecanoic acid and octadecanoic acids. Branched chain fatty acids are produced by microbial action during digestion in the rumen of the cow and appear to be taken up unchanged into the blood stream and consequently appear unchanged in the milk. Usually the branch consists of a single methyl group, either on the penultimate (iso) or on the antepenultimate (anteiso) carbon atom (Fig. 1.1).

Figure 1.1 Iso and Anteiso Branched Chain Fatty Acids

Table 1.3 shows the typical fatty acid composition of triacylglycerols of butter oil, including saturated, mono-unsaturated and branched fatty acids on a weight percent basis.

Table 1.3 Fatty Acid Composition of Total Fatty Acid Methyl Esters in Butter Oil

		Saturates	Monoenes		Branched	
Fatty Acid	Systematic Name		Cis	Trans	Iso	Anteiso
C4:0	Butanoic	3.25	-	-	-	-
C6:0	Hexanoic	2.32	-	-	-	-
C8:0	Octanoic	1.85	-	-	-	-
C10:0	Decanoic	4.02	-	-	-	-
C12:0	Dodecanoic	4.15	0.03	-	0.01	-
C14:0	Tetradecanoic	11.05	0.47	-	0.08	-
C15:0	Pentadecanoic	0.95	0.08	-	0.23	0.42
C16:0	Hexadecanoic	26.15	1.25	0.03	0.32	-
C17:0	Heptadecanoic	0.70	0.32	0.01	0.33	0.40
C18:0	Octadecanoic	9.60	20.40	5.34	0.15	-

Figures expressed as weight percent
Jensen and Clark (1988)

Free fatty acids are relatively uncommon in natural products and their presence in significant concentrations in commodities denotes that some degradation of fat may have taken place. The presence of medium chain fatty acids in milk is due to the fact that they provide a rapid energy source for the young. Medium chain fatty acids are absorbed directly from the small intestine and transported directly to the liver via the portal blood supply (Gurr & Harwood 1991[2], Babayan and Rosenau 1991). The relative proportions of free fatty acids in milk and butter are given in Table 1.4. The relative proportions of the individual fatty

acids are not substantially different from those of the esterified fatty acids in butter oil (Table 1.3).

Table 1.4 Relative Proportions of Free Fatty Acids in Milk and Butter

Fatty Acid	Systematic Name	Milk Weight %	Butter Weight %
C4:0	butanoic acid	6.43	0.98
C6:0	hexanoic acid	2.79	0.33
C8:0	octanoic acid	2.66	0.47
C10:0	decanoic acid	3.76	2.22
C10:1	decenoic acid	0.52	0.18
C12:0	dodecanoic acid	4.35	4.00
C14:0	tetradecanoic acid	7.59	7.71
C14:1	tetradecenoic acid	1.47	1.85
C15:0	pentadecanoic acid	0.95	1.27
C16:0 ^{br}	methyl-pentadecanoic acid	0.09	0.18
C16:0	hexadecanoic acid	19.99	21.67
C16:1	hexadecenoic acid	2.57	2.73
C17:0	heptadecanoic acid	0.46	0.62
C18:0 ^{br}	methyl-heptadecanoic acid	0.37	0.51
C18:0	octadecanoic acid	8.51	11.82
C18:1	octadecenoic acid	31.50	36.25
C18:2	octadecadienoic acid	3.28	3.89
C18:3	octadecatrienoic acid	2.72	3.31

figures are expressed as weight percent of total free fatty acids
^{br} - branched
Anon (1991)

In milk about one third of the free fatty acids are found in the aqueous phase, one third in the membrane of the fat globule and one third in the core of the fat globule (Anon 1991). Fatty acids are derived by hydrolysis or lipolysis of mono-, di- or triacylglycerols. Both enzymatic and non-enzymatic hydrolysis can take place. The starter organisms (bacterial and fungal) used in the production of cheese have lipolytic activity. Triacylglycerols (Fig. 1.2) are insoluble in water. They are hydrolysed by lipases (triacylglycerol acyl hydrolase EC 3.1.1.3) to give di- and monoacylglycerols and free fatty acids. Lipases act specifically at oil and water interfaces (Tombs 1990). In mould-ripened cheese the extent of lipolysis is due to production of extracellular

lipase enzymes by the mould. In white mould-ripened cheeses, the extent of lipolysis is 3 - 5 % of the total acids. In blue cheese, the extent is 18 - 25 % of the total fatty acid content (Choisy *et al.* 1984[1]). The general process of lipolysis is given in Figure 1.3.

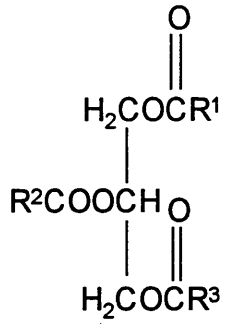


Figure 1.2 1, 2, 3- triacylglycerol

Triacylglycerol → 1,2 - or 1,3 - diacylglycerol → 2, monoacylglycerol → etc.[a]
↓ ↓
Free fatty acids Free fatty acids

[a] - Lipolysis may continue to give complete hydrolysis of the triacylglycerols.

Figure 1.3 Hydrolysis of Triacyglycerols to give Free Fatty Acids (FFA's)

1.2.1 Fatty Acid Breakdown

The normal biological route for fatty acid breakdown is by the β- oxidation pathway (Gurr and Harwood 1991[1]). Fatty acids are degraded by 2 carbon atom units, with the concomitant production of one molecule of acetyl Coenzyme A. Acetyl Co A can then be utilised as a building block in the synthesis of new cellular material or in the production of energy for use in anabolic or catabolic reactions. Some fungi however can modify the β-oxidation pathway for fatty acid breakdown (Hawke 1966, Kinderlerer and Kellard 1984). They can convert (usually medium chain) fatty acids to volatile methyl ketones containing one carbon less than the parent fatty acid (Fig. 1.4)

(Dartey and Kinsella 1973, Kellard *et al.* 1985, Yagi *et al.* 1989, Kinderlerer 1993). Thus 2- undecanone will be produced from dodecanoic acid, 2-nonanone from decanoic acid and 2- heptanone from octanoic acid. Enzymes from both the spores and mycelium of the mould are involved in these bioconversions (Fan *et al.* 1976).

The production of aliphatic methyl ketones is important in the flavour of all blue cheeses (Kinsella and Hwang 1976[1]). Methyl ketones are less important as flavour compounds in the white mould-ripened cheeses but they are present. 2- nonanone and 2- heptanone are the most abundant methyl ketones in all cheeses (Schwartz and Parks 1963, Hardy 1984). The levels of methyl ketones in blue cheese range from 80 - 600 μmoles/ 100 g fat (Schwartz *et al.* 1963, Kinsella and Hwang 1976[1]). In Camembert cheese, the levels are of the order 25 - 60 μmoles/ 100 g fat (Schwartz *et al.* 1963). The methyl ketones are further reduced by enzymes produced by the Penicillium moulds, to give secondary alcohols, which also contribute to the flavour of the cheese.

$$\text{Saturated fatty acid } (C_{2n}) \rightarrow \text{Methyl ketone } (C_{2n-1}) + CO_2$$

Figure 1.4 The Process of Methyl Ketone Production by β- Oxidation

1.3 SAFETY OF CHEESE

Food must be safe for the consumer. This statement implies that there is no risk to the consumer from eating food. A major hazard in food is the presence of food-borne pathogens. Pathogens are micro-organisms, which if either they or some products of their metabolism are ingested, will result in an illness. Vegetative bacterial cells will be killed by most cooking processes, thereby rendering the food safe. Cheese is an example of a food which is eaten without

being cooked. It's safety depends on controlling the environmental factors (both extrinsic and intrinsic ones) in food so that if micro-organisms are present, they do not multiply. In cheese, many factors are used to control the growth of micro-organisms. They include : (1) hydrogen ion concentration (pH), (2) water activity (a_w), (3) absence of food-borne pathogens, (4) temperature. A fifth factor which may be important is the presence of natural or added antimicrobial compounds. In recent years the safety of soft-ripened cheese has been questioned.

1.3.1 Incidence of *Listeria monocytogenes* in Mould-Ripened Cheeses

Two epidemic outbreaks of listeriosis have occurred world wide which were attributed to the consumption of soft mould-ripened cheeses. These outbreaks set the scene for concern about the presence of *Listeria monocytogenes* in the food chain (WHO 1988, DoH 1989). The first outbreak of cheese-borne listeriosis occurred in the Vaud region of Switzerland between 1983 - 1987 where 140 cases were attributed to the consumption of Vacherin Mont d'Or cheese (Bille and Glauser 1988). In 1985 142 cases of listeriosis were reported in California, United States, where one brand of Mexican-style fresh cheese was implicated as the vehicle of infection (Linan *et al.* 1988). Listeriosis remains a rare disease in England and Wales. There were only 108 cases of listeriosis reported in 1992, of which not all were of food-borne origin (Newton *et al.* 1993). This figure is low when compared with 27,500 cases of Salmonellosis and 29,000 cases of campylobacter infection in 1991 (Anon 1991).

Only two isolated cases of listeriosis have been reported in England and Wales in 1986 and 1988 due to the consumption of soft-ripened cheese (Lund 1990, McLauchlin *et al.* 1990). This evidence was the reason why the Department of Health issued a press release in February 1989 to advise the public to avoid eating high risk foods such as soft-ripened cheese. This advice was given to

the groups of people who were most at risk; the elderly, the young or those with a compromised immune system (DoH press release 1989). In a recent survey of cheese purchased in England and Wales, high counts of the pathogen *Listeria monocytogenes* were found in soft-ripened cheeses (Table 1.5).

Table 1.5 Survey for the Presence of *Listeria monocytogenes* in Cheese made from Cow's Milk in England and Wales Between November 1988 - October 1990

Cheese Type	Number sampled	Number Lm present	%
soft-ripened	766	63	8.2
soft-unripened	366	4	1.1
hard	66	1	1.5

Soft-ripened cheese included mould-ripened and blue veined cheeses.
The counts of Lm in 13 samples was > 10^3 cfu/g and in 3 samples was 10^5 cfu/g

Greenwood *et al.* (1991)

1.3.2 The Fate of *Listeria monocytogenes* in Soft-Ripened Cheeses

The fate of *Listeria monocytogenes* was investigated in various experimental cheeses. *L. monocytogenes* survived and multiplied when inoculated into the milk of laboratory made Camembert cheeses (Back *et al.* 1993). Higher rates of growth were observed at the surface of the cheese compared to the centre. At the surface there was a 100- fold increase in cell number after storage at 3 or 6 °C, whilst no growth was seen in the centre of the cheese at these temperatures. At 15 °C in the centre, an increase in pH was detected from 4.5 to 7.3 along with increased growth of *L. monocytogenes*. Thus lower storage temperatures restricted, but did not inhibit growth of *L. monocytogenes*. This agrees with the results of Ryser and Marth (1987). These authors found that there was generally a 10- to 100- fold increase in numbers of *Listeria monocytogenes* at the surface compared to the interior of the cheese, towards the end of the ripening period. An increase in the number of listerias paralleled

the increase in the pH of the cheese. In comparison, *L. monocytogenes* in blue cheese increased in numbers during the first 24 hours of production, but then declined as the pH increased to 6.0 towards the end of the ripening period. In the blue cheeses, *Listeria monocytogenes* survived but did not multiply (Papageorgiou and Marth 1989).

1.4 CONTROL OF GROWTH OF PATHOGENIC ORGANISMS IN CHEESE

A number of intrinsic and extrinsic factors affect the growth of potential pathogens in cheese. Most pathogenic organisms do not grow at pH's less than 5.0 and a water activity (a_w) less than 0.92 (Choisy *et al.* 1984[1]).

1.4.1 Water Activity and pH

Water activity in cheese depends on cheese type. Hard cheeses have a lower water content than soft cheeses (Table 1.2). The fat content affects the water activity. The higher the fat content, the less water will be available for microbial growth in the cheese. Again the higher the salt content, the lower the water activity. As we have seen the pH of a cheese depends on the concentration of lactic acid present. Marcos and Esteban (1991) in a study of mould-ripened cheeses investigated the range of pH and water activity in many varieties of such cheeses. A summary of their results is given in Table 1.6.

Table 1.6 Water Activity (a_W), pH, and Aqueous Concentration of Ash, NaCl, NPN and SN (g/100 g moisture) for Blue and White Mould-Ripened Cheeses

Cheese Type	n	Ash	NaCl	NPN	SN	pH	a_W
Brie	14	5.63	3.57	1.33	-	6.93	0.96
Camembert	14	5.44	3.28	1.28	-	7.01	0.97
Roquefort	5	13.02	8.57	3.63	5.21	6.19	0.92
Bleu de Bresse	2	7.63	4.51	3.11	3.68	6.85	0.95

NPN - Non protein nitrogen
SN - Soluble nitrogen

Marcos and Esteban (1991)

From this Table it can be seen that mould-ripened cheeses have a high pH and a relatively high water activity. If food-borne pathogens were present in the cheese, then they could grow.

1.4.2 Temperature

To control growth of potential pathogens in milk, the milk should be stored at 5 °C at the dairy (Food Hygiene [Amendment] Regulations 1990). Storage of milk at temperatures above 5 °C would allow an increase in numbers of any micro-organisms which were present. Pasteurisation (heat treatment) of cheese milk aims to destroy the micro-organisms or enzyme systems which would otherwise be harmful to the cheese process or more importantly to the consumer. There are two legal heat treatments for milk in the United Kingdom ; high temperature, short hold time (HTST) and low temperature, long hold time (LTLT). Only HTST is used in the pasteurisation of cheese milk. The milk is heated to a minimum of 71.7 °C (maximum ≈ 78 °C) held at that temperature for 15 seconds. After an efficient heat treatment the number of organisms should be reduced by 95 - 99 %.

1.4.3 Inhibition of Micro-Organisms by Medium Chain Fatty Acids

For the control of *L. monocytogenes* in refrigerated products it has become necessary to incorporate barriers including preservatives (Ryser and Marth

1991). Nowadays many food additives are lipophilic acids which prevent the growth of micro-organisms, probably by preventing transport of substrate molecules into the cell (Freese *et al.* 1973, 1978). Fatty acids are lipophilic acids which have no known toxicity to humans. Inhibitory fatty acids must be sufficiently water soluble to reach an effective concentration in aqueous solution, but sufficiently hydrophobic to interact with the hydrophobic protein or lipid on the bacterial cell surface. Ionisation of fatty acids causes a reduction in their antibacterial activity, therefore the molecules must be associated, causing pH to be a controlling factor (Gershon and Shanks 1978).

Medium chain fatty acids have been known to have antibacterial activity for almost a century. Early observations were based on studies of soaps (Lamar 1911). Saturated fatty acids are known to inhibit Gram positive micro-organisms (Nieman 1954, Kabara 1978). Work carried out in 1933 by Tetsumoto showed the effect of medium chain fatty acids on *Clostridium perfringens* (Table 1.5). Hexanoic (C6:0), octanoic (C8:0) and decanoic acids (C10:0) were more effective inhibitors at pH 6.5 than 7.5. However, the level of dodecanoic acid (C12:0) needed to inhibit *C. perfringens* decreased as the pH was raised. This can be seen in Table 1.7. At pH 7.5 dodecanoic (lauric) acid was the most inhibitory saturated medium chain fatty acid.

Table 1.7 MIC (mg/100 ml) of Medium Chain Fatty Acids against *Clostridium perfringens*

Fatty Acid	pH 6.5	pH 7.5
C6:0	1160	5810
C8:0	721	3610
C10:0	172	862
C12:0	1000	200
C14:0	2280	457

Tetsumo (1933)
MIC - Minimum inhibitory concentration

Wang and Johnson (1992) observed the growth of *Listeria monocytogenes* (Lm) in Beef Heart Infusion (BHI) when various fatty acids were added. They found that *Listeria monocytogenes* was sensitive to certain fatty acids. Lauric

acid was the most active saturated fatty acid investigated (with greater inhibition seen at pH 5.0 than 6.0). They suggested that there was reduced hydrophobicity and increased solubility of the shorter chain length fatty acids rather than longer chain fatty acids in the aqueous phase. A wealth of evidence suggests that of the medium chain fatty acids dodecanoic acid is the most inhibitory acid against a range of organisms including *Listeria monocytogenes* (Nieman 1954, Kabara 1978). In an *in vitro* system hexanoic and octanoic acids were listeriocidal at pH 5.0 and 5.5. The degree of inhibition depended on the concentration of the unionised acid. (Kinderlerer and Lund 1992).

1.4.4 Inhibition of Micro-Organisms by Monoacylglycerols

Monoacylglycerols (MAG) have been investigated as antimicrobial agents. In an *in vitro* system, a range of MAG's was studied (MC_8 - MC_{14}) for inhibitory properties against *Listeria monocytogenes.* MC_{12} was the most inhibitory at 37 °C and pH 6.0 (Wang *et al.* 1993). Kinderlerer (Report to the Dairy Trades Federation 1994) found that more conversion of triacylglycerols occurred in blue mould-ripened cheese than in white mould-ripened cheese, to give mono- and diacylglycerols.

1.5 CHEESES INVESTIGATED

Four mould-ripened cheeses were studied in this investigation. Two cheeses were blue mould-ripened cheeses, namely Fourme d'Ambert and Bleu d'Auvergne and two were surface-ripened cheeses, Brie de Pays and Vacherin Mont d'Or. These cheeses were investigated because Prince Charles in his address to the Association of France-Grande Bretagne in Paris in 1992, warned that the above cheeses would cease to be produced if the minimum

hygiene standards for cheese (and other foods) proposed in Brussels, to eliminate potentially harmful bacteria, came into force (The Times 1992).

1.5.1 Fourme d'Ambert

This cheese is produced in the Livradois region in the Auvergne in France. It is protected by an *appellation d'origine*. Fourme d'Ambert is made from pasteurised cow's milk. The cheese resembles a tall Stilton, having a similar rough brown-grey crust. Fourme d'Ambert has a dark blue veining marbled throughout the cheese. It was originally blued naturally, however it is now often injected with the blue *Penicillium roquefortii* mould. When the cheese is wrapped in foil at factories, the crust is inferior to the one formed when the traditional methods are used. Fourme d'Ambert is only lightly pressed, so the smooth, creamy white paste should be firm and moist.

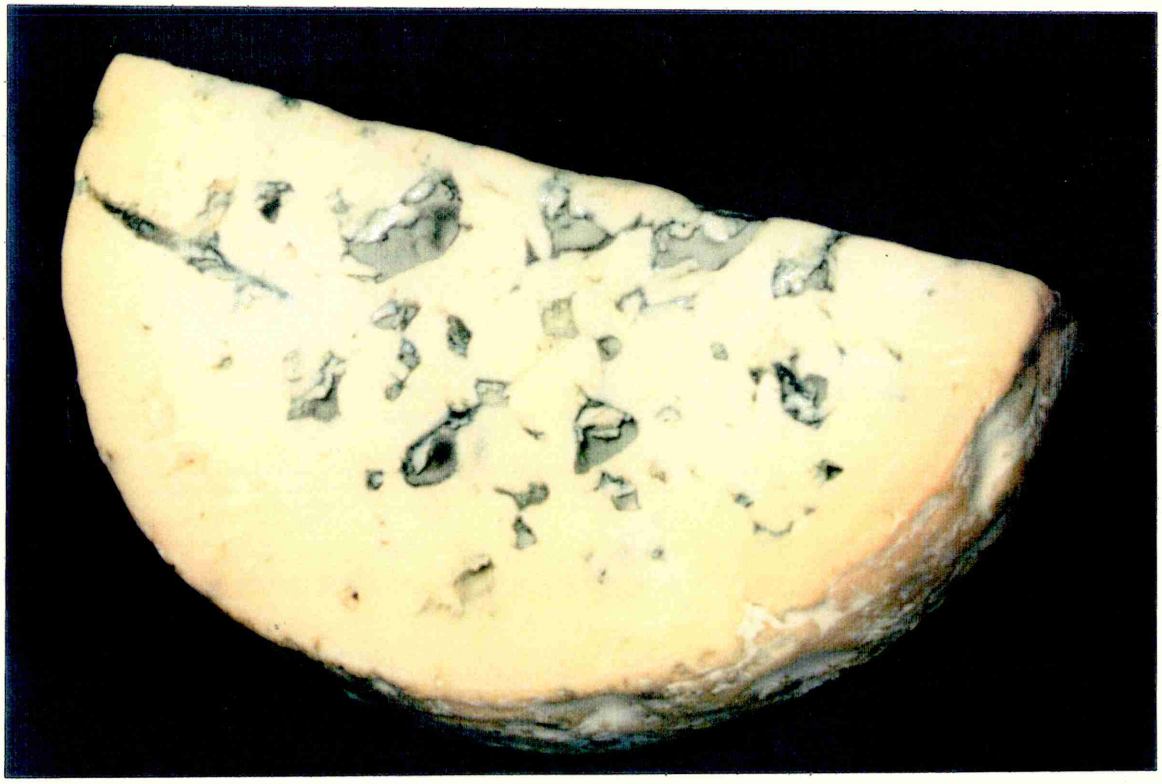


Figure 1.5 Fourme d'Ambert Cheese

1.5.2 Bleu d'Auvergne

Bleu d'Auvergne is produced in the Massif Central region of Auvergne in France. It is still made on mountain farms by traditional methods. Commercial dairies produce the cheese from pasteurised cow's milk. Bleu d'Auvergne is a lightly pressed, semi-hard scalded cheese. *Penicillium roquefortii* is usually added at the renneting stage, so the cheese is only lightly pressed to allow the fungus to grow from the inside to the edge. The cheese is moulded into tall cylinders and wrapped in foil to mature slowly for approximately 2 months. An ideal Bleu d'Auvergne ought to be a creamy cheese with a pale paste. It should taste salty and sharp, and a dark blue veining should be evident throughout the cheese.

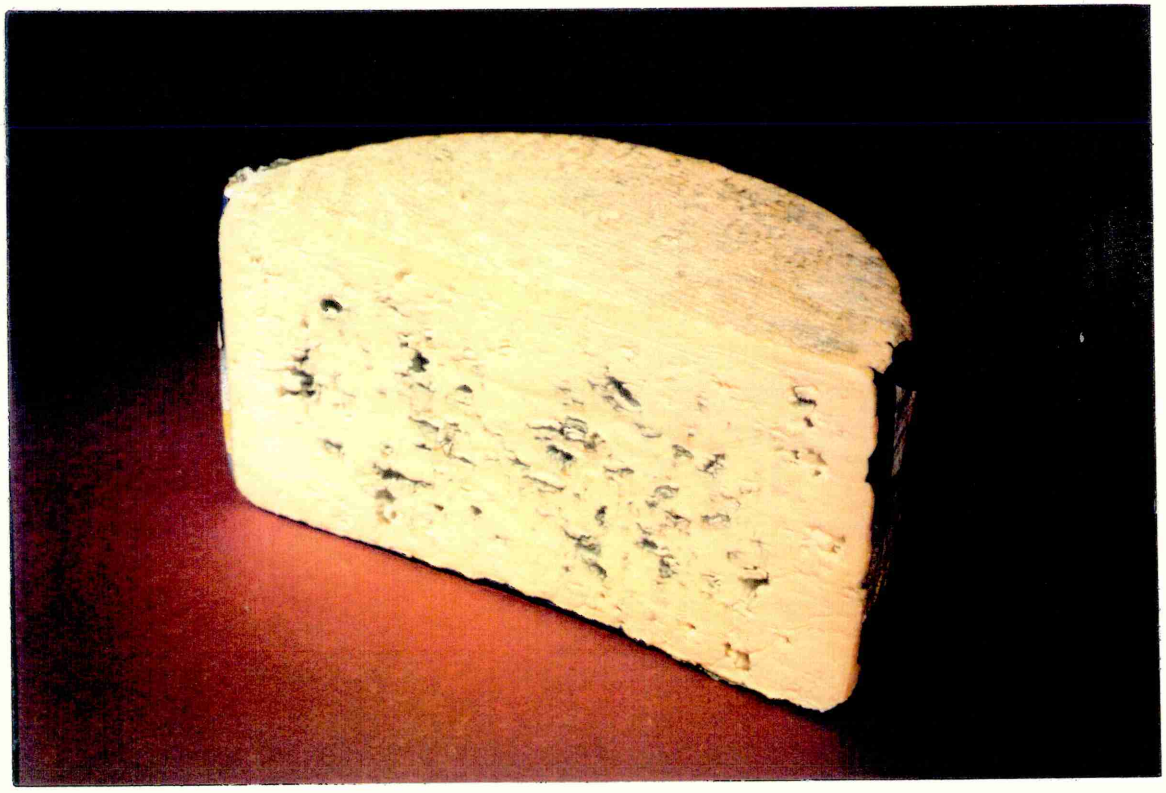


Figure 1.6 Bleu d'Auvergne Cheese

1.5.3 Brie de Pays

The group name for Brie is *Pâtés molles à croute Fleurie. Pâtés molles* is a cheese which undergoes further fermentations in addition to the first lactic acid fermentation. *Croute Fleurie* literally means 'rind in bloom' or mouldy rind. Brie is traditionally made in the Normandy region of France, although nowadays it is produced all over France and in many other countries. Brie is a soft, unpressed naturally drained cow's milk cheese. Some are still made in the traditional way, from unpasteurised milk. The cheese is moulded into large flat discs (35 - 37 cm diam., 2.5 - 3 cm thick). It is sprayed with the white mould *Penicillium camembertii*, which grows from the surface to the centre, during the 2 - 4 week maturation stage. An ideal Brie has a rich straw coloured paste. The paste should not be chalky, as this is the sign of an under ripe cheese. The rind should be firm (not hard) and tender. The cheese should smell clean. Two signs of an over ripe cheese are a runny paste and an ammoniacal odour.

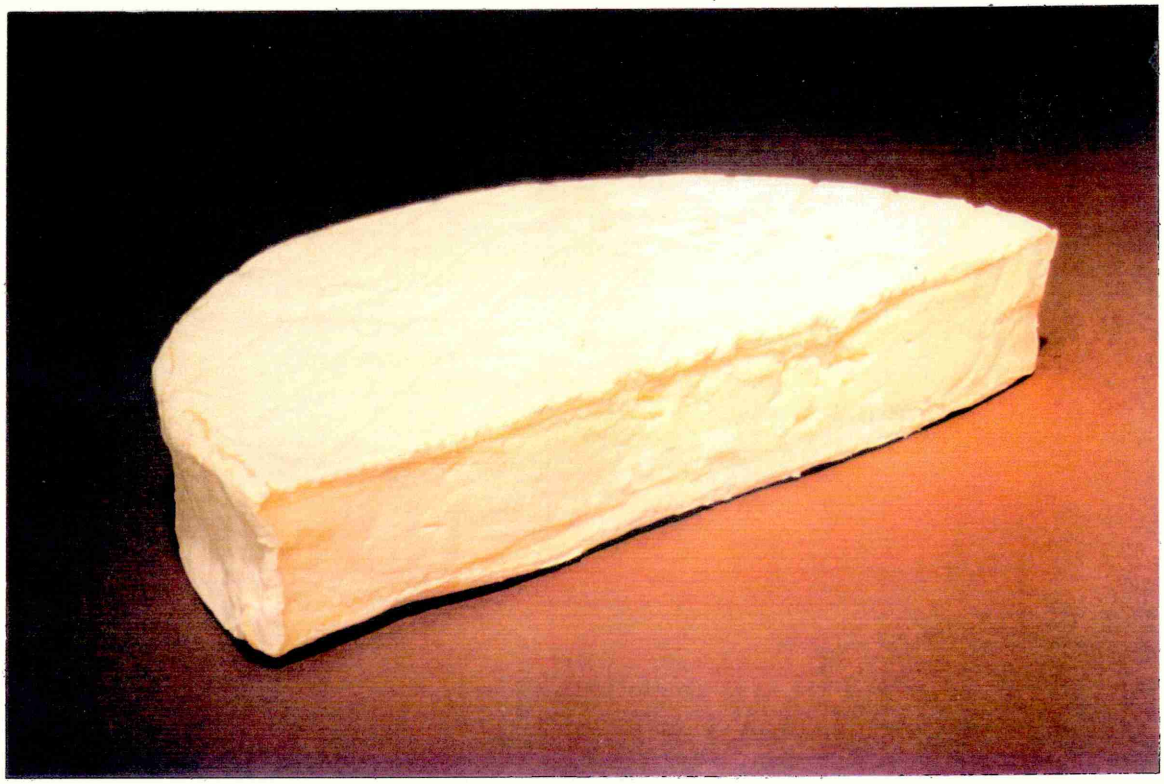


Figure 1.7 French Brie Cheese

1.5.4 Vacherin Mont d'Or

This cheese is made in the Vallée du Joux, Switzerland, near the border with France. The French produce the same cheese, but due to legal arguments have been forced to change the name of their cheese to Vacherin du Haut Doubs, although it is commonly known as Mont d'Or. Vacherin Mont d'Or is only produced in the winter, from milk of the last hay crop, when the fat content is higher than usual. The flat, circular cheese is moulded in a strip of pine bark and cured in a humid atmosphere for three months. It is sold in wooden boxes, in which it is commonly served. The crust is a pale reddish-brown which becomes slightly crumpled when the cheese is *à point*. The paste is a dull yellow, with the consistency of thick clotted cream. To serve this cheese, the crust is removed in one piece and the inside scooped out with a spoon.

Figure 1.8 Vacherin Mont d'Or Cheese

There are many differences in the manufacture and characteristics of blue and white mould-ripened cheeses, which with particular reference to Bleu d'Auvergne and Brie are highlighted in Table 1.8.

Table 1.8 Differences in the Production and Characteristics of a White Mould-ripened Cheese (made from raw milk) and a Blue Mould-ripened Cheese (made from pasteurised milk)

Blue mould ripened cheese (Bleu d'Auvergne)	White mould ripened cheese (Brie)
pasteurised milk	raw milk
mould added to milk prior to renneting	mould added to surface of cheese
curd cut and stirred	curd not cut
curd scalded	curd not scalded (increased temperature)
whey drained prior to putting in mould	whey drained once cheese is in mould
lightly pressed to remove whey	not pressed
matured for 1 - 4 months	matured for 2 - 4 weeks
mould grows from centre to edge	mould grows from surface to centre
moulded into a tall cylinder	moulded into a thin, large cylinder
higher salt content (≈ 10 %)	lower salt content (≈ 4 %)
ripened at 7 - 12 °C	ripened at 11 - 13 °C

1.5.5 Bioconversions Which Take Place During the Ripening of Surface and Internally Moulded Cheeses

The presence of the Penicillium mould leads to a more complex ripening process than in other varieties of cheese with a simple flora (Gripon 1987). The composition, structure, appearance and colour of the cheese are modified, whilst the flavour and aroma develop in mould-ripened cheeses (Choisy *et al.* 1984[1]). The mould produces enzymes which neutralise the acidity of the cheese and affect the breakdown processes which dominate ripening. The three important bioconversions which occur are : (1) protein degradation (proteolysis) to give amino acids and ammonia, (2) fat degradation (lipolysis) which releases di- and monoacylglycerols and free fatty acids, (3) lactic acid utilisation (Lenoir 1974). The processes of protein degradation and lactic acid utilisation result in a pH increase to between 6 -7.

The protein matrix of the cheese determines the texture of the cheese. Two types of proteolytic enzymes are produced by the Penicillium moulds. Two endopeptidases (proteases) hydrolyse the protein to peptides. These peptides are broken down by a further enzyme, an exopeptidase (amino, carboxy and di-peptidases), to give amino acids which are subsequently deaminated to give ammonia (Choisy *et al.* 1984[1]). These degradative processes cause the body of the cheese to become soft and smooth rather than firm and tough. A rise in pH is observed due to the release of ammonia. Godinho and Fox (1982) noted that proteolysis is more limited in the outer parts than the centre of Camembert. They suggested that sodium chloride inhibits Penicillium growth, and hence it's proteolytic action.

The fat or lipid fraction of the curd is also acted upon by the Penicillium moulds. *Penicillium camembertii* has one extracellular lipase (optimum pH 9), whilst *P. roquefortii* has two extracellular lipases (optimum pH's 6 and 9) for the hydrolysis of triacylglycerols (Choisy *et al.* 1984[1]). The triacylglycerols are broken down to give free fatty acids, mono- and diacylglycerols. More lipolytic activity is observed in the blue cheese than the white cheese, causing more fatty acids to be released from the triacylglycerols.

1.5.6 External Factors Which Affect Ripening

The external factors controlling storage conditions during ripening of cheese are significant for two reasons. They affect growth and activity of the microbial flora, and the production of active enzymes. Temperature, humidity, movement & composition of the air are all controlled and regulated. The temperature of ripening is 11 - 13 °C for surface mould-ripened cheeses and 7 - 12 °C for internal mould-ripened cheeses. The temperature is maintained slightly below the optimum temperature for mould growth. An increase in temperature by 1 or 2 °C can reduce the ripening time, however it unfortunately brings about

changes in the organoleptic properties of the cheese. A reduction in temperature to the same extent extends the ripening time (Weber and Ramet 1984). The humidity of the ripening room affects the water activity. Ripening is always carried out below 100 % Relative Humidity (RH). The humidity for surface ripened cheeses is of the order 85 - 90 %, with the cheese losing water to the atmosphere (Weber and Ramet 1984).

1.5.7 Differences in the Maturing Stage of Blue and White Mould-Ripened Cheeses

Soft cheeses such as Brie ripen quickly, due to the high water content and rapid growth of the mould on the surface. The cheese is transferred to the ripening room where it is placed upon supports which allow for the best possible ventilation to allow the mould to grow. Reed and bamboo trays were originally used, but due to the more rigorous hygiene standards now in practice, stainless steel trays with thin rods spaced at intervals are used. After 3 or 4 days, the cheese is turned to ensure that the mould grows evenly on both faces and the water distributes evenly. After 6 days of ripening a covering of the white mould (*Penicillium camembertii*) can be seen on the surface of the cheese. The cheese may be turned once more after another 3 or 4 days. After day 15 - 20, the mould will have utilised the lactic acid causing de-acidification of the cheese (pH $\approx$ 6). The cheese is left for a total of 2 - 4 weeks to mature. An aerophilic acid sensitive bacterial flora becomes established on the surface of the cheese after this time (Weber and Ramet 1984).

In internally-ripened cheeses the mould (*Penicillium roquefortii*) must obviously grow within the mass of the cheese. A heterofermentative lactic acid starter which includes *Leuconostoc* is added to the milk as carbon dioxide is produced, which causes fissures in the cheese. Prior to ripening the cheese is 'pierced' to allow air to enter the cheese, and the mould to grow. To achieve the optimum circulation of air for this growth, the cheese is placed upon flat surface

slabs or perforated metal trays. *Penicillium roquefortii* can however tolerate low oxygen and high carbon dioxide levels. During the one to four month ripening period, the cheeses are turned and the surfaces scraped to prevent micro-organisms from growing in the air gaps and blocking them. Further piercing may also be carried out. Salting reduces the total flora at the surface but not at the centre, because it takes ten days for the sodium chloride to diffuse into the centre of the cheese (Choisy *et al.* 1984[2], Weber and Ramet 1984).

1.6 METHODS OF ANALYSIS

Analysis of lipids and free fatty acids in a food is always difficult. These two groups of compounds are insoluble in an aqueous solution but are soluble in organic solvents. The traditional solvents used for extraction of lipids are chloroform : methanol (1 :2 v/v) or more non-polar solvents such as petroleum ether or hexane. Triacylglycerols are usually hydrolysed and the fatty acids converted to the methyl ester prior to analysis by gas chromatography. Free fatty acids are difficult to analyse. They are more soluble in organic solvents in the unionised form. Many methods of analysis have been developed which involve adsorption onto a silica gel column, elution and conversion to the methyl ester prior to analysis by gas chromatography. In this study a direct solvent extraction using chloroform / methanol was used. This method had been developed by Unilever for the analysis of free fatty acids in chocolate. The total lipid fraction was extracted using a Soxhlet extraction into petroleum ether. Confirmation of the identity of fatty acids was made using gas chromatography coupled to a mass spectrometer.

1.7 OUTLINE OF THE PROJECT

The aim of this project was to compare the free medium chain fatty acids and total fatty acid composition in blue and white mould-ripened cheeses at the point of sale and after storage under simulated ripening conditions. Both the regions with considerable mould growth (blue veins of the blue cheeses and surface of the soft-ripened cheeses) and regions with no obvious mould growth (white region of the blue cheeses and centre of the soft-ripened cheeses) were examined. Four cheeses were investigated, two blue mould-ripened cheeses, Bleu d'Auvergne and Fourme d'Ambert and two white mould-ripened cheeses, Brie and Vacherin Mont d'Or.

CHAPTER 2

MATERIALS AND METHODS

2.1 FREE MEDIUM CHAIN FATTY ACIDS

2.1.1 Procedure for the Extraction of Free Fatty Acids

Extraction of free fatty acids from the cheese was carried out in duplicate using a method developed at Colworth House, Unilever by Dr Brian Jeffrey (Personal Communication). The different regions of the Bleu d'Auvergne and Brie cheeses were sampled and separated as described in section 2.5.3. Cheese (400 mg) was weighed accurately into a screw top culture tube (10 ml) and nonanoic acid (10-20 µl) internal standard, was weighed into the tube. Chloroform : methanol 1:1 v/v (1 ml) and hydrochloric acid 2 M (200 µl) were added. The sealed tube was heated in a water bath (60 °C) for 5 minutes. During this time, the contents were triturated twice for 20 seconds with a Whirlimixer (Fisons) at full speed. Isooctane (5 ml) was added with a Gilson pipette and the contents mixed by the Whirlimixer for a further 20 seconds. The tube was reheated (60 °C) for a further 5 minutes during which time it was shaken as before. The tube was immediately cooled in ice. The lower (organic) layer was transferred with a Pasteur pipette to a conical flask containing anhydrous sodium sulphate (≈ 5 g). The flask was left at 4 °C overnight. The solvent was removed by evaporation to ≈ 0.5 ml with nitrogen gas, transferred to a vial (2.5 ml) and stored at 4 °C until analysed.

2.1.2 Analysis of Free Medium Chain Fatty Acids by Gas Chromatography

Gas chromatographic analysis was carried out on a Varian 3400 gas chromatograph with a flame ionisation detector. The fatty acid extracts were manually injected (0.2 µl) onto a 2 m x 2 mm i.d. glass packed column (5 %

DEGS, 0.1 % phosphoric acid on WHP 80 -100 mesh). Nitrogen was the carrier gas with a fixed flow of 20 ml min^{-1}. The injector block was heated to 220 °C and the detector to 300 °C, with a range of 10^{-10} and attenuation of 4. The fatty acids were run on one of two column programmes; the first a temperature programme (140 -175 °C at a rate of 3 °Cmin^{-1}, 175 - 185 °C at a rate of 10 °C min^{-1}) and the second at an isothermal (constant) temperature (165 °C). The chromatograms were recorded on a Pye Unicam PU 4810 integrator. The gas chromatograph was calibrated on a set of fatty acids C6:0, C7:0, C8:0, C10:0, C12:0, C14:0 (20 mg ml^{-1}) and C9:0 internal standard (40 mg ml^{-1}). The response factors for the series of fatty acids were automatically determined by use of a fixed programme in the integrator with respect to the internal standard nonanoic acid. The fatty acids were identified by comparison with the retention times of the standard fatty acids (Tables 2.1 and 2.2). The concentration of each acid in the sample was reported by use of the scale factor (which corrected for the weight of cheese extracted) and the calculated response factor. Results were expressed as mg fatty acid per g fresh weight cheese. The following equations (Figure 2.1) were used to calculate the response factor, scale factor and concentration (mg/ g oil) for the fatty acids, by the integrator :

$$RF = \frac{conc_s}{area_s} \times \frac{area_{is}}{conc_{is}}$$

$$XF = \frac{1}{w} \times 1000 \rightarrow mg\ /g\ oil$$

$$CONC\ (mg/g\ oil) = \frac{RF_s}{RF_{is}} \times \frac{area_s}{area_{is}} \times XF \times \frac{IS}{SA}$$

RF = response factor (1 for IS)
conc = concentration (mg ffa per g oil)
XF = scale factor
w = mass of oil (mg)
IS = internal standard in sample (mg)
SA = sample amount (grams of cheese)
s = sample
is = internal standard

Figure 2.1 Equations used by the Integrator to Calculate Response Factor, Scale Factor and Concentration (mg/ g oil) for the Fatty Acids

Table 2.1 Retention Times, Relative Retention Times and Response Factors for the Standard Free Fatty Acids Analysed by Gas Chromatography

Pk No	Peak Identification	Retention Time t_R(mins)	Rel. Retention Time t_{RR}	Response Factor
1	Solvent front	0.51	0.070	-
2	Hexanoic acid	2.87	0.396	0.962
3	Heptanoic acid	4.01	0.554	0.983
4	Octanoic acid	5.47	0.756	0.830
5	Nonanoic acid	7.24	1.000	1.00
6	Decanoic acid	9.30	1.285	0.955
7	Dodecanoic acid	13.82	1.909	1.012
8	Tetradecanoic acid	18.30	2.528	1.341

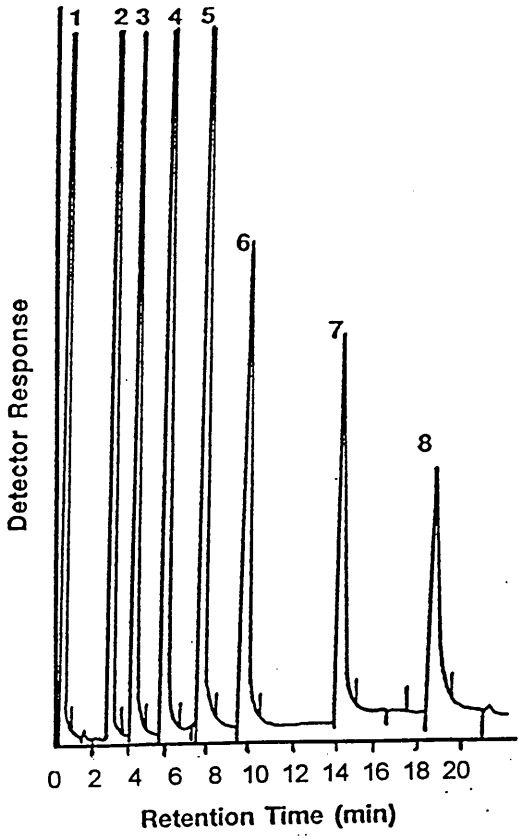
Temperature programme 140-175, 3 °Cmin^{-1} then 175-185, 10 °Cmin^{-1}
This data refers to Figure 2.2

Table 2.2 Retention Times, Relative Retention Times and Response Factors for the Standard Free Fatty Acids Analysed by Gas Chromatography

Pk No	Peak Identification	Retention Time t_R (mins)	Rel. Retention Time t_{RR}	Response Factor
1	Solvent front	0.97	0.124	-
2	Hexanoic acid	3.37	0.430	0.918
3	Octanoic acid	5.84	0.745	0.867
4	Nonanoic acid	7.84	1.000	1.00
5	Decanoic acid	10.70	1.365	1.182
6	Dodecanoic acid	22.12	2.566	1.848
7	Tetradecanoic acid	38.31	4.882	2.238

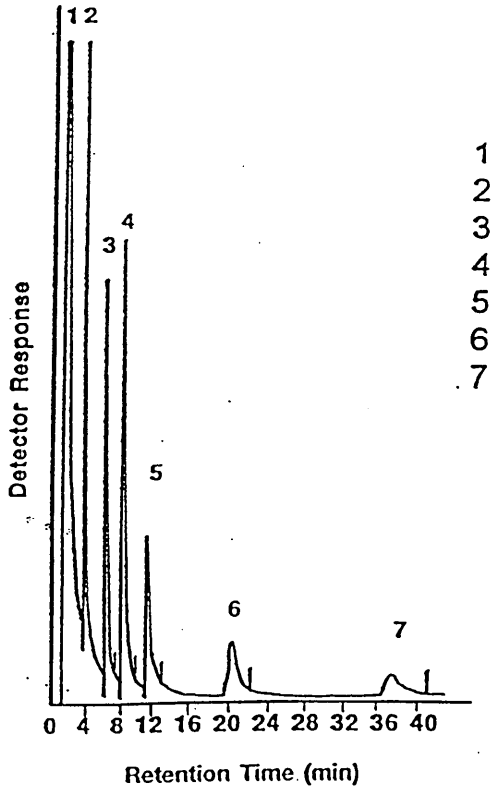
Isothermal temperature at 165 °C of the standard free fatty acid solution
This data refers to Figure 2.3

Figure 2.2 demonstrates a chromatogram of the standard free fatty acid solution when analysed under the temperature programme conditions and Figure 2.3 demonstrates a chromatogram of the standard free fatty acid solution under the isothermal conditions described above.



1 Solvent Front
2 Hexanoic acid
3 Heptanoic acid
4 Octanoic acid
5 Nonanoic acid (IS)
6 Decanoic acid
7 Dodecanoic acid
8 Tetradecanoic acid

Figure 2.2 Standard Free Fatty Acids Analysed by GC on the Temperature Programme, 140-175 °C at 3 °Cmin^{-1} then 175-185 °C at 10 °Cmin^{-1}



1 Solvent Front
2 Hexanoic acid
3 Octanoic acid
4 Nonanoic acid (IS)
5 Decanoic acid
6 Dodecanoic acid
7 Tetradecanoic acid

Figure 2.3 Standard Free Fatty Acids Analysed by GC Isothermally at 165 °C

2.1.3 Gas Chromatography / Mass Spectrometry (GC/MS) Analysis for the Identification of Free Medium Chain Fatty Acids (MCFA's)

The samples were run on a VG Trio -1 Mass Spectrometer with helium as the carrier gas. The injector temperature was held at 250 °C and the source (electron impact) at 200 °C. The sample was manually injected onto the capillary column (All Tech) SE 30 25 m x 0.32 i.d., film thickness 0.25 µm. The column was programmed from 100 - 220 °C with a 10 °Cmin^{-1} temperature ramp. Figure 2.4 demonstrates the standard solution of free fatty acids run on the GC/MS under the above conditions with the retention times and relative retention times for the free fatty acids of the standard solution in Table 2.3.

Table 2.3 Retention Times and Relative Retention Times for the Standard Free Fatty Acids Analysed by Gas Chromatography / Mass Spectrometry

Peak No.	Peak Identification	Retention time t_R (mins)	Rel. Retention time t_{RR}
1	Solvent front	1.62	0.444
2	Hexanoic acid	2.08	0.570
3	Octanoic acid	2.75	0.753
4	Nonanoic acid (IS)	3.65	1.000
5	Decanoic acid	4.60	1.260
6	Dodecanoic acid	6.78	1.858
7	Tetradecanoic acid	8.98	2.461

Temperature programme 100 - 220 °C, 10 °Cmin^{-1} ramp
This data refers to Figure 2.4

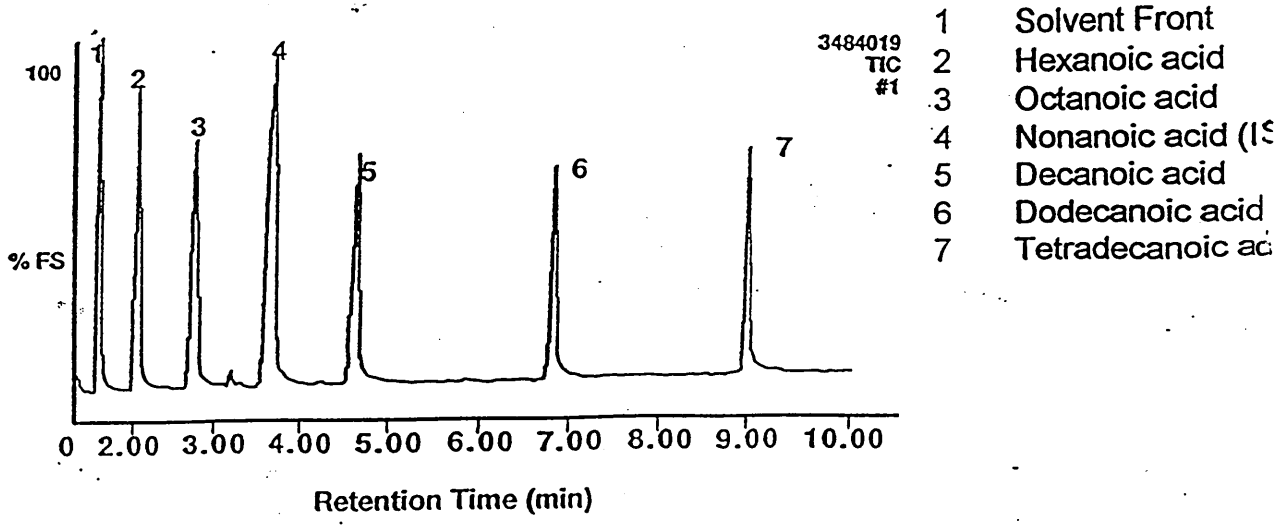


Figure 2.4 Standard Free Fatty Acids Analysed by GC/MS on the Temperature Programme 100-220 °C, at 10°Cmin^{-1}

2.2 TOTAL FATTY ACID CONTENT AS METHYL ESTERS

2.2.1 Procedure for the Extraction of Acylglycerols from Cheese

Duplicate samples were taken from each region of the cheese as described in section 2.5.3. The cheese (3-5 g) was carefully dissected out with a scalpel and weighed into a tared thimble (Whatman 33 × 10 mm diameter) to which Celite (1g) was added, to aid extraction. The cheese and Celite were mixed carefully with a spatula. Great care was taken to avoid losing any material. The glass receiving tank for the Soxhlet apparatus (Tecator soxtec system HT 6) was heated (70 °C) for half an hour, cooled in a dessicator and weighed. The sample was extracted for half an hour with 40-60 °Bp petroleum ether (50 ml) at 85 °C, followed by a 2 hour rinse phase and a final 20 minute evaporation stage. The receiving flask was re-weighed to give the mass of fat extracted. The fat was removed with a Pasteur pipette and put into a glass vial (2.5 ml), which was flushed with nitrogen gas and stored at -18 °C prior to further analysis.

2.2.2 Method for the Conversion of Acylglycerols to Fatty Acid Methyl Esters

Fatty acid methyl esters were produced by the complete esterification method of Hitchcock and Hammond (1980). The extracted oil was weighed (40 mg) into a pear shaped flask (100 ml), using a positive displacement Gilson pipette (50 µl). Undecanoic acid (10-20 mg) was added as internal standard. The esterification reagent (sulphuric acid : toluene : methanol 1:10:20 v/v) (5 ml) was added and the mixture allowed to reflux for one hour. The flask was cooled to room temperature when water (5 ml) and diethyl ether (5 ml) were added. The flask was removed from the condenser, sealed with a glass stopper and shaken (100 times). It was left for 5 minutes to allow the two layers to separate. The lower aqueous layer was aspirated by vacuum and the remaining upper

organic layer dried with anhydrous sodium sulphate overnight at 4 °C. The organic layer was put into a glass sample vial (2.5 ml) and stored at 4 °C.

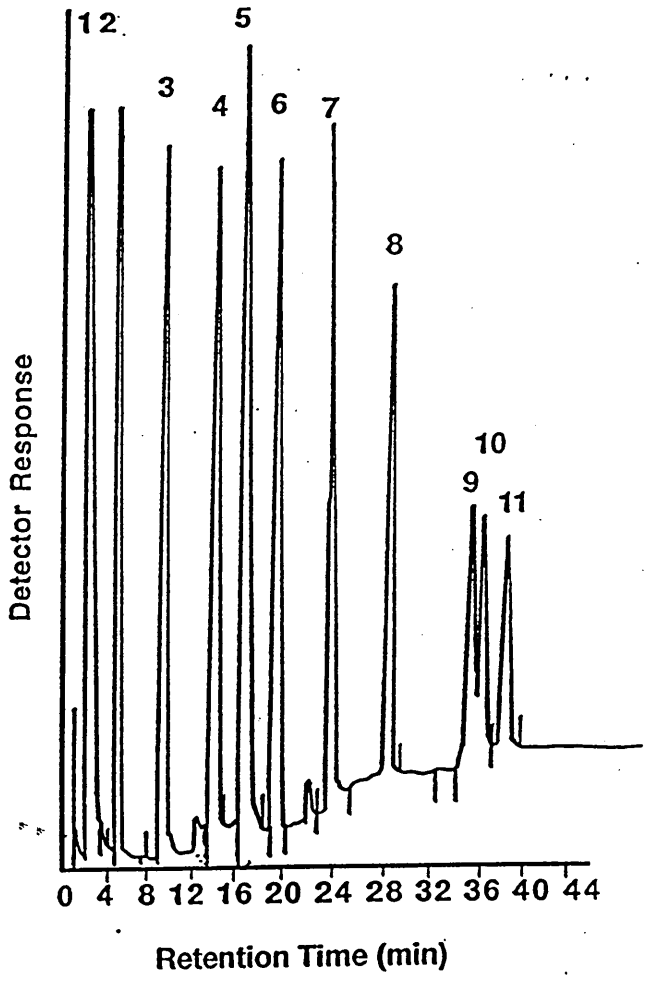
2.2.3 Analysis of Fatty Acid Methyl Esters (FAME's) by Gas Chromatography

Gas chromatographic analysis was carried out on a Varian 3400 gas chromatograph, with a flame ionisation detector. The esterified fat extracts were manually injected (0.2 µl) onto a 2 m × 2 mm i.d. glass packed column (10 % FFAP on chromosorb WAW 80-100 mesh). Nitrogen was the carrier gas with the flow rate fixed at 20 ml min^{-1}. The injector block was heated to 220 °C and the detector to 300 °C, with a range of 10^{-10} and attenuation of 4. The column was programmed from 110° to 220 °C at a rate of 5 °Cmin^{-1}, with an initial hold of 2 minutes. The chromatograms were recorded on a Pye Unicam PU 4810 integrator. The gas chromatograph was calibrated on a set of fatty acid methyl esters C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C18:1, C18:2 (40 mg ml^{-1} each) and C11:0 internal standard (80 mg ml^{-1}). The response factors of the homologous series of fatty acid methyl esters were automatically determined by use of a fixed programme in the integrator with respect to C11:0. The fatty acids were identified by comparison to the retention times of the standard methyl esters. The concentration of each acid in the sample was expressed as mg fatty acid per g of oil or as weight percent. Figure 2.5 demonstrates the standard fatty acid methyl ester solution when analysed by GC under the conditions described above.

Table 2.4 Retention Times, Relative Retention Times and Response Factors for the Standard Fatty Acid Methyl Esters Analysed by Gas Chromatography

Pk No	Peak Identification	Retention time t_R (mins)	Rel. retention time t_{RR}	Response factor
1	Solvent front	1.97	0.12	-
2	Hexanoic acid methyl ester	4.99	0.305	1.075
3	Octanoic acid methyl ester	9.12	0.557	0.994
4	Decanoic acid methyl ester	13.95	0.852	1.002
5	Undecanoic acid methyl ester	16.37	1.000	1.000
6	Dodecanoic acid methyl ester	18.73	1.144	1.007
7	Tetradecanoic acid methyl ester	23.17	1.415	1.045
8	Hexadecanoic acid methyl ester	27.75	1.695	1.044
9	Octadecanoic acid methyl ester	34.76	2.123	1.238
10	Octadecenoic acid methyl ester	35.72	2.182	1.161
11	Octadecadienoic acid methyl ester	37.96	2.319	1.158

Temperature programme 110 - 220 °C, ramp 5 °Cmin^{-1}
This data refers to Figure 2.5



1 Solvent Front
2 Hexanoic acid
3 Octanoic acid
4 Decanoic acid
5 Undecanoic acid (IS)
6 Dodecanoic acid
7 Tetradecanoic acid
8 Hexadecanoic acid
9 Octadecanoic acid
10 Octadecenoic acid
11 Octadecadienoic acid

Figure 2.5 Standard Fatty Acid Methyl Esters Analysed by GC on the Temperature Programme 110-220 °C, at 5°Cmin^{-1}

2.2.4 Extraction of the Acylglycerols from Cheese for Gas Chromatography / Mass Spectrometry Analysis

The method described by Bligh and Dyer (1959) was adopted. Cheese (4 g) was weighed (in duplicate) into a 250 ml conical flask. Chloroform (4 ml) and methanol (8 ml) were added to each flask and the contents homogenised for 2 minutes at full-power with a Polytron. A further 4 ml chloroform was added and the mixture homogenised for 30 seconds before addition of water (10 ml) followed by homogenisation (30 s). The mixture was filtered through a Whatman No 1 filter paper (under vacuum) and the residue washed with 3 x 10 ml $CHCl_3$. The lower organic layer was separated and dried over anhydrous sodium sulphate at 4 °C overnight. The solvent was removed under vacuum at 40 °C and the sample taken to dryness with nitrogen gas. The aqueous layer from the chloroform : methanol extraction was acidified with HCl (2M) to give a final pH 2.5 and then extracted with 3 x 25 ml ethyl ether. The upper organic layer was separated, dried overnight with sodium sulphate at 4 °C and reduced to 250 µl with N_2 gas. All samples were stored in glass vials at 4 °C.

2.2.5 Method for the Conversion of the Acylglycerols to Fatty Acid Methyl Esters for Analysis by Gas Chromatography / Mass Spectrometry

The method was adapted from Hitchcock and Hammond (1980). Lipid (0.5 g) and methylating agent 50 ml (H_2SO_4 98%: Toluene: Methanol 1:10:20 v/v) were refluxed for 1 hour in a round bottom flask (250 ml), fitted with a condenser. Distilled water (100 ml) was added. The solution was extracted with 3 x 50 ml diethyl ether in a separating funnel. The upper ether layer was dried with anhydrous sodium sulphate at 4 °C and reduced in volume (≈ 10 ml) with nitrogen gas.

2.2.6 Analysis of Fatty Acid Methyl Esters (FAME's) by Gas Chromatography / Mass Spectrometry

The capillary column (All Tech) SE 30 30 m x 0.32 mm i.d. film thickness 0.25 µm, was run from 100°C to 200°C at 10°Cmin^{-1}. The source (electron

impact) was at 200°C and the carrier gas was helium. A 0.2 µl sample was manually injected with a split injector held at 250°C. Table 2.5 shows the retention times and relative retention times of the fatty acid methyl esters when analysed under the above conditions. Figure 2.6 demonstrates the separation of the standard solution.

Table 2.5 Retention Times and Relative Retention Times for the Standard Fatty Acid Methyl Esters Analysed by GC/MS

Pk No	Peak Identification	Retention Time t_R (mins)	Rel. Retention Time t_{RR}
1	Solvent front	1.03	0.199
2	Hexanoic acid methyl ester	1.45	0.280
3	Octanoic acid methyl ester	2.38	0.459
4	Decanoic acid methyl ester	4.08	0.788
5	Undecanoic acid methyl ester (IS)	5.18	1.000
6	Dodecanoic acid methyl ester	6.30	1.216
7	Tetradecanoic acid methyl ester.	8.49	1.639
8	Hexadecanoic acid methyl ester	10.70	2.066
9	Octadecadienoic acid methyl ester	12.30	2.375
10	Octadecanoic acid methyl ester	12.72	2.456

Temperature programme 100°C - 200°C with a 10°Cmin^{-1} ramp
This data refers to Figure 2.6

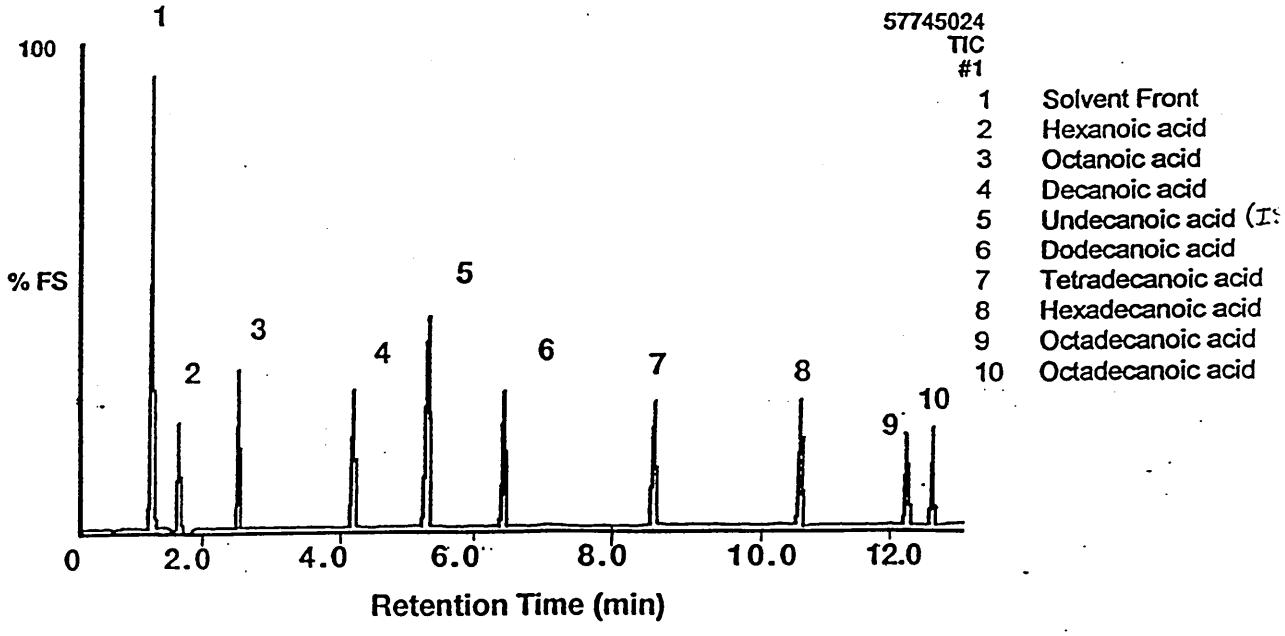


Figure 2.6 Standard Fatty Acid Methyl Esters Analysed by GC/MS on the Temperature Programme 100-200 °C with a 10 °Cmin^{-1} ramp

2.3 METHOD FOR THE DETERMINATION OF pH OF CHEESE

To measure the pH of the different regions of the cheese a Philips combined electrode, Pye Unicam (CE2, 4 mm) was placed directly into the cheese sample for 30 seconds to equilibrate, then a reading was taken. Care was taken to ensure that the probe was wet before insertion into the cheese. For each cheese piece five readings were taken from each region (blue and white regions for Bleu d'Auvergne and surface and centre for the Brie and Vacherin-type cheeses). The probe was washed with glass distilled water and re-calibrated with pH 7.0 and 4.0 buffer solutions after 5 readings. After use the probe was cleaned in pepsin/HCl to prevent the protein in the cheese from blocking the membrane of the pH probe. The average of the five readings was calculated.

2.4 METHOD FOR THE DETERMINATION OF THE FAT CONTENT OF CHEESE

The BS 696 Gerber method was used to determine the fat content in each region of cheese. The analysis was carried out in duplicate. Sulphuric acid (10 ml) was placed into a cheese butyrometer with a graduated pipette. Distilled water (6 mm layer) was added carefully. The cheese sample (3.0 ± 0.001 g) was ground to a pulp in a beaker then placed in the butyrometer, followed by amyl alcohol (1 ml) and distilled water (5 ml). The butyrometer was sealed and shaken in a stand to mix the contents thoroughly and then placed in a water bath (65 °C) for five minutes. The fat layer was separated by centrifugation (1100 ± 100 revs min^{-1}) for 12 minutes. The butyrometer was heated in the water bath (65 °C) for five minutes, inverted and the percentage fat read directly from the scale. If the fat level was off scale, additional water was added

to bring it on to scale. Readings were checked after heating for an additional three minutes. The average of the four readings was calculated.

2.5 EXPERIMENTS UNDERTAKEN

Analysis of the free medium chain fatty acids was carried out for two blue mould-ripened cheeses (Fourme d'Ambert and Bleu d'Auvergne), and two white mould-ripened cheeses (Brie de Pays and Vacherin-type cheeses). The pH and fat content (percent) were also determined. These cheeses were analysed at the point of sale. The work was continued with a simulated ripening experiment where the pH, fat content, free medium chain fatty acids and total fatty acid composition were determined. The cheeses were incubated at 12 °C for 8 weeks. One blue cheese (Bleu d'Auvergne) and one white cheese (Brie de Pays) were analysed.

The analysis of **free medium** chain fatty acids at the point of purchase includes **tetradecanoic acid**, although it is realised that this is technically a longer chain fatty acid.

2.5.1 Experiment to Analyse Cheeses at the Point of Purchase

a) Fourme d'Ambert

Three slices (130 g each) of Fourme d'Ambert were purchased from a supermarket on the 28/ 6/92 and 30/ 6/92. A third sample, a half cheese (≈ 950 g) was purchased from a wholesale supplier. All pieces of cheese were removed from their polyethylene packaging. The crust of the Fourme d'Ambert cheeses was brown. The slightly yellow or creamy-coloured cheeses had dark green veining thoughout. The clean, milky smell of Blue cheese was especially evident in the Fourme d'Ambert cheeses.

b) Bleu d'Auvergne

One half moon shaped piece of cheese (1180 g) was purchased on 26/11/92 from a wholesale supplier in London (use by date 1/ 1/93). Upon removal of the foil covering, the mould (*Penicillium roquefortii*) could be seen growing sporadically throughout the mass of the cheese. It had a firm, hard texture and very little odour, except for a pleasant milky smell.

c) Brie de Pays

Three 500 g pieces of Traditional French Brie were purchased on 10/12/92 (use by date 20/12/92). A fourth 500 g piece was purchased on 27/ 2/93 (use by date 7/ 3/93). All cheeses which had been made from unpasteurised milk were purchased from the same supermarket in Sheffield. The surface of each wedge was densely covered in *P. camembertii.* They had a firm cream coloured texture, and the milky fresh odour associated with Brie cheese was detected.

d) Vacherin Mont d'Or

Two whole cheeses (2 kg) were purchased on 4/12/92 and 29/ 1/93 from a Delicatessen in Sheffield. Each arrived encased in a circular wooden box. A 4 mm layer of pine bark was found on the perimeter of the cheese. The first cheese was slightly under-ripe. The surface of this cheese was covered in a beige coloured, slightly crumpled-looking crust. The centre of the cheese consisted of a creamy-coloured paste, which upon cutting was very runny. The cheese also had a pleasant earthy, nutty, mushroom-like odour. The second cheese was much riper than the first. The surface was covered in a harsher brown/orange crust, whilst the paste was again creamy and very runny. The cheese had an extremely unpleasant odour of stale vegetables and natural fertilizer.

e) Vacherin du Haut Doubs (Mont d'Or)

A third cheese was analysed. It is essentially the same as Vacherin Mont d'Or but due to legal reasons, as it is produced on the French side of the France / Switzerland border and not in Switzerland, it can not be called Vacherin Mont

d'Or. The half cheese (1083 g) was purchased from the International Cheese Centre in London on 13 /11/92. It was covered in two layers of polyethylene film, which were removed to expose the layer of pine bark. The surface of this over-ripe cheese consisted of a beige/orange crust. The paste was again creamy and liquidy upon cutting into it. The cheese had an over-powering odour, which emphasised the extreme over-ripeness of the cheese.

2.5.2 Experiment to Analyse Cheeses following Extended Ripening

a) Bleu d'Auvergne

Two half Bleu d'Auvergne cheeses (1442 and 1435 g) were purchased from a wholesale supplier in London on 28/ 6/93. Each cheese was unwrapped on receipt and placed on plastic gauze standing on 3 Petri-dishes inside a plastic cake box. The lid was placed on each box which was put in an incubator at 12 °C ± 0.5 °C. The first cheese was sampled upon purchase and after 7 and 14 days. The second cheese was sampled after 27 and 56 days.

b) Brie de Pays

Three 500 g pieces of French Brie were purchased from a local supermarket in Sheffield on 15/ 3/93 (use by date 31/ 3/93). Each cheese was unwrapped on receipt and treated as above. The first cheese was analysed upon purchase and after 7 days. The second was analysed after 14 and 27 days and the third cheese after 56 days.

2.5.3 Sampling the Cheese

Two regions of each cheese were analysed - the region of obvious mould growth and the region of no obvious mould growth. This was carried out to observe any differences in the cheese itself which may be due to the presence of the mould, whether it be on the surface or in the mass of the cheese.

a) Fourme d'Ambert and Bleu d'Auvergne

A portion of the cheese was cut away using a scalpel. The veined regions of the cheese (blue) containing spores of *Penicillium roquefortii* was separated from the remaining cheese with no obvious mould growth (white).

b) Brie

A cork borer (number 5) was inserted vertically into the cheese and a cylinder of cheese removed. It included the obvious mould growth on the surface and the creamy centre. The surface and centre were separated to give the two regions of analysis for the French Brie cheese.

c) Vacherin Mont d'Or

This cheese type was also analysed at the surface and centre. The crust was removed from the cheese with a scalpel. The creamy paste was scooped out. The crust contained obvious mould growth, whilst the paste did not.

2.6 CHEMICALS

Sigma grade fatty acid and fatty acid methyl esters were obtained from Sigma Chemical Company Ltd (Fancy Road, Poole, Dorset). Solvents were purchased from Fisons Scientific Equipment (Bishop Meadow Rd, Loughborough, Leicestershire), BDH Chemicals Ltd (Poole, Dorset), Fahrenheit Laboratory Supplies (Bishop Meadow Rd, Loughborough, Leicestershire) and Romil Chemicals Ltd. (Ashby Rd, Shepshed, Loughborough). Buffers were obtained from Fisons Scientific Equipment (Bishop Meadow Rd, Loughborough, Leicestershire). GC columns were obtained from Phase Separations (Deeside Industrial Park, Deeside), and GC/MS column was obtained from All Tech (Carnforth, Lancashire).

CHAPTER 3

IDENTIFICATION OF FATTY ACID METHYL ESTERS AND FREE FATTY ACIDS BY GAS CHROMATOGRAPHY / MASS SPECTROMETRY

3.1 IDENTIFICATION OF TOTAL FATTY ACID METHYL ESTERS FOLLOWING ESTERIFICATION OF THE LIPID SOLUBLE MATERIAL IN BLEU D'AUVERGNE AND BRIE CHEESE

Figures 3.1 and 3.2 show the total ion chromatogram of all components present in the esterified lipid soluble fraction of Bleu d'Auvergne and Brie cheese, demonstrating a complex fraction. When the mass spectra of the individual peaks were compared to the standard mass spectra, the components could be divided into three major groups. The identification of the peaks is given below. The groups were :-

i) saturated straight chain aliphatic fatty acid methyl esters of odd and even chain length,
ii) monoenoic aliphatic fatty acid methyl esters with the double bond at the 4th or 9th carbon,
iii) methyl branched saturated aliphatic fatty acid methyl esters.

3.1.1 Identification of Saturated Fatty Acid Methyl Esters by GC/MS

Identification of a compound is usually based upon the characteristic fragmentations (seen as peaks) which have occurred during analysis in the mass spectrometer. The following characteristic peaks for saturated alphatic methyl esters could be seen. Firstly, M^+ the apparent molecular ion of the saturated fatty acid methyl ester was seen in both the standard solution and the two lipid extracts. This also indicated the relative molecular mass of the individual fatty acid methyl esters.

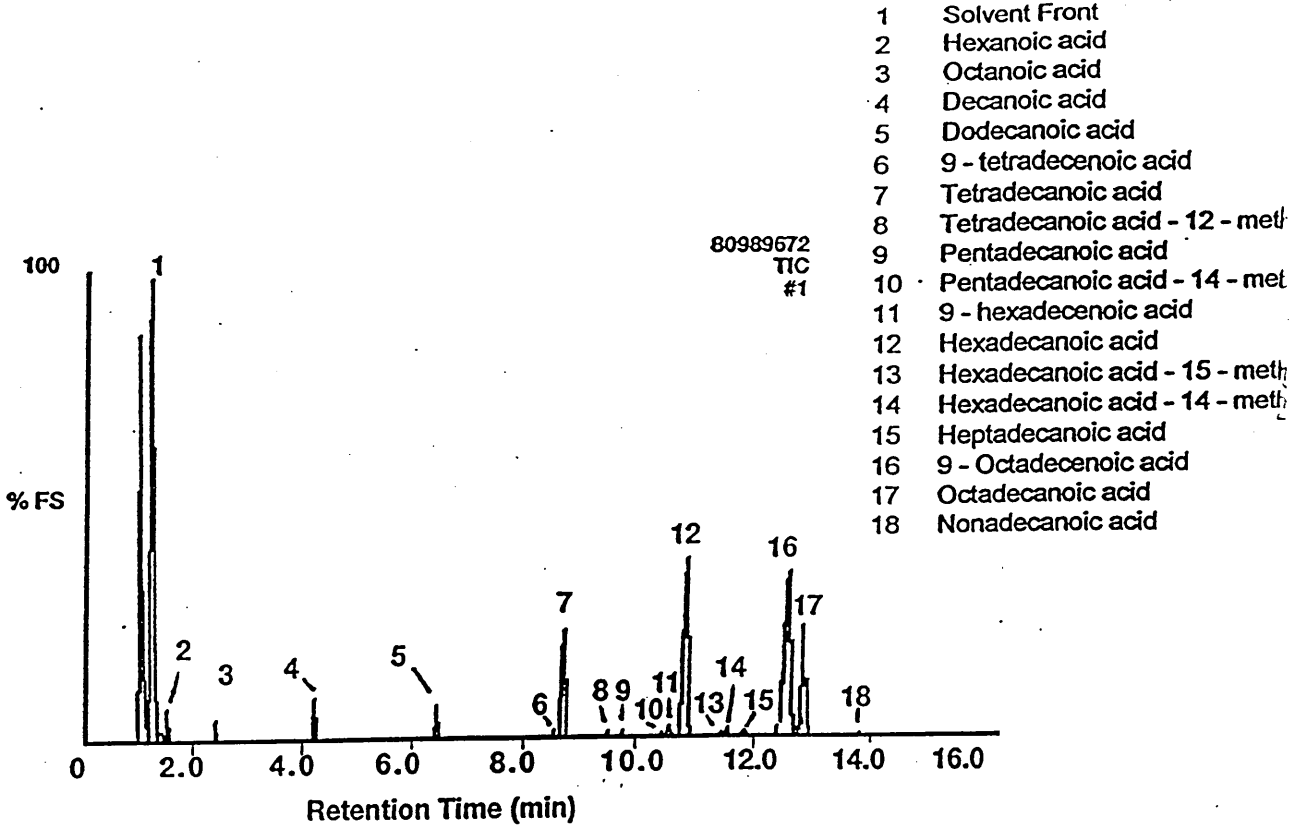


Figure 3.1 Total Ion Chromatogram of the Lipid Extract from Bleu d'Auvergne Cheese Analysed by GC/MS at 100 - 200 °C, with a 10 °Cmin^{-1} ramp

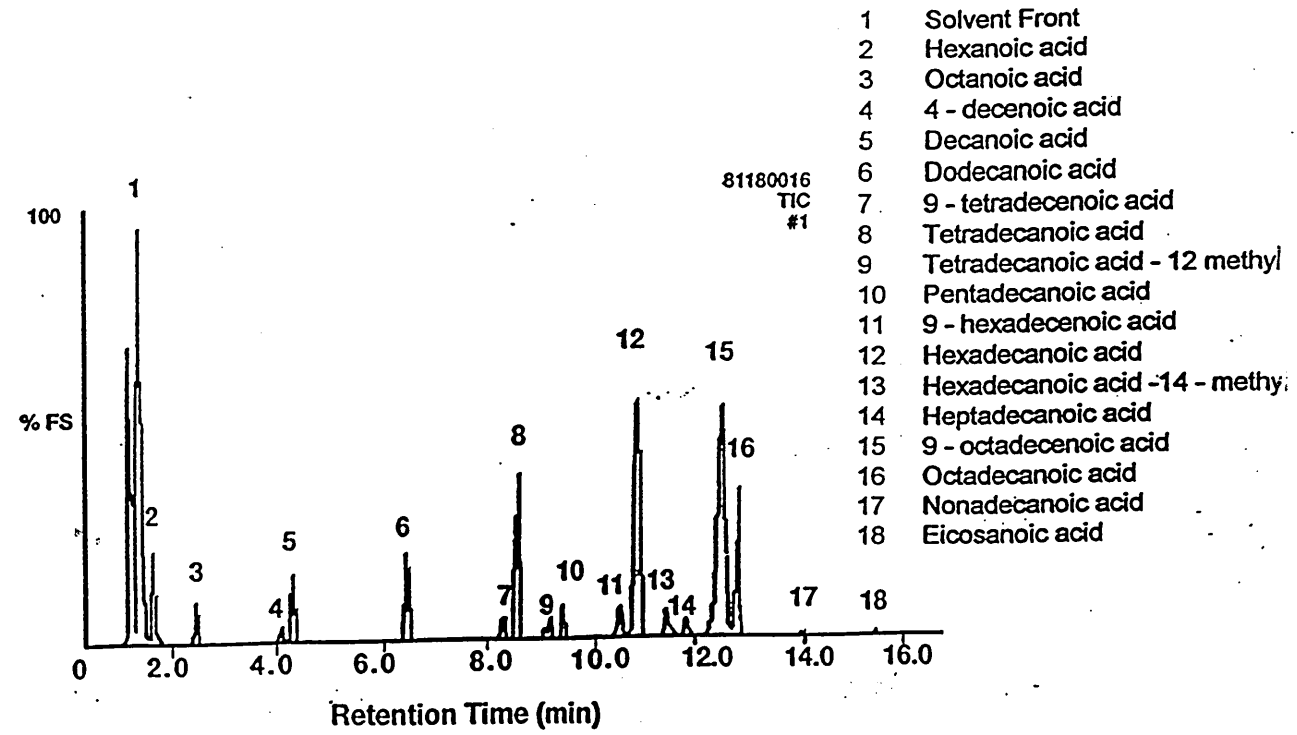


Figure 3.2 Total Ion Chromatogram of the Lipid Extract from Brie Cheese Analysed by GC/MS at 100 - 200 °C, with a 10 °Cmin $^{-1}$ ramp

Three other fragmentation peaks were of significance in identification of the saturated fatty acid methyl esters. They appeared at m/z 74, M^+- 31 and m/z 59. The first peak, m/z 74, with an even mass number indicated that a rearrangement of the molecule had occurred. Indeed it was the product of a McLafferty rearrangement. This is one of the key peaks in identification of saturated fatty acid methyl esters :-

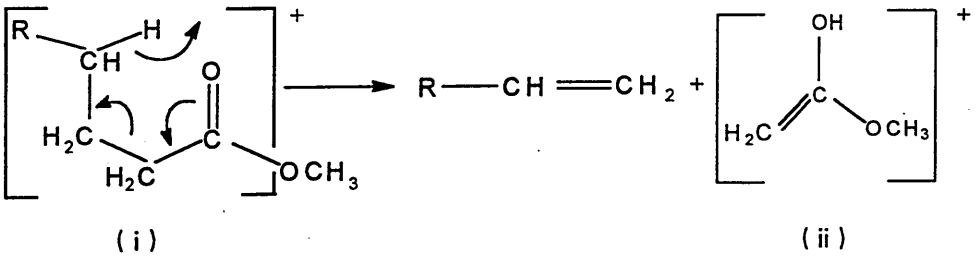
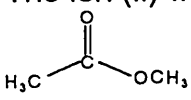


Figure 3.3 Mc Lafferty Rearrangement of a Fatty Acid Methyl Ester, Resulting in a Peak of m/z 74

The ion (ii) was very stable, but could have undergone resonance to give 

. The peak at m/z 74 was seen as the base peak, the most abundant peak on the chromatogram, for all saturated fatty acid methyl esters. The second characteristic peak at M^+- 31 was due to the loss of the fragment $-OCH_3$ and the third at m/z 59, due to the fragment $-CO_2CH_3$. In addition, peaks at M^+-29 $-(CH_2)_n$, indicated the presence of the aliphatic carbon chain, but would not identify saturated fatty acid methyl esters alone. As the fatty acid methyl esters increased in size more fragmentations occurred, hence the larger fragments were not observed or seen as very small peaks. Tables 3.1 and 3.2 show the identification of the saturated fatty acid methyl ester peaks in Bleu d'Auvergne and Brie. In both cheeses the range of saturated FAME's identified was from hexanoic to eicosanoic acids. The most abundant peaks were hexadecanoic, octadecanoic and tetradecanoic acids. Odd-number FAME's were also detected, namely pentadecanoic, heptadecanoic and nonadecanoic fatty acid methyl esters.

The absence of a peak at M^+- 32 (expected to only be seen in monounsaturated fatty acids methyl esters) (Section 3.1.2) in the spectra of saturated fatty acid methyl esters gave further evidence for these peaks to be saturated.

Table 3.1 Identification of Saturated Fatty Acid Methyl Esters by GC/MS, in the Lipid Extract of Bleu d'Auvergne Cheese

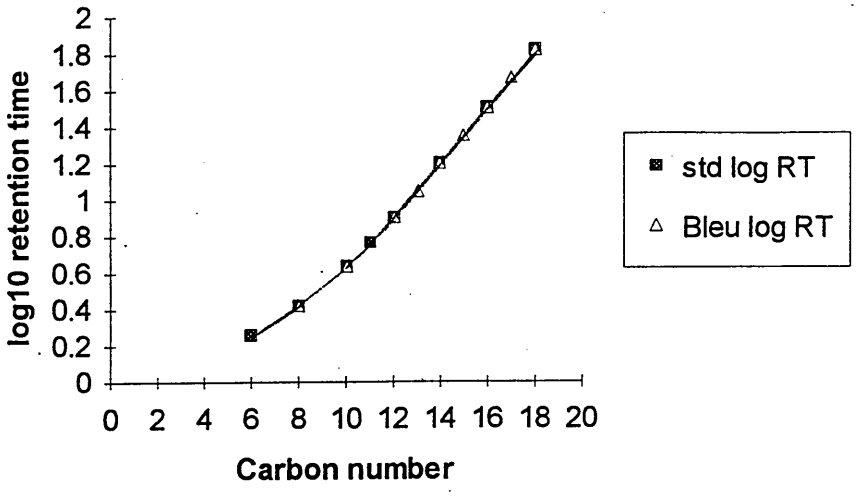
Pk no †	M^+	M^+ -31	M^+- 29 -$(CH_2)_n$	t_R std	t_R peak	library % id	Peak Identity
2	-	99	101,87,73	1.45	1.45	92	Hexanoic acid
3	-	127	129,115,101,87	2.37	2.35	98	Octanoic acid
4	186	155	157,143,129,115, etc	4.08	4.08	91	Decanoic acid
5	214	183	185,171,157,143,129, etc	6.30	6.28	90	Dodecanoic acid
7	242	211	213,199,185,171,157, etc	8.57	8.57	93	Tetradecanoic acid
9	256	225	227,213,199,185,171, etc	-*	9.62	93	Pentadecanoic acid
12	270	239	241,227,213,199,185, etc	10.7	10.73	81	Hexadecanoic acid
15	284	23	255,241,227,213,199, etc	-*	11.68	89	Heptadecanoic acid
17	298	267	269,255,241,213,199, etc	12.73	12.73	no id	Octadecanoic acid
18	312	281	269,227,199,143,129, etc	-*	13.87	77	Nonadecanoic acid

† - refers to peaks in figure 3.1

Table 3.2 Identification of Saturated Fatty Acid Methyl Esters by GC/MS, in the Lipid Extract of Brie Cheese

Pk no ‡	M^+	M^+ -31	M^+- 29 -$(CH_2)_n$	t_R std	t_R peak	library % id	Peak Identity
2	-	99	101,87	1.45	1.47	94	Hexanoic acid
3	-	127	129,115,101,87	2.37	2.35	97	Octanoic acid
5	-	155	157,143,129,115,101,87	4.08	4.08	90	Decanoic acid
6	214	183	185,171,157,43,129, etc	6.30	6.30	88	Dodecanoic acid
8	242	211	213,199,185,171,157, etc	8.57	8.60	93	Tetradecanoic acid
10	256	225	227,213,199,185,171, etc	-*	9.63	93	Pentadecanoic acid
12	270	239	241,227,213,199,185, etc	10.70	10.78	80	Hexadecanoic acid
14	284	253	255,241,199,185,143, etc	-*	11.68	89	Heptadecanoic acid
16	298	-	129,101	12.73	12.82	no id	Octadecanoic acid
17	312	281	269,227,213,199,185, etc	-*	13.88	84	Nonadecanoic acid
18	326	295	297,283,241,227,199, etc	-*	15.37	88	Eicosanoic acid

* - These fatty acid methyl esters were not contained in the standard solution
‡ - refers to peaks in figure 3.2
Temperature programme 100 - 200 °C, with a 10 °Cmin $^{-1}$ ramp



Isothermal analysis at 180 °C

Figure 3.6 Graph of Log_{10} Retention Time against Carbon Number for Standard Fatty Acid Methyl Esters and Bleu d'Auvergne Cheese to Identify the Odd-Chain Methyl Esters

As highlighted in the footnote at the bottom of the tables, the odd-chain fatty acid methyl esters were not included in the standard solution of FAME's. To establish that these peaks were in fact odd-chain fatty acid methyl esters, the FAME's were analysed on the gas chromatograph under isothermal conditions at 180 °C. A graph was then plotted of the log_{10} retention time versus carbon length of the standard even chain fatty acid methyl esters. This plot allowed the expected retention time for the odd-chain length fatty acid methyl esters to be read directly from the graph.

The mass spectrum of hexadecanoic acid methyl ester analysed by GC/MS at 100 - 200 °C, with a 10 °Cmin^{-1} ramp from a standard solution and the Bleu d'Auvergne cheese are given in Figures 3.4 and 3.5, to illustrate the characteristic peaks discussed above.

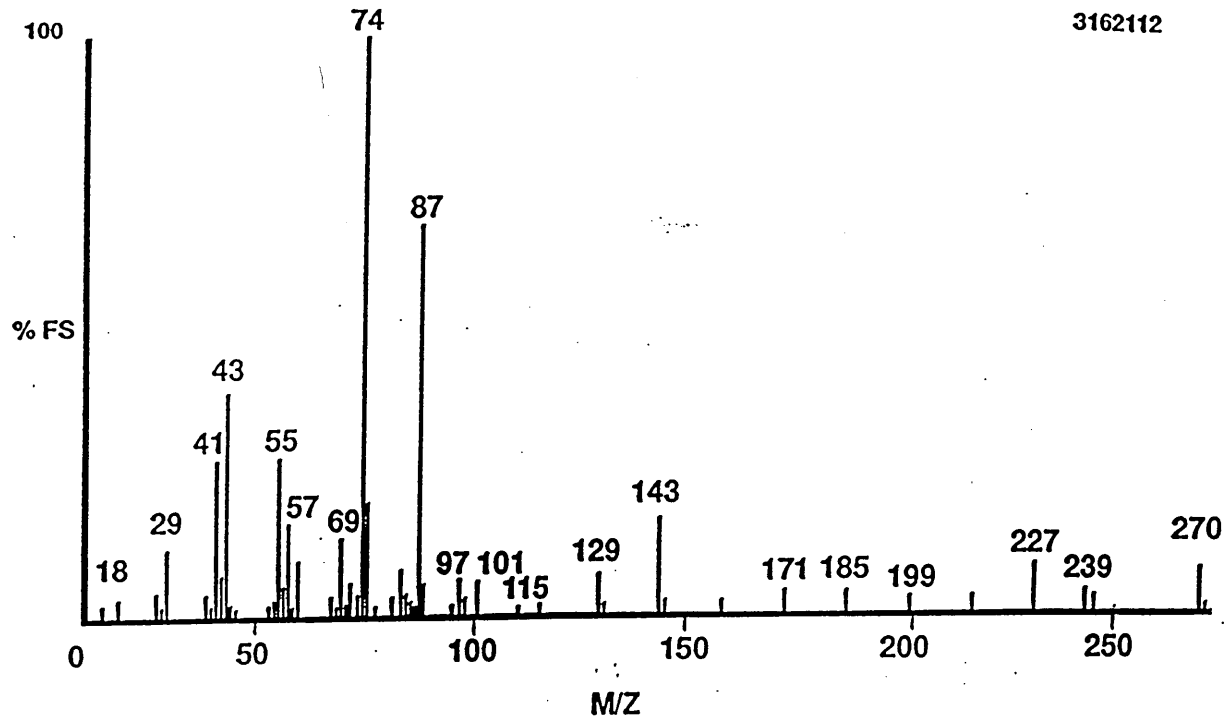


Figure 3.4 Standard Hexadecanoic Acid Methyl Ester Analysed by GC/MS from 100 - 200 °C, with a 10 °Cmin^{-1} ramp

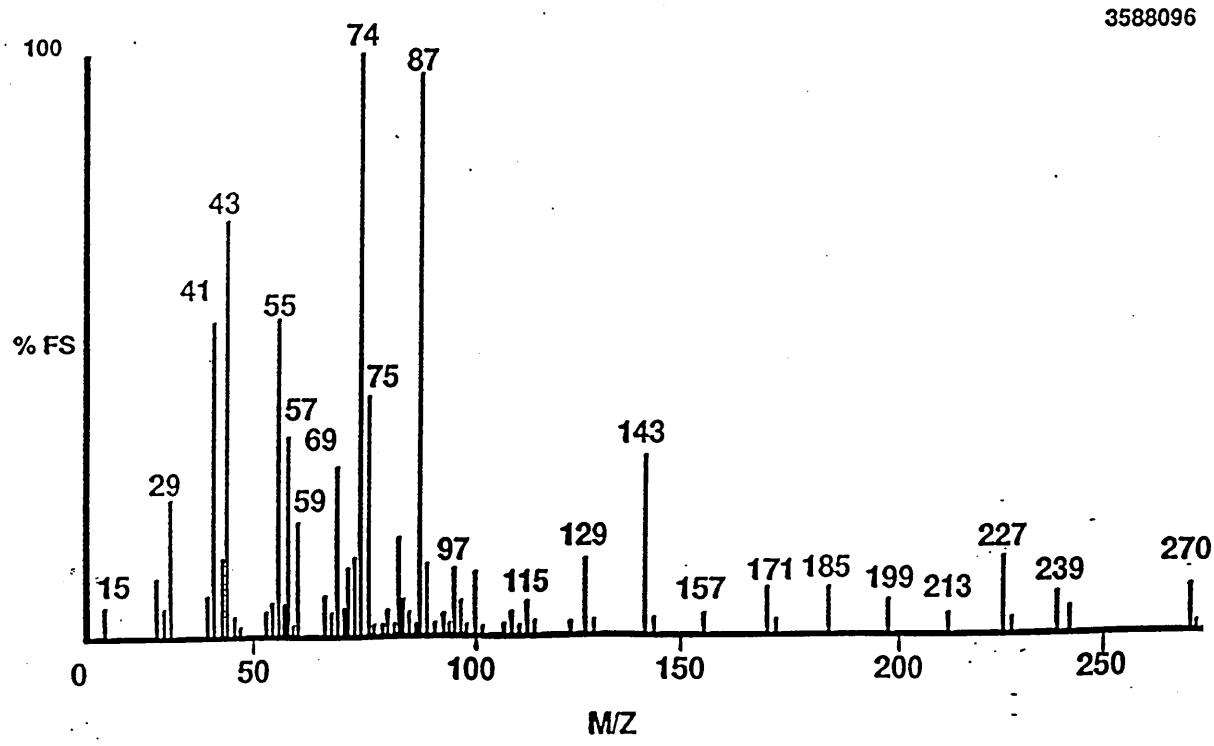
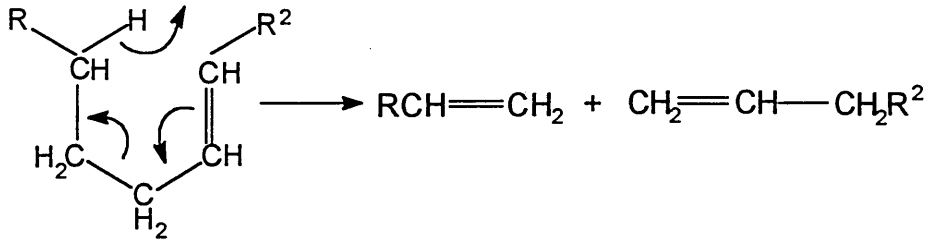


Figure 3.5 Hexadecanoic Acid Methyl Ester Detected in the Lipid Extract of Bleu d'Auvergne Cheese when Analysed by GC/MS at 100 - 200 °C, with a 10 °Cmin^{-1} ramp

3.1.2 Identification of Monoenoic Fatty Acid Methyl Esters by GC/MS

The mass spectrum of 9 -octadecenoic acid from a standard solution and the lipid fraction of Bleu d'Auvergne cheese are included to illustrate the following characteristic peaks of monounsaturated fatty acid methyl esters (Figures 3.7 and 3.8). The monoenoic fatty acid methyl esters were eluted before their saturated counterparts due to the greater polarity of the C=C on the non-polar stationary phase (OV-1). There were 6 significant peaks in identification of monoenoic fatty acid methyl esters : The base peak at m/z 55, M^+, M^+- 31, M^+- 32, M^+- 74 and m/z 74.

The base peak for all monounsaturated acids at m/z 55, may have been formed by addition of a hydrogen atom to the very reactive aliphatic carbon adjacent to the double bond. This peak also appeared in the spectra of saturated fatty acid methyl esters, wever it was not the base peak there, so a difference was seen between the saturated and unsaturated methyl esters. The mass ion (M^+) was two mass units less than that of the corresponding saturated fatty acid methyl esters due to the absence of 2 hydrogen atoms. The peak M^+- 31 was present due to the loss of -OCH_3, whilst the peak M^+- 32 was seen due to the loss of -$HOCH_3$. The peak (M^+- 32) was a significant peak in differentiating the monounsaturated from the saturated fatty acid methyl esters as discussed in Section 3.1.1. The hydrogen atom could have been released during one of the many fragmentations which occurred because of the double bond, and then picked up by the ester group (-$HOCH_3$) . Finally, the peaks m/z 74 and M^+- 74 (H_3C–C(=O)–OCH_3) were also seen. The peak m/z 74 was probably produced during a McLafferty rearrangement similar to that described in Figure 3.4. Many other rearrangements could have taken place. For example, a different McLafferty rearrangement leading to the following fragments :-



where $R = (CH_2)_4CH_3$
and $R_2 = (CH_2)_7CO_2CH_3$

Figure 3.9 A further Example of a McLafferty Rearrangement

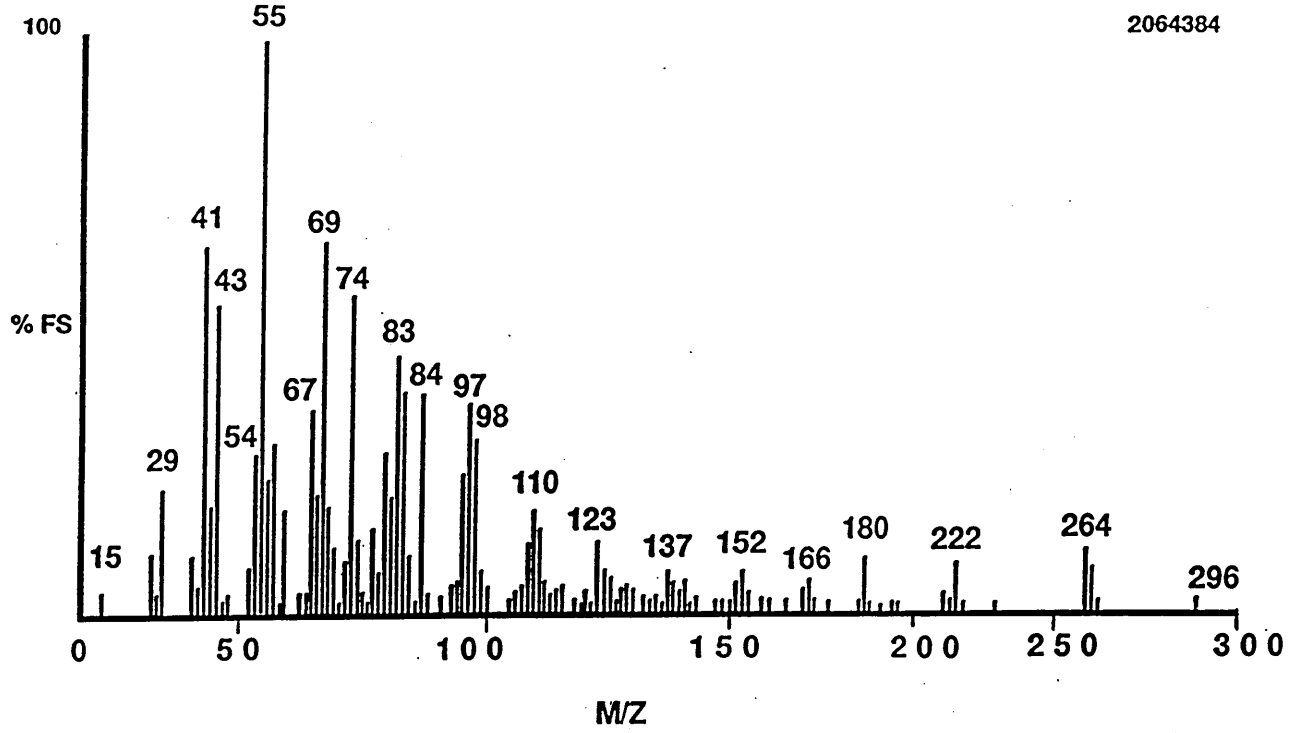


Figure 3.7 Standard Octadecenoic Acid Methyl Ester Analysed by GC/MS from 100 - 200 °C, with a 10 °Cmin^{-1} ramp

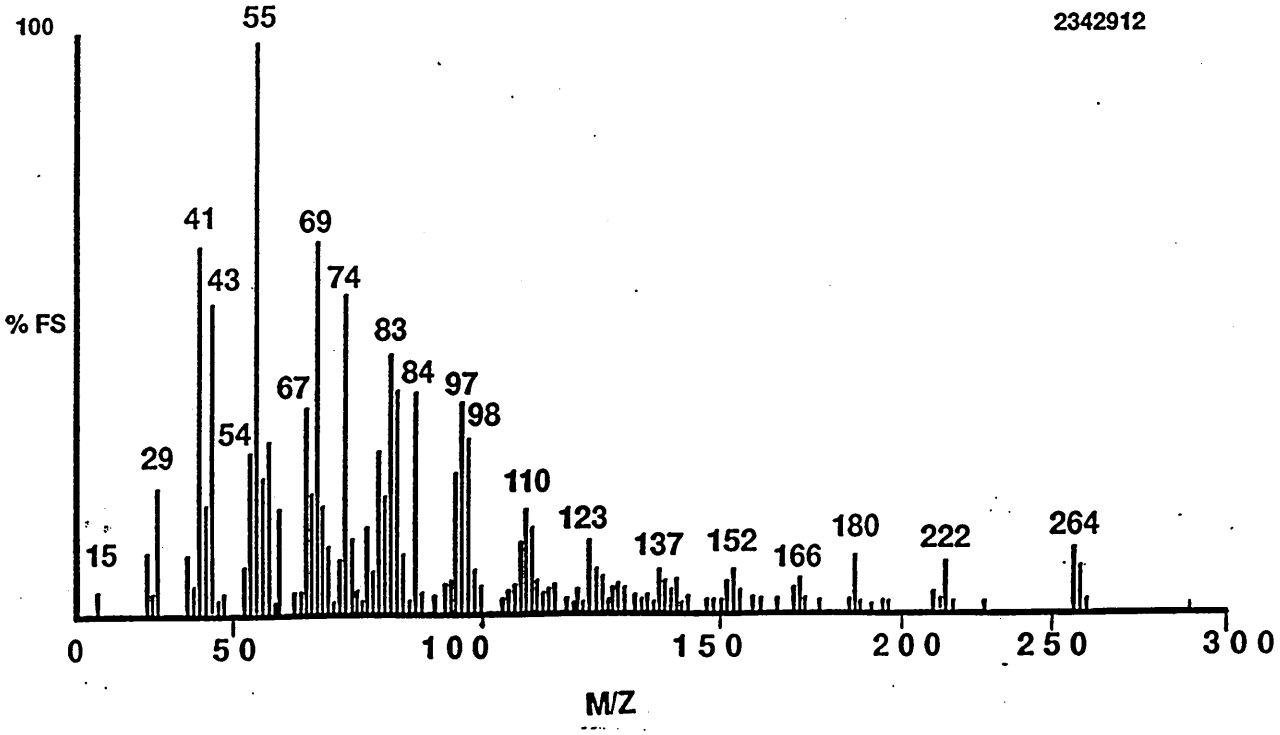


Figure 3.8 Octadecenoic Acid Methyl Ester Detected in the Lipid Extract of Bleu d'Auvergne Cheese Analysed by GC/MS, at 100 - 200 °C, with a 10 °Cmin $^{-1}$ ramp

Tables 3.3 and 3.4 illustrate the identification of mono-unsaturated fatty acid methyl esters in Bleu d'Auvergne and Brie. In both cheeses, the most abundant monounsaturated fatty acid methyl ester was definitely 9- octadecenoic acid, whilst 9- tetradecenoic acid and 9- hexadecenoic acids were present as much smaller peaks. No other monounsaturated FAME's were detected in the Bleu d'Auvergne cheese, but 4- decenoic acid was detected in the Brie cheese, probably a product of the breakdown of 9- octadecenoic acid. The monounsaturated fatty acid methyl esters which were detected, were in the common cis configuration rather than the trans configuration (computer identification library).

Table 3.3 Identification of the Mono-Unsaturated (monoenoic) Fatty Acid Methyl Esters by GC/MS, in the Lipid Extract of Bleu d'Auvergne Cheese

Pk No†	M^+	M^+ -31	M^+ -32	M^+ -74	M^+- 74 -$(CH_2)_n$	RT std	RT peak	library % id	Peak identity
6	240	209	208	166	152,138,124,96,82, etc	- *	8.35	no id	cis-9-tetradecenoic acid
11	268	237	236	194	166,152,138,124,110,etc	- *	10.42	93	cis-9-hexadecenoic acid
16	296	265	264	222	222,180,166,152,138 etc	12.30	12.43	98	cis-9-octadecenoic acid

Table 3.4 Identification of the Mono-Unsaturated (Monoenoic) Fatty Acid Methyl Esters by GC/MS in the Lipid Extract of Brie Cheese

Pk No‡	M^+	M^+ -31	M^+ -32	M^+ -74	M^+- 74 -$(CH_2)_n$	RT std	RT peak	library % id	Peak identity
4	-	153	152	110	96, 82, 68, 54, 40	- *	3.95	70	4-dececenoic acid
7	240	209	208	166	152,138,124,110,96,82	- *	8.37	no id	cis-9-tetradecenoic acid
11	268	237	236	194	166,152,138,124,110,96	- *	10.43	95	cis-9-hexadecenoic acid
15	296	265	264	222	180,166,152,138,124	12.30	12.48	98	cis-9-octadecenoic acid

* - These components were not included in the standard solution
† - refers to the peaks in figure 3.1
‡ - refers to the peaks in figure 3.2
100-200 °C, with a 10 °Cmin $^{-1}$ ramp

3.1.3 Identification of Branched Chain Saturated Fatty Acid Methyl Esters

The mass spectrum of each monobranched alkyl fatty acid was identical to the equivalent unbranched fatty acid methyl ester. Both the saturated branched and unbranched components had the same molecular ion, due to the branch

always being a methyl group. The basis for identification of these branched chain saturated fatty acid methyl esters was retention time only (Table 3.5). The much smaller branched peaks were eluted before the corresponding unbranched peaks because they had a more tightly packed structure which increased their volatility. Tetradecanoic acid 12- methyl methyl ester and hexadecanoic acid 14- methyl methyl ester were observed in both cheeses. Pentadecanoic acid 14- methyl methyl ester and hexadecanoic acid 15- methyl methyl ester were seen in Bleu d'Auvergne only. The computer library of the mass spectrometer identified these peaks with the following probabilities (Table 3.5). To definitely identify these peaks, analysis by an alternative technique e.g. nuclear magnetic resonance (nmr) would need to be carried out. Although no chromatograms of standard branched methyl esters were obtained, the indication of the branched nature of the esters is provided by the retention time. These branched chain fatty acid methyl esters have also been identified in cheeses by other authors (Christie 1982).

Table 3.5 Percent Probability of Identification and Retention Time of the Branched Chain Fatty Acids

	Bleu d'Auvergne		*Brie*	
Fatty acid methyl ester	**% id**	**t_R**	**% id**	**t_R**
Tetradecanoic acid 12-methyl	91	9.32	92	9.25
Pentadecanoic acid 14-methyl	71	10.30	-	-
Hexadecanoic acid 15-methyl	74	11.32	-	-
Hexadecanoic acid 14-methyl	88	11.40	88	11.47

3.1.4 Identification of Dienoic Unsaturated Fatty Acid Methyl Esters

Octadecadienoic acid (C18:2) was expected to be seen in both the standard solution and lipid extracts from the cheeses. Due to the non-polar nature of the liquid phase (OV-1), octadecadienoic acid would have been eluted before octadecanoic acid and octadecenoic acid. However, the peak was probably masked by the wide C18:1 peak.

3.2 IDENTIFICATION OF FREE FATTY ACIDS IN THE CHLOROFORM / METHANOL EXTRACTION OF CHEESE BY GC/MS

The total ion chromatogram of all components present in the chloroform /methanol extracts of Bleu d'Auvergne and Brie cheese are shown in Figures 3.10 and 3.11. Identification of free fatty acids in the lipid extract was carried out by comparison of standard mass spectra to those obtained from the two cheese extracts. The mass spectra of dodecanoic acid from a standard solution and the lipid fraction of Bleu d'Auvergne cheese are included to illustrate the characteristic peaks for identification of free fatty acids : M^+, m/z 60, m/z 45 (Figures 3.12 and 3.13). Firstly, the apparent mass ion, M^+ , was seen in all cases except for hexanoic acid in the standard solution. The second peak (iii) at m/z 60, the base peak for most free fatty acids was produced by the following rearrangement resulting in a very stable ion :-

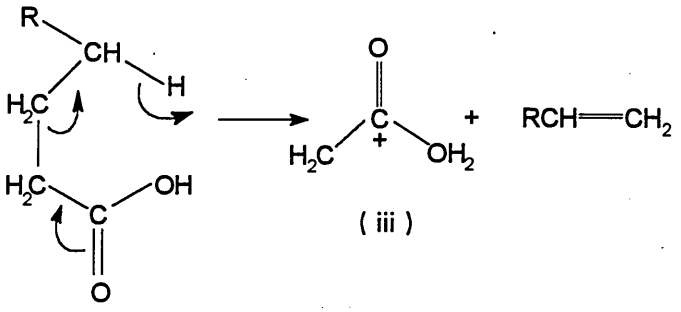


Figure 3.14 McLafferty Rearangement of a Fatty Acid to give a Peak with m/z 60

The third peak at m/z 45 was due to the loss of the carboxylic group, -COOH. Fragments were also seen at M^+- 29 - $(CH_2)_n$, due to the loss of CH_2CH_3 - (characteristic of an aliphatic chain). Tables 3.6 and 3.7 show the identification of free fatty acids in the extracts of Bleu d'Auvergne and Brie cheese. In both cheeses, hexanoic acid was not detected, either because it was masked by earlier peaks, or due to it being highly soluble in the aqeous phase, it was not present in the lipid extract.

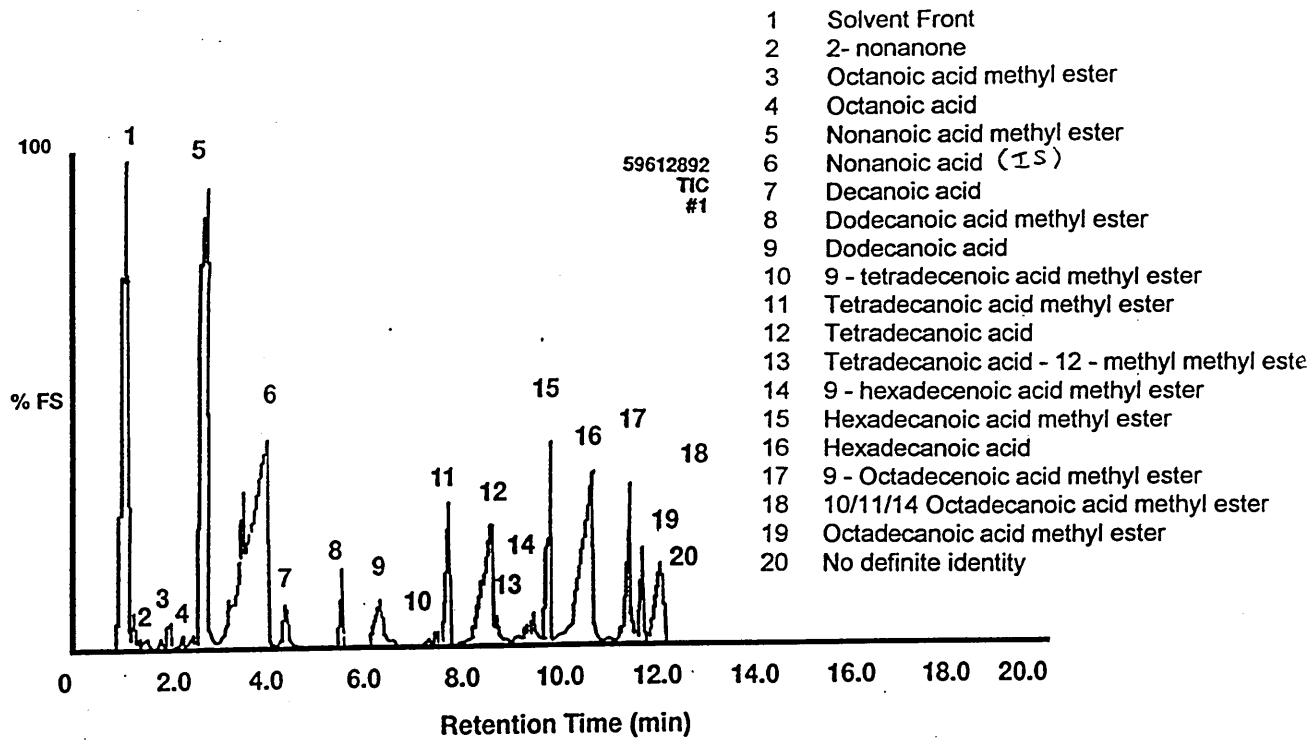


Figure 3.10 Total Ion Chromatogram of Components in the Lipid Extract of Bleu d'Auvergne Cheese by GC/MS at 110 - 220 °C with a 10 °Cmin^{-1} ramp

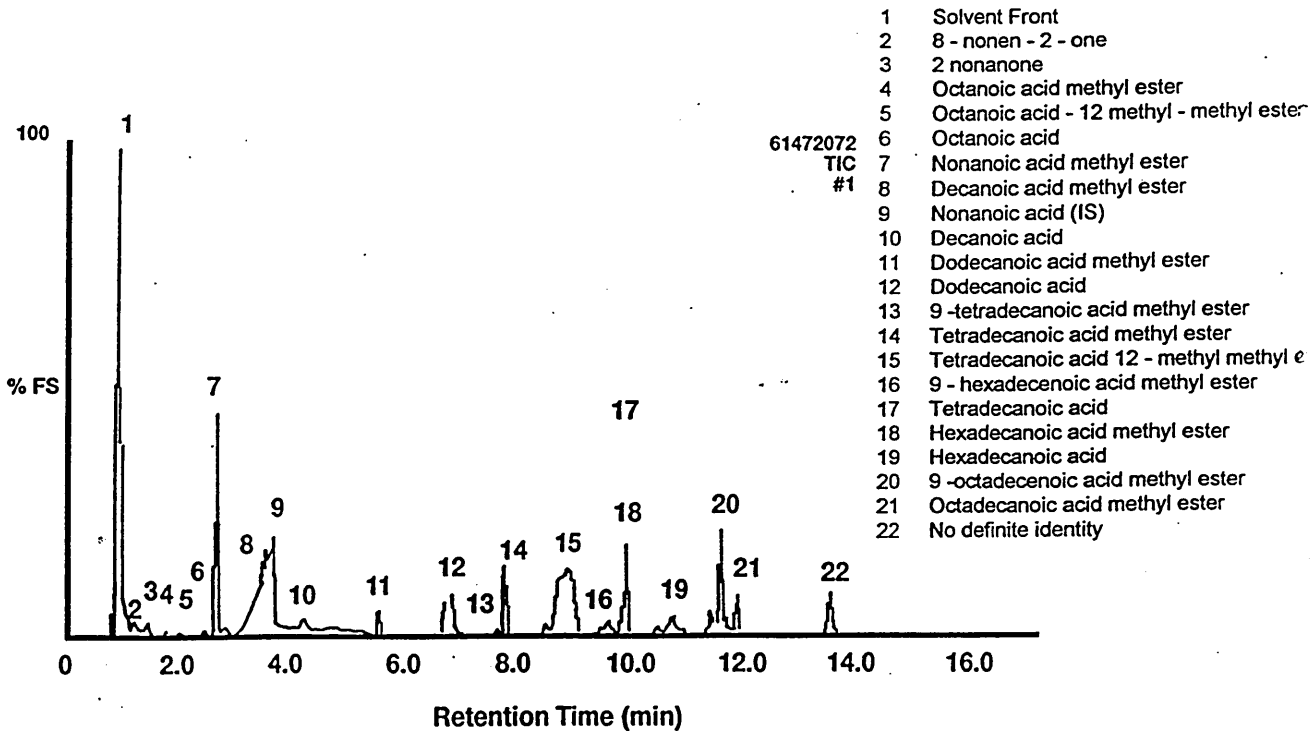


Figure 3.11 Total Ion Chromatogram of all Components Detected in the Lipid Extract of Brie Cheese by GC/MS at 110 - 220 °C with a 10 °min $^{-1}$ ramp

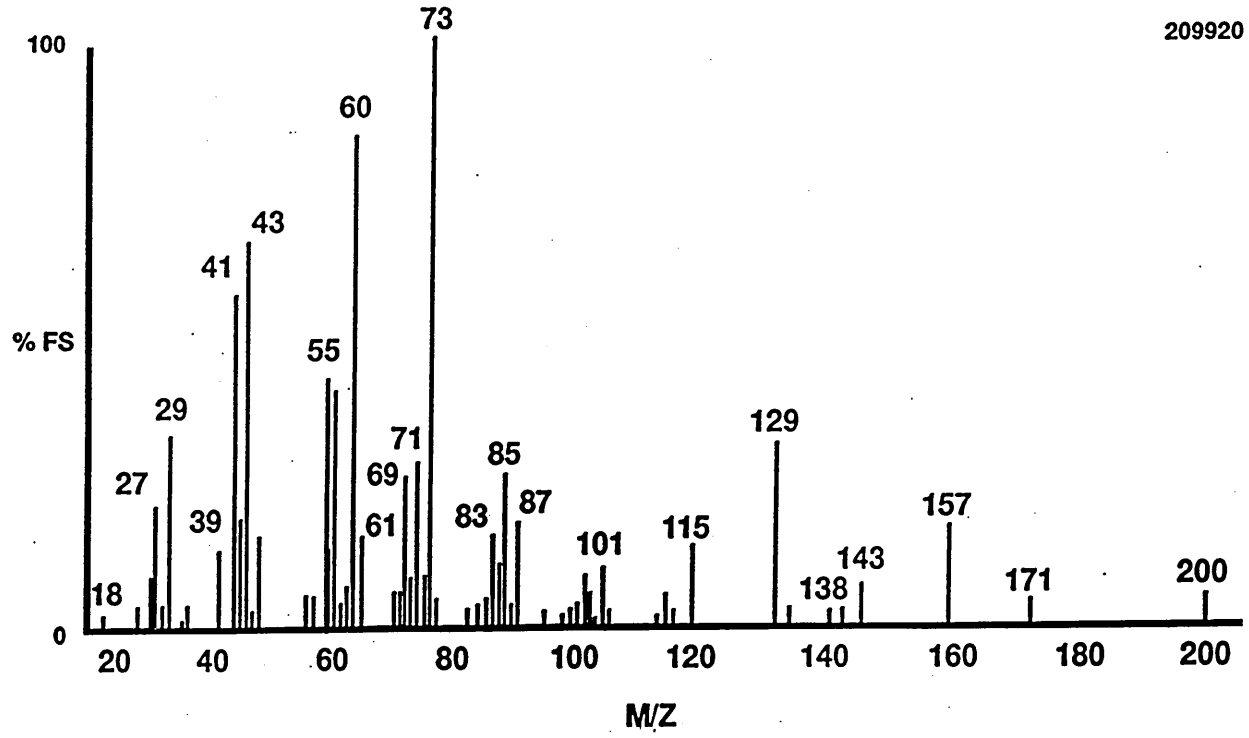


Figure 3.12 Standard Dodecanoic Acid Analysed by GC/MS at 110 - 220 °C with 10 °Cmin^{-1} ramp

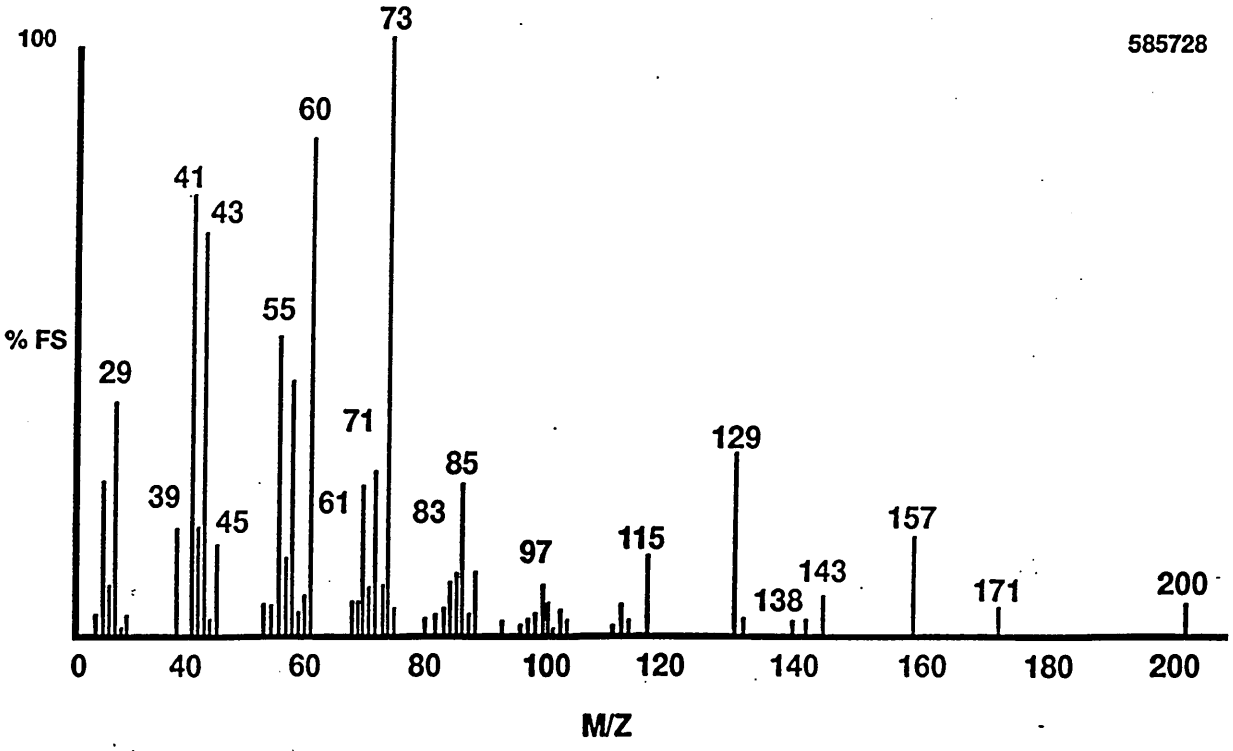


Figure 3.13 Dodecanoic Acid Detected in the Lipid Extract of Bleu d'Auvergne Cheese Analysed by GC/MS at 110 - 220 °C with 10 °Cmin^{-1} ramp

The extract from the Bleu d'Auvergne cheese was more concentrated, so it was easy to distinguish that hexadecanoic and tetradecanoic acids were the most abundant free fatty acids. In the Brie cheese, the peaks of the total ion chromatogram were smaller than for the Bleu d'Auvergne cheese. However, the most abundant peak here was dodecanoic acid with hexadecanoic and tetradecanoic acids appearing to be present in much lower concentrations.

Table 3.6 Identification of Saturated Free Fatty Acids by GC/MS, in the Lipid Extract of Bleu d'Auvergne Cheese

Pk No†	M^+	M^+- 29 -$(CH_2)_n$	t_R std	t_R peak	t_{RR}	library % id	Peak identity
4	-	115,101,87,73,59	1.62	2.28	0.69	79	Octanoic acid
6	158	143,129,115,101,87,73,59	2.75	3.32	1.00	91	Nonanoic acid (IS)
7	172	143,129,115,101,87 etc	4.60	4.23	1.27	91	Decanoic acid
9	200	171,157,143,129,115 etc	6.78	6.22	1.87	97	Dodecanoic acid
12	228	199,185,171,157,143 etc	8.98	8.52	2.57	92	Tetradecanoic acid
16	256	227,213,199,185,171, etc	11.13	10.57	3.18	83	Hexadecanoic acid

† - refers to peaks in figure 3.10

Table 3.7 Identification of Saturated Free Fatty Acids by GC/MS, in the Lipid Extract of Brie Cheese

Pk no‡	M^+	M^+- 29 -$(CH_2)_n$	t_R std	t_R peak	t_{RR}	library % id	Peak identity
6	-	115,101,87,73,59	1.62	2.43	0.65	82	Octanoic acid
9	158	129,115,101,87,73	2.75	3.72	1.00	92	Nonanoic acid (IS)
10	172	143,129,115,101,87 etc	4.60	4.25	1.14	91	Decanoic acid
12	200	171,157,143,129,115 etc	6.78	6.90	1.85	96	Dodecanoic acid
17	228	213,199,185,171,157, etc	8.98	9.90	2.66	87	Tetradecanoic acid
19	256	227,213,199,185,171, etc	11.13	10.78	2.90	-	Hexadecanoic acid

‡ - refers to peaks in figure 3.11

Peaks were also identified on the total ion chromatogram, which corresponded to fatty acid methyl esters. Tables 3.8 - 3.11 identify the saturated and monounsaturated fatty acid methyl esters, relating their identification to the information in Section 3.1. During the extraction of the free fatty acids, any lipase enzymes present in the cheese could have catalysed or initiated reactions. The presence of methanol in the extraction liquid could have brought about the esterification of the free, succeptible fatty acids. Therefore, to prevent such reactions from occurring, extraction of free fatty acids from the cheeses

was carried out in the presence of excess acid (hydrochloric acid) and cooled rapidly at a low temperature (0°C). Nonanoic acid was the internal standard for all of the following peaks.

Table 3.8 Identification of Saturated Fatty Acid Methyl Esters by GC/MS in the Lipid Extract of Bleu d'Auvergne Cheese

Pk No†	M^+	M^+ -31	M^+- 29 -$(CH_2)_n$	t_R std	t_R peak	t_{RR} mins	library % id	Peak identity
3	-	127	101,87,59	2.37	1.85	0.56	91	Octanoic acid
5	200	169	143,129,115,101,87	-	2.53	0.81	90	Nonanoic acid
8	214	183	185,171,157,143,129, etc	6.30	5.38	1.62	90	Dodecanoic acid
11	242	211	213,199,185,171,157, etc	8.57	7.60	2.29	94	Tetradecanoic acid
15	270	239	241,227,213,199,185, etc	10.70	9.72	2.93	84	Hexadecanoic acid
19	298	267	269,255,241,213,199, etc	12.73	11.64	3.51	-	Octadecanoic acid

Table 3.9 Identification of Saturated Fatty Acid Methyl Esters by GC/MS, in the Lipid Extract of Brie Cheese

Pk No‡	M^+	M^+ -31	M^+- 29 -$(CH_2)_n$	t_R std	t_R peak	t_{RR}	library % id	Peak identity
4	-	127	129,115,101,87	2.37	1.97	0.53	95	Octanoic acid
7	172	141	143,129,115,101,87		2.67	0.72	90	Nonanoic acid
8	-	155	157,143,101,87,73 etc	4.08	3.53	0.95	86	Decanoic acid
11	214	183	185,171,157,143,129,etc	6.30	5.65	1.52	89	Dodecanoic acid
14	242	211	213,199,185,157,143 etc	8.57	7.88	2.12	94	Tetradecanoic acid
18	270	239	241,227,213,199,185,etc	10.70	10.02	2.69	83	Hexadecanoic acid
21	298	267	269,255,241,213,199,etc	12.73	11.95	3.21	-	Octadecanoic acid

Table 3.10 Identification of Mono-Unsaturated Fatty Acid Methyl Esters by GC/MS in the Lipid Extract of Bleu d'Auvergne Cheese

Pk No†	M^+	M^+ -31	M^+ -32	M^+ -74	M^+- 29 -$(CH_2)_n$	t_R peak	t_{RR}	library % id	Peak identity
10	240	209	208	166	129,115,101,87	7.37	2.22	-	9-Tetradecenoic acid
14	268	237	236	194	143,129,115,101,87	9.38	2.83	90	9-Hexadecenoic acid
17	296	265	264	222	157,143,101,87,73	11.68	3.52	95	9-Octadecenoic acid

† - refers to figure 3.10

Table 3.11 Identification of Mono-Unsaturated Fatty Acid Methyl Esters by GC/MS, in the Lipid Extract of Brie Cheese

Pk No‡	M^+	M^+ -31	M^+ -32	M^+ -74	M^+- 29 -$(CH_2)_n$	t_R* peak	t_{RR}	library % id	Peak identity
13	240	209	208	166	129,115,101,87	7.68	2.06	-	9-Tetradecenoic acid
16	268	237	236	194	143,129,115,101,87	9.72	2.61	89	9-Hexadecenoic acid
20	296	265	264	222	157,143,101,87,73	11.68	3.14	95	9-Octadecenoic 0acid

Standards were not run for these peaks
‡ - refers to figure 3.11

Other compounds of interest detected in the Brie and Bleu d'Auvergne cheeses, were branched saturated methyl esters and ketones.

Table 3.12 Volatile Compounds Detected in the Lipid Extract of Bleu d'Auvergne and Brie Cheeses

	Bleu d'Auvergne		*Brie*	
Compound	t_R	ID	t_R	ID
2 methyl octanoic acid methyl ester	-	-	2.20	93
12 methyl tetradecanoic acid methyl ester	8.62	86	7.88	94
2- nonanone	1.62	81	1.78	96
8- nonen-2- one	-	-	1.72	74

These compounds have also been detected by other authors in cheese (Kinderlerer 1994).

From Figures 3.1 and 3.2 and Tables 3.1 and 3.2, it can be seen that the fatty acid composition of the lipids in both the Bleu d'Auvergne and Brie cheeses was extremely complex with a wide range in the molecular weight of the individual fatty acids. The major fatty acids were C16:0, C18:0 and C18:1. There was no significant difference in the fatty acids composition of the lipids in the Bleu d'Auvergne and Brie cheeses.

The free medium chain fatty acids, C6:0 - C12:0 were identified in both the Bleu d'Auvergne and Brie cheeses as well as the longer chain fatty acids C14:0 and C16:0. There appeared to be much less tetradecanoic and hexadecanoic acids in the Brie rather than the Bleu d'Auvergne. Even though the concentration of the Bleu d'Auvergne sample was much greater, there seemed to be similar quantities of dodecanoic acid in each cheese.

CHAPTER 4

POINT OF PURCHASE EXPERIMENT

4.1 FREE MEDIUM CHAIN FATTY ACIDS

The analysis of **free medium** chain fatty acids at the point of purchase includes tetradecanoic acid, although it is realised that this is technically a long chain fatty acid.

4.1.1 Analysis of Free Medium Chain Fatty Acids in the Surface and Centre of Fourme d'Ambert Cheese at the Point of Purchase

The results of analysis of the free medium chain fatty acids, pH and fat content (%) at the surface and centre of nine Fourme d'Ambert cheeses are shown in Table 4.1.

Table 4.1 Free Medium Chain Fatty Acids, pH and Fat Content (%) in the Surface and Centre of Fourme d'Ambert Cheese at the Point of Purchase

FFA	Systematic name	Surface	Centre
C 6:0	Hexanoic acid	0.41 ± 0.49	0.47 ± 0.37
C 8:0	Octanoic acid	0.62 ± 0.23	0.81 ± 0.72
C 10:0	Decanoic acid	1.50 ± 0.82	1.12 ± 1.27
C 12:0	Dodecanoic acid	3.46 ± 1.42	2.2 ± 2.2
C 14:0	Tetradecanoic acid	6.81 ± 2.21	4.24 ± 3.58
TOTAL		**12.80 ± 0.98**	**8.84 ± 1.63**
pH		6.74 ± 0.47	6.55 ± 0.56
Fat (%)		29 ± 0.83	28 ± 1.08

Medium chain fatty acid results expressed as mg per g fresh weight cheese
Data is the mean ± standard deviation of 2 extractions and 4 analyses by GC for each cheese
pH and fat content are the mean ± standard deviation of 5 and 4 measurements respectively

It can be seen that many fungal species grow on the outside of this cheese (Figure 1.1). The presence of the mixed mycoflora on the surface of the cheese may have contributed to the apparently higher concentration of the total free medium chain fatty acids at the surface (Student t-test : values for the total fatty

acids were significantly different at the 0.01 probability level). Higher lipolytic activity due to the mixed mycoflora and not just *Penicillium roquefortii*, could have catalysed the hydrolysis of acylglycerols to give free fatty acids (Table 4.1). The standard deviations of free fatty acid concentrations reported in this Table were high. One explanation for the large standard deviations in these values could be due to the cheese being non-homogeneous. Variations in the composition of the cheese would occur from region to region. The two regions analysed were surface and centre, not blue and white regions. The fungal mycelium were present in both regions which were sampled.

No significant differences in the pH and fat content (%) between the surface and the centre of Fourme d'Ambert cheese were observed (Student t-test : values were not significantly different at the probability level 0.05) (Table 4.1).

4.1.2 Analysis of Free Medium Chain Fatty Acids in the Blue and White Regions of Bleu d'Auvergne at the Point of Purchase

Table 4.2 gives the results of the analysis of free medium chain fatty acids, pH and fat content (%) in the blue and white regions of one Bleu d'Auvergne cheese.

Table 4.2 Medium Chain Fatty Acids, pH and Fat Content (%) in the Blue and White Regions of Bleu d'Auvergne Cheese at the Point of Purchase

FFA	Systematic name	Blue	White
C 6:0	Hexanoic acid	0.172 ± 0.19	nd
C 8:0	Octanoic acid	0.500 ± 0.09	0.347 ± 0.09
C 10:0	Decanoic acid	0.427 ± 0.19	0.053 ± 0.01
C 12:0	Dodecanoic acid	1.773 ± 0.23	0.469 ± 0.15
C 14:0	Tetradecanoic acid	2.541 ± 0.92	0.376 ± 0.12
TOTAL		**5.413 ± 1.85**	**1.246 ± 0.18**
pH		7.92 ± 0.15	7.41 ± 0.18
Fat (%)		32 ± 0.00	27 ± 0.00

Results expressed as mg per g fresh weight cheese
Data is the mean ± sd of two extractions and 6 analyses by GC for the one cheese
pH and fat content are the mean ± standard deviation of 5 and 4 measurements respectively
nd - not detected

Table 4.2 highlights the clear differences in concentration of free medium chain fatty acids in the blue and white regions of Bleu d'Auvergne cheese (Student t-test : values were significantly different at the 0.01 probability level for C10:0, C12:0 and C14:0). The conidia spores in the blue region have considerable lipolytic activity and can hydrolyse triacylglycerols to give free fatty acids (Kinsella and Hwang 1976[2]). The presence of large numbers of blue-green conidia spores in the blue region of the cheese, may have therefore been responsible for increased hydrolysis of the acylglycerols to give free medium chain fatty acids. A higher concentration of the longer chain length medium chain fatty acids was detected in both regions of the Bleu d'Auvergne cheese than decanoic, octanoic and hexanoic acids. Tetradecanoic acid was present in the highest concentration in the blue region whilst dodecanoic acid was detected in the highest concentration in the white region of Bleu d'Auvergne. Four times the concentration of medium chain fatty acids were found in the blue region of the cheese compared to the white, but the difference from acid to acid varied markedly (Table 4.2). Both the pH and fat content (%) were higher in the blue region of the cheese than the white (Student t-test : both the pH and fat content (%) values were significantly different at the 0.01 probability level).

4.1.3 Analysis of Free Medium Chain Fatty Acids at the Surface and Centre of French Brie Cheese at Point of Purchase

The results of analysis of free medium chain fatty acids, pH and fat content (%) at the surface and centre of four Brie de Pays cheeses, are given in Tables 4.3 and 4.4.

Table 4.3 Free Medium Chain Fatty Acids, pH and Fat Content (%) at the Surface and Centre of French Brie Cheese at the Point of Purchase

		Surface	Centre	Surface	Centre
FFA	Systematic name	Brie[a]	Brie[a]	Brie[b]	Brie[b]
C 6:0	Hexanoic acid	nd	0.099 ± 0.03	nd	0.093 ± 0.15
C 8:0	Octanoic acid	0.432 ± 0.03	0.490 ± 0.05	0.486 ± 0.09	0.569 ± 0.10
C 10:0	Decanoic acid	0.057 ± <0.01	0.069 ± 0.01	0.057 ± <0.01	0.081 ± 0.03
C 12:0	Dodecanoic acid	nd	nd	nd	nd
C 14:0	Tetradecanoic acid	nd	nd	nd	nd
TOTAL		0.496 ± 0.04	0.630 ± 0.15	0.543 ± 0.09	0.743 ± 0.25
pH		7.76 ± 0.05	7.16 ± 0.24	8.09 ± 0.05	7.84 ± 0.11
Fat (%)		29.13 ± 0.25	27.25 ± 0.50	27.63 ± 0.48	26.63 ± 0.48

Table 4.4 Free Medium Chain Fatty Acids, pH and Fat Content (%) at the Surface and Centre of French Brie Cheese at the Point of Purchase

		Surface	Centre	Surface	Centre
FFA	Systematic name	Brie[c]	Brie[c]	Brie[d]	Brie[d]
C 6:0	Hexanoic acid	nd	nd	0.048 ± 0.01	0.030 ± 0.02
C 8:0	Octanoic acid	0.528 ± 0.04	0.400 ± 0.04	0.655 ± 0.09	0.677 ± 0.05
C 10:0	Decanoic acid	0.079 ± 0.02	0.042 ± <0.01	0.074 ± 0.02	0.047 ± 0.08
C 12:0	Dodecanoic acid	nd	nd	nd	nd
C 14:0	Tetradecanoic acid	nd	nd	nd	nd
TOTAL		0.693 ± 0.11	0.479 ± 0.10	0.777 ± 0.12	0.753 ± 0.07
pH		8.01 ± 0.10	7.51 ± 0.12	7.65 ± 0.13	6.64 ± 0.26
Fat (%)		28.13 ± 0.25	27.75 ± 0.50	27.5 ± 0.71	23.5 ± 0.71

Medium chain fatty acid results expressed as mg per g fresh weight cheese
Data is the mean ± sd of two extractions and 6 analyses by GC for each cheese
pH and fat content are the mean ± standard deviation of 5 and 4 measurements respectively
nd - not detected
a,b,c,d are four different cheeses

In each of the four Brie cheeses analysed, there was no significant difference between the concentration of the individual free medium chain fatty acids in the centre and at the surface (Student t-test : values were not significantly different at the 0.05 probability level). Dodecanoic and tetradecanoic acids were not detected in either region of the cheese. At both the centre and surface of the Brie cheeses, octanoic acid was the dominant fatty acid. Hexanoic and decanoic acids were present in low, or undetectable concentrations in the two regions of the cheeses.

The pH range was consistently greater at the surface (7.6 - 8.1) than the centre (6.6 - 7.8) for all cheeses (Student t-test : pH values were significantly different at the 0.01 probability level for all cheeses). The fat content (%) was higher at the surface (27.5 - 29.1 %) than centre (23.5 - 27.8 %) for two of the four cheeses (Student t-test : values were significantly different at the 0.01 probability level for all cheeses).

4.1.4 Analysis of Free Medium Chain Fatty Acids in the Surface and Centre of Vacherin Mont d'Or and Mont d'Or Cheeses at Point of Purchase

The results of analysis at the surface and centre of two **different** Swiss Vacherin Mont d'Or cheeses and one French Mont d'Or cheese are given in Tables 4.5, 4.6 and 4.7.

Table 4.5 Free Medium Chain Fatty Acids, pH and Fat Content (%) at the Surface and Centre of the Vacherin Mont d'Or Cheese Extracted without Acid, at the Point of Purchase

		Surface	Centre
FFA	**Systematic name**	**Vacherin Mont d'Or**	**Vacherin Mont d'Or**
C 6:0	Hexanoic acid	nd	nd
C 8:0	Octanoic acid	0.406 ± 0.04	0.576 ± 0.07
C 10:0	Decanoic acid	0.057 ± 0.01	0.102 ± 0.06
C 12:0	Dodecanoic acid	nd	nd
C 14:0	Tetradecanoic acid	nd	nd
TOTAL		**0.463 ± 0.04**	**0.675 ± 0.11**
pH		8.34 ± 0.09	7.74 ± 0.34
Fat (%)		28.25 ± 0.30	26 ± 0.00

Table 4.6 Free Medium Chain Fatty Acids, pH and Fat Content (%) at the Surface and Centre of the Vacherin Mont d'Or Cheese Extracted with Acid at the Point of Purchase

		Surface	Centre
FFA	**Systematic name**	**Vacherin Mont d'Or**	**Vacherin Mont d'Or**
C 6:0	Hexanoic acid	0.053 ± 0.01	0.039 ± < 0.01
C 8:0	Octanoic acid	0.752 ± 0.06	0.593 ± 0.08
C 10:0	Decanoic acid	0.230 ± 0.04	0.225 ± 0.12
C 12:0	Dodecanoic acid	0.388 ± 0.04	0.435 ± 0.16
C 14:0	Tetradecanoic acid	1.008 ± 0.34	0.775 ± 0.40
TOTAL		**2.358 ± 0.32**	**2.067 ± 0.75**
pH		8.04 ± 0.07	7.39 ± 0.08
Fat (%)		28 ± 0.00	27 ± 0.00

Table 4.7 Free Medium Chain Fatty Acids, pH and Fat Content (%) in the Surface and Centre of Mont d'Or Cheese Extracted with Acid at the Point of Purchase

		Surface	Centre
FFA	**Systematic name**	**Mont d'Or**	**Mont d'Or**
C 6:0	Hexanoic acid	nd	0.028 ± 0.05
C 8:0	Octanoic acid	0.680 ± 0.09	0.633 ± 0.10
C 10:0	Decanoic acid	0.136 ± 0.07	0.175 ± 0.05
C 12:0	Dodecanoic acid	0.319 ± 0.23	0.466 ± 0.34
C 14:0	Tetradecanoic acid	nd	0.213 ± 0.21
TOTAL		**1.295 ± 0.28**	**1.476 ± 0.29**
pH		8.14 ± 0.08	7.67 ± 0.07
Fat (%)		27.3 ± 0.50	25.5 ± 0.58

Medium chain fatty acid results expressed as mg per g fresh weight cheese
Data is the mean ± standard deviation of two extractions and 6 analyses by GC
pH and fat content are the mean ± standard deviation of 5 and 4 measurements respectively
nd - not detected

Two different Swiss Vacherin Mont d'Or cheeses were analysed. Hydrochloric acid (2 M HCl) was added during the extraction of the second cheese to demonstrate the improvement in recovery and detection of the fatty acids upon it's inclusion in the extraction liquid. From the data in Tables 4.5 and 4.6, the addition of acid did increase the concentration of free fatty acids which were recovered. Subsequent extractions were therefore carried out with the addition of acid.

Relatively little difference between the concentration of medium chain fatty acids was detected in the centre and at the surface of the individual Vacherin Mont d'Or cheeses. Higher concentrations of octanoic and tetradecanoic acids were present than hexanoic, decanoic and dodecanoic acids in the second cheese. The major difference between the surface and the centre of both cheeses was the higher pH and fat content (%) at the surface.

The concentrations of octanoic, decanoic and dodecanoic acids detected in the French Mont d'Or cheese, were remarkably similar to those detected in the second Swiss Vacherin Mont d'Or cheese (Student t-test : values were not significantly different at the 0.05 probability level for C8:0, C10:0 and C12:0).

Hexanoic and tetradecanoic acids were not detected at the surface of the French cheese, which accounted for the smaller total fatty acid concentration reported in that region, compared to the second Swiss cheese. In the centre of the Mont d'Or cheese, a lower concentration of tetradecanoic acid was detected compared to the second Swiss cheese which contributed to the lower total fatty acid concentration in that region. Both the pH and percent fat content were higher at the surface than the centre.

The average total concentration of medium chain fatty acids (C6:0, C8:0, C10:0, C12:0 and C14:0) for the three cheeses was between 0.4 and 2.4 mg fatty acid/ g cheese at a pH in excess of 7.0. This concentration was too low to have a bactericidal effect in the cheese when compared to the data of Wang and Johnson (1992), who found that the concentration of dodecanoic acid needed to have any listericidal effect in broth was 10 mg/ l (0.01 mg/g) at pH 5.0 and 200 mg/ l (0.2 mg/g) at pH 6.0. Most of the acids in the cheese would have been present in the dissociated form at a pH in excess of 7.0. Using the following equation :-

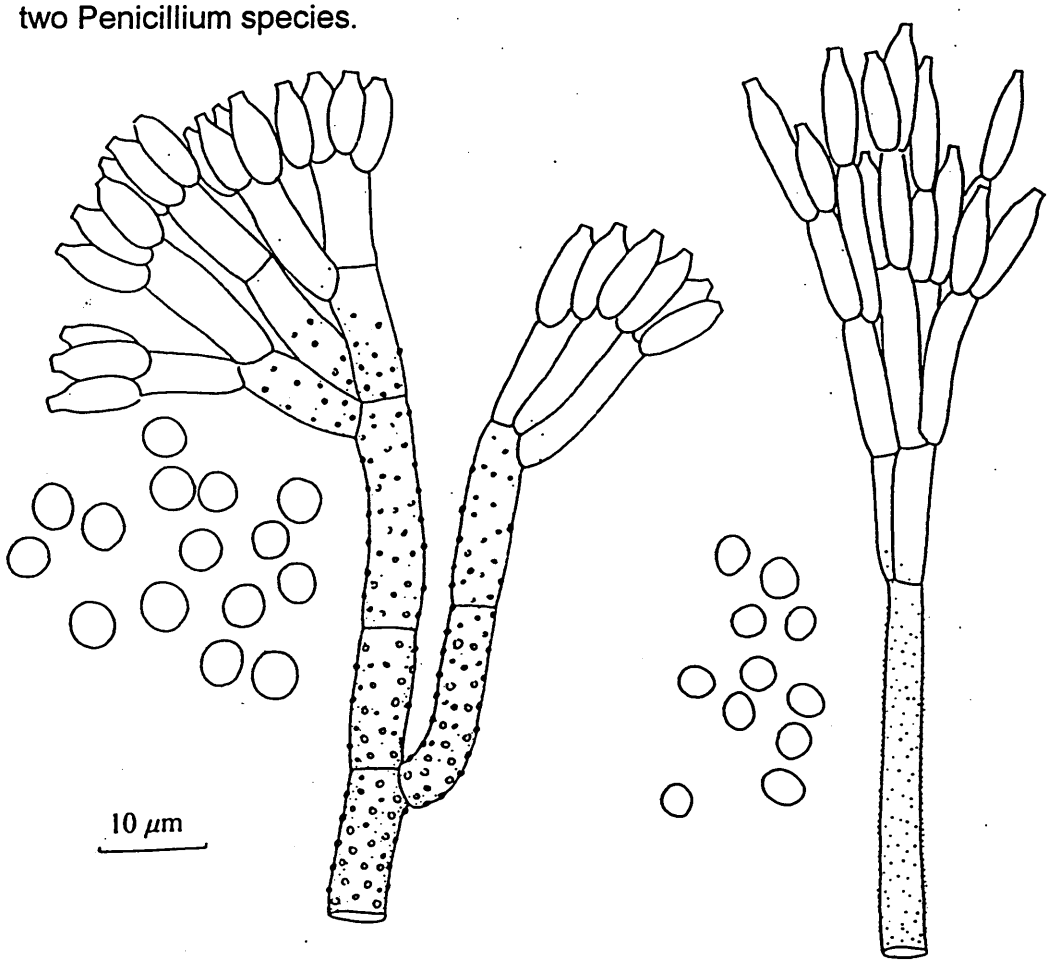
$$\log \frac{S}{A} = pH - pK_a$$

where $S = RCOO^-$
$A = RCOOH$
$pK_a = 4.85$ for octanoic acid (Freese *et al.* 1973)

the value of undissociated octanoic acid present at the surface of the Mont d'Or cheese was 0.00035 mg fatty acid per gram cheese, or 0.35 mg / kg cheese. Therefore less than 1 % of the fatty acids would have been present in the undissociated form at pH 8.14. It is extremely unlikely that these acids could act as natural preservatives, as the concentration of the undissociated acid is too low to have a significant effect at the high pH's of these cheeses.

4.1.5 Comparison of Free Medium Chain Fatty Acids in the Blue and White Mould-Ripened Cheeses

Figures 4.2, 4.3, 4.4, 4.5 and 4.6 give the concentrations of free hexanoic, octanoic, decanoic, dodecanoic and tetradecanoic acids in one blue-veined cheese, Bleu d'Auvergne and three surface-ripened cheeses Brie, Vacherin Mont d'Or and Mont d'Or analysed at the point of purchase. In the blue-veined cheese, *Penicillium roquefortii* grows along veins and cracks in the cheese. It is in these regions that the blue-green conidia spores are produced. In the surface-ripened cheeses, the *Penicillium camembertii* grows from the outside to the centre of the cheese during ripening. The conidia spores are found on the surface of such cheeses. Figure 4.1 gives a drawing of the conidiophore of the two Penicillium species.



Penicillium roquefortii ***Penicillium camembertii***

Figure 4.1 Conidiophore of *Penicillium roquefortii* and *P. camembertii*

Samson R.A. and van Reenen-Hoekstra E.S. (1988)

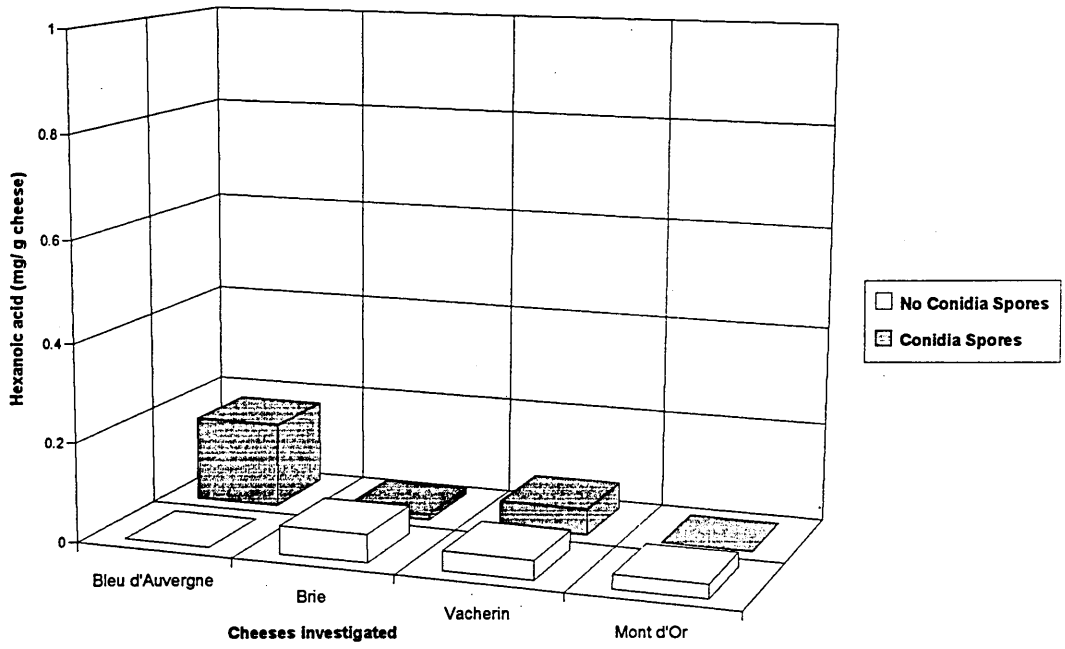


Figure 4.2 Concentration of Hexanoic Acid in Bleu d'Auvergne, Brie and Vacherin-type Cheeses (mg /g cheese)

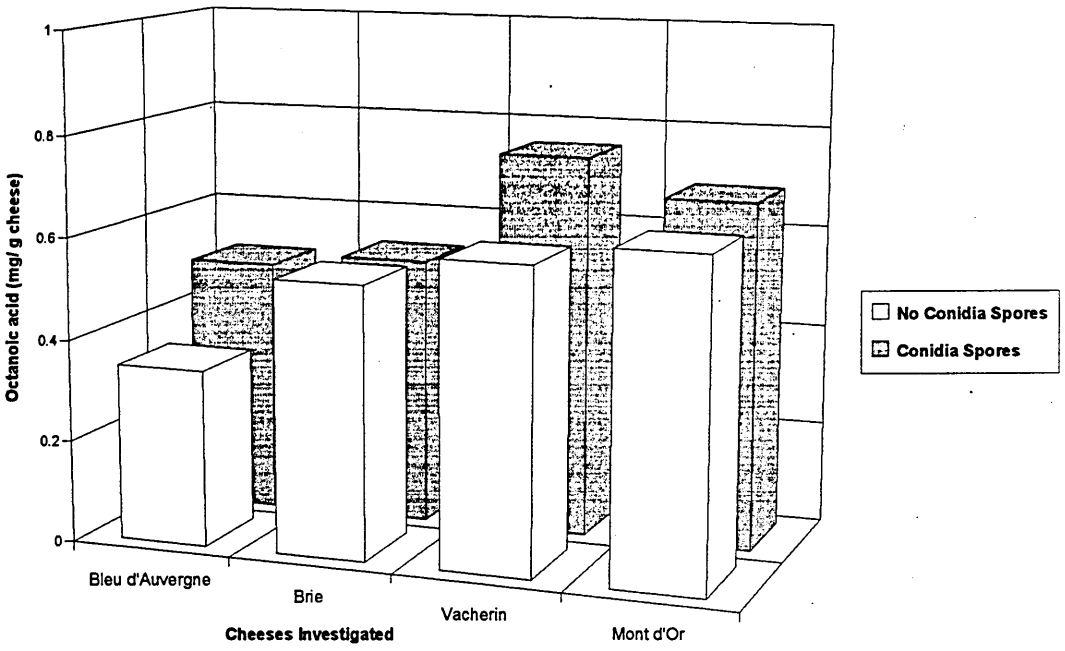


Figure 4.3 Concentration of Octanoic Acid in Bleu d'Auvergne, Brie and Vacherin-type Cheeses (mg /g cheese)

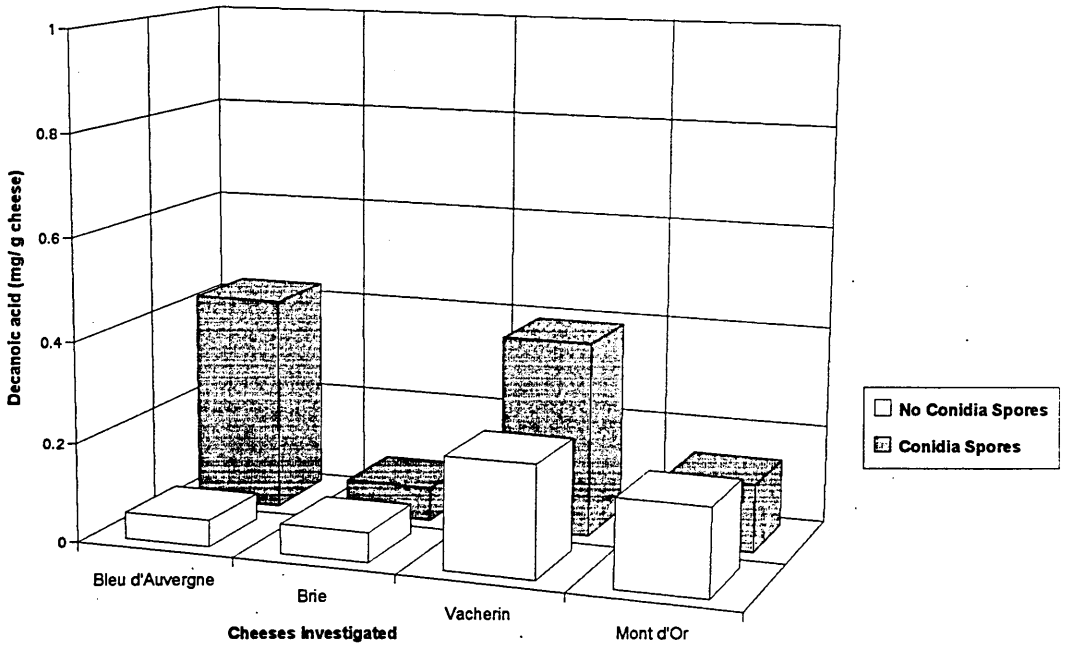


Figure 4.4 Concentration of Decanoic Acid in Bleu d'Auvergne, Brie and Vacherin-type Cheeses (mg /g cheese)

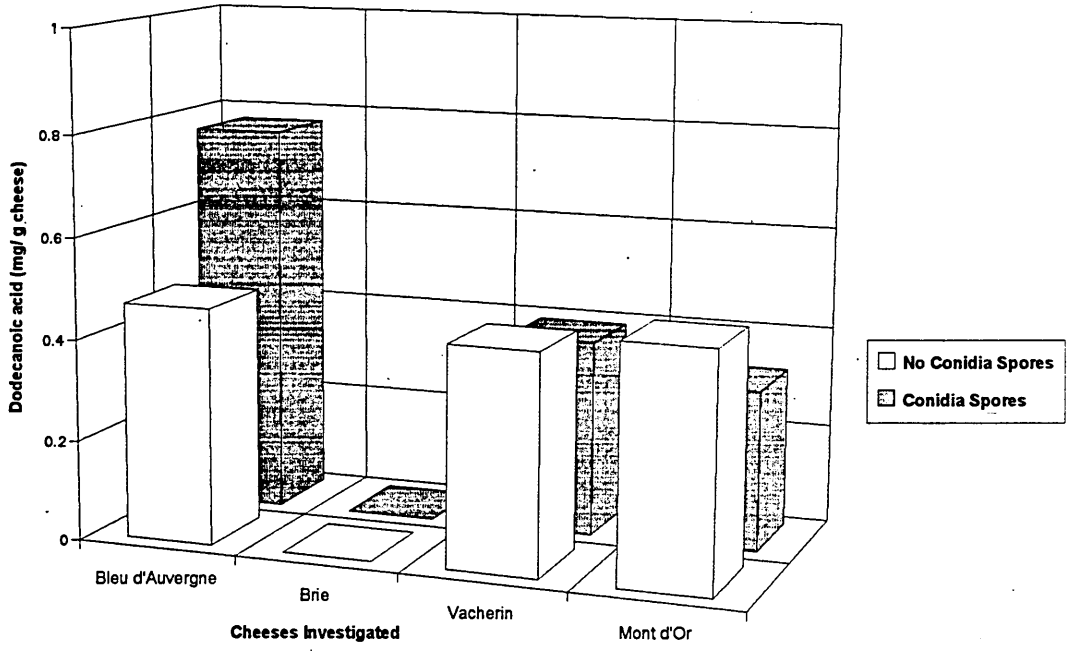


Figure 4.5 Concentration of Dodecanoic Acid in Bleu d'Auvergne, Brie and Vacherin-type Cheeses (mg /g cheese)

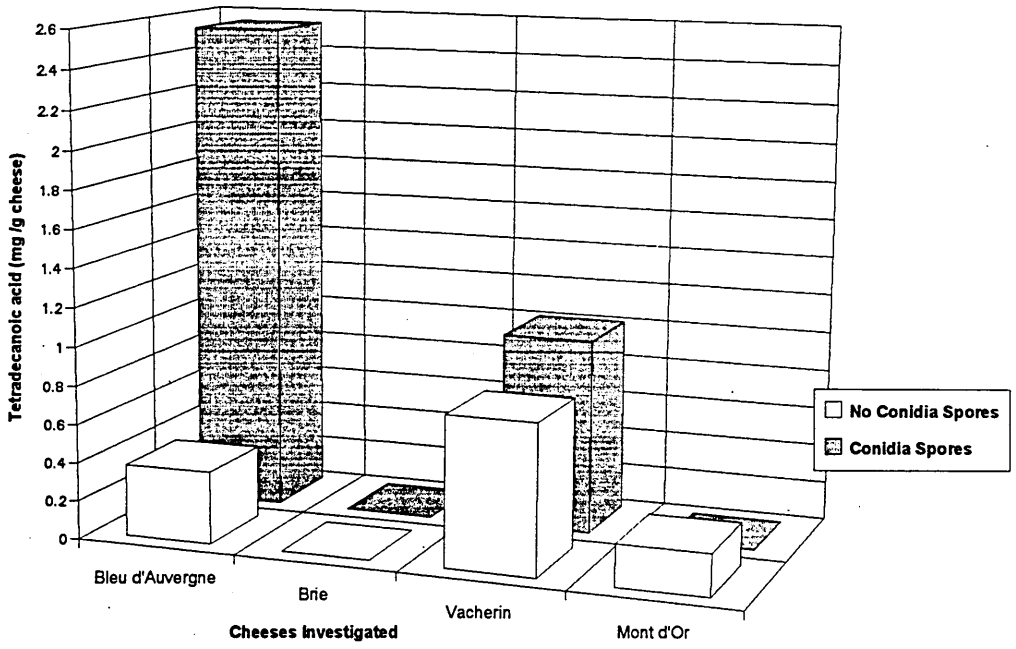


Figure 4.6 Concentration of Tetradecanoic Acid in Bleu d'Auvergne, Brie and Vacherin-type Cheeses (mg /g cheese)

The Figures (4.2, 4.3, 4.4, 4.5 and 4.6) demonstrate clearly that in the Bleu d'Auvergne cheese, consistently higher concentrations of each medium chain fatty acid were found in the region containing large numbers of conidia spores (blue region). There appeared to be an increase in concentration of the fatty acids with an increase in the chain length of the acid in this cheese. In the surface-ripened cheeses there was no significant difference in the concentration of medium chain fatty acids between the surface and centre. In Brie cheese, only octanoic acid was present in significant concentrations whilst in the Vacherin type, free C6:0, C8:0, C10:0, C12:0 and C14:0 were detected.

4.1.6 Discussion

De la Fuente *et al.* (1993) studied the free fatty acid composition of many cheeses, including Camembert and Cabrales, a blue cheese made primarily from cow's milk, but also from goat's and ewe's milk. The total medium chain

fatty acid concentration of the cheeses were 1.592 mg FFA / g cheese for Camembert and 11.890 mg FFA / g cheese for Cabrales. The data in Tables 4.2, 4.3 and 4.4 gives similar values (1.34 g/ kg cheese for Brie, and 7.57 g/ kg cheese for Bleu d'Auvergne). The Spanish authors did not distinguish between different regions of the cheeses when sampling. In the experiments described in this thesis, the cheeses were sampled specifically in regions which coincided with the presence of Penicillium conidia spores (i.e. the blue region in the blue mould-ripened cheeses or the surface of soft-ripened cheeses), and areas where the conidia spores would be absent.

Both Brie and Vacherin-type cheeses have been implicated as the vehicle of infection in listeriosis outbreaks (Bille and Glauser 1988, Greenwood *et al.* 1991). Dodecanoic acid has been found to inhibit the growth of many bacteria including listeria spp. (Kabara 1984). Figure 4.5 highlights the difference in concentration of dodecanoic acid in these two cheeses. There was no dodecanoic acid detected in the Brie cheese, whilst it was detected in the Vacherin-type cheeses. If dodecanoic acid is effective, this data suggests that *Listeria monocytogenes* could perhaps survive in Brie cheese, whilst it's growth be inhibited in Vacherin Mont d'Or cheese.

CHAPTER 5

STORAGE EXPERIMENT

5.1 pH AND FAT CONTENT (%)

5.1.1 Changes in pH and Fat Content (%) in the Blue and White Regions of Bleu d'Auvergne Cheese during Storage at 12 °C

The average pH and fat content (%) for the blue and white regions of Bleu d'Auvergne during the 8 week storage period at 12 °C are given in Table 5.1.

Table 5.1 Average pH and Fat Content (%) in the Blue and White Regions of Bleu d'Auvergne Cheese during Storage at 12 ± 0.5°C

	pH[1]		Fat content (%)[2]	
Time (days)	Blue	White	Blue	White
0	**6.69 ± 0.15**	**6.52 ± 0.05**	29.75 ± 0.50	29.13 ± 0.25
6	**7.12 ± 0.22**	**6.96 ± 0.22**	30.13 ± 1.03	29.00 ± 0.00
14	**7.17 ± 0.16**	**7.16 ± 0.11**	31.00 ± 0.00	31.00 ± 1.15
27	**6.82 ± 0.19**	**6.73 ± 0.34**	36.30 ± 0.52	31.10 ± 0.82
56	**6.43 ± 0.31**	**6.91 ± 0.37**	33.50 ± 0.84	36.50 ± 1.76

1 - Mean ± standard deviation of 5 readings in each region
2 - Mean ± standard deviation of 4 measurements in each region

No significant differences in pH were observed over time in the blue and white regions of Bleu d'Auvergne cheese. In both regions the pH's were similar (Figures 5.1 and 5.2). In the blue region, the fat content (%) increased with time but then appeared to decrease at 56 days. This decrease could have been due to fungal metabolism of the triacylglycerols. Two reactions are involved in metabolism of triacylglycerols. First, the triacylglycerols are hydrolysed to give free fatty acids. Secondly these free fatty acids are esterified to give acyl Coenzyme A derivatives. The fate of the acyl Coenzyme A may vary. Medium chain acyl Coenzyme A derivatives may undergo decarboxylation and deacylation to give the methyl ketones one carbon atom less than the parent fatty acid. With long chain acyl Coenzyme A derivatives, the acyl group may be metabolised to give acetyl Coenzyme A and NADH.

In the white region, a gradual increase in fat content (%) up to 27 days was followed by a further apparent rise at 56 days. This was probably due to the extensive loss of water during storage.

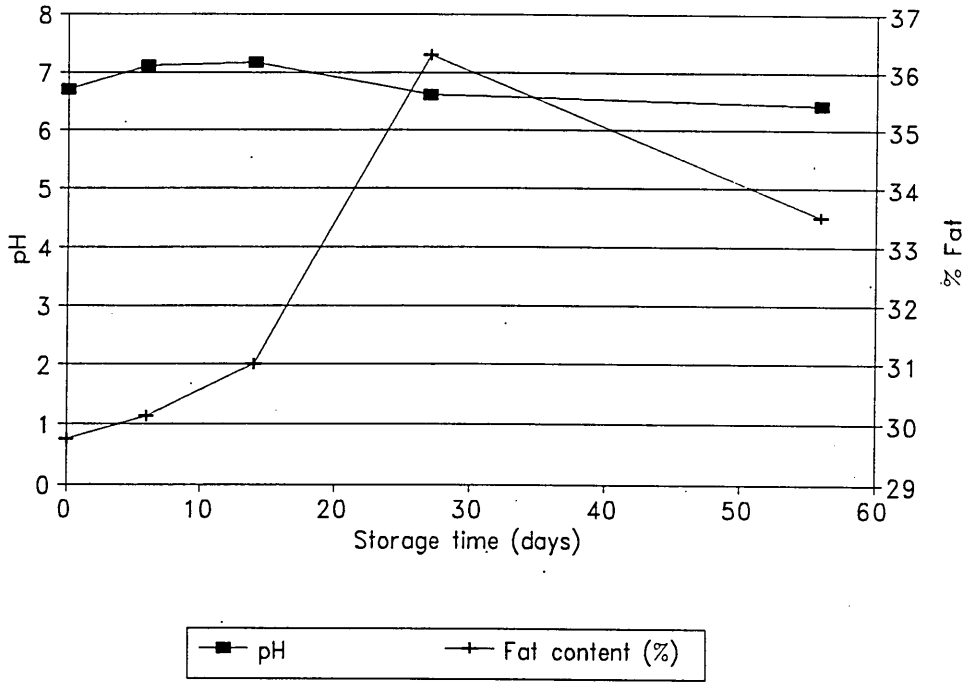


Figure 5.1 pH and Fat Content (%) in the Blue Region of Bleu d'Auvergne Cheese during Storage at 12 °C

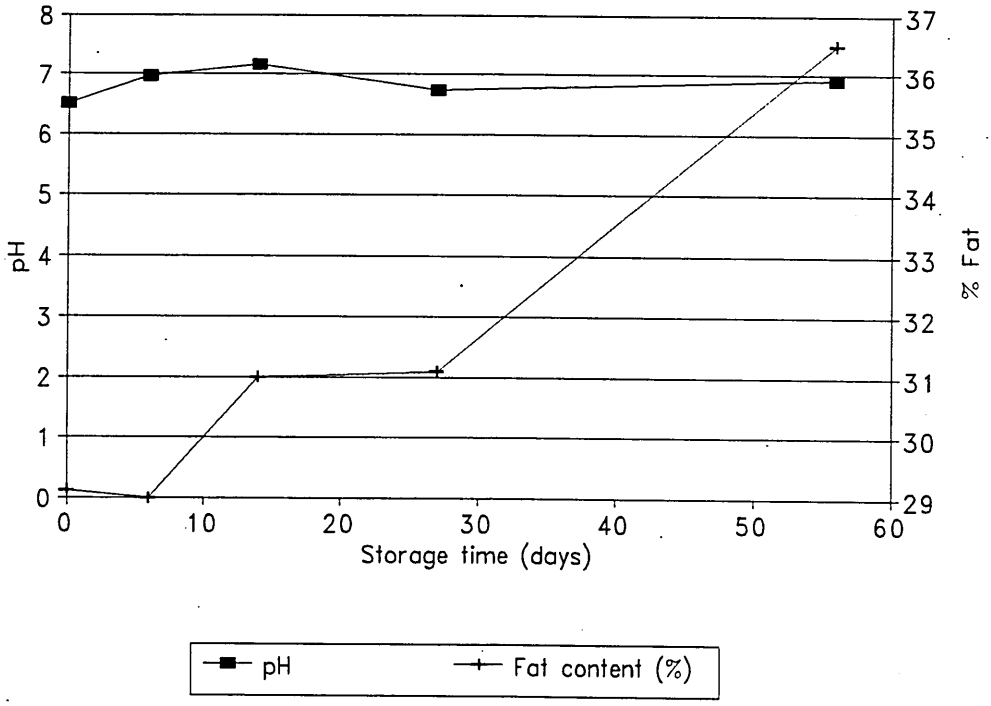


Figure 5.2 pH and Fat Content (%) in the White Region of Bleu d'Auvergne Cheese during Storage at 12 °C

5.1.2 Changes in pH and Fat Content (%) at the Surface and Centre of Brie Cheese during Storage at 12 °C

The average pH and fat content (%) at the surface and centre of French Brie cheese during the 8 week storage period are shown in Table 5.2.

Table 5.2 Average pH and Fat Content (%) at the Surface and Centre of Brie Cheese during Storage at 12 ± 0.5°C

	pH[1]		Fat content (%)[2]	
Time (days)	Surface	Centre	Surface	Centre
0	7.17 ± 0.05	6.12 ± 0.10	27.90 ± 0.12	24.90 ± 0.20
7	6.98 ± 0.07	5.72 ± 0.17	31.00 ± 0.00	25.00 ± 0.00
14	7.64 ± 0.29	7.01 ± 0.23	29.50 ± 0.60	26.00 ± 0.00
27	7.72 ± 0.11	7.57 ± 0.08	28.90 ± 0.25	25.00 ± 0.00
56	7.78 ± 0.06	7.84 ± 0.08	25.75 ± 0.35	23.00 ± 0.00

1- Mean ± standard deviation of 5 readings for each region
2- Mean ± standard deviation of 4 measurements for each region

The pH at the surface and the centre of Brie cheese differed. Initially the pH at the surface was higher than in the centre (7.17 : 6.12), but with time the pH in the centre increased until the value was similar to that at the surface after 56 days storage. The minimum pH recorded was 6.98 at the surface and 6.12 in the centre of the Brie cheese (Figures 5.3 and 5.4). The increase in pH was probably due to the production of ammonia during proteolysis, and the utilisation of lactic acid by microbial enzymes.

The fat content (%) was consistently higher at the surface than the centre of the cheese (Figures 5.3 and 5.4). The fat content (%) at the surface apparently increased at 7 days, but was followed by a continuous decrease from 29 % to 25.5 % at 56 days. In the centre of the cheese the fat content (%) was fairly constant until 56 days, when it decreased. These decreases were probably due to fungal metabolism of the fat. A greater loss of fat (≈ 4 %) was seen at the outside than the inside of the cheese (≈ 2 %) after 56 days storage. Unlike the Bleu d'Auvergne cheese, no visible evidence of water loss was seen in the Brie cheese during ripening.

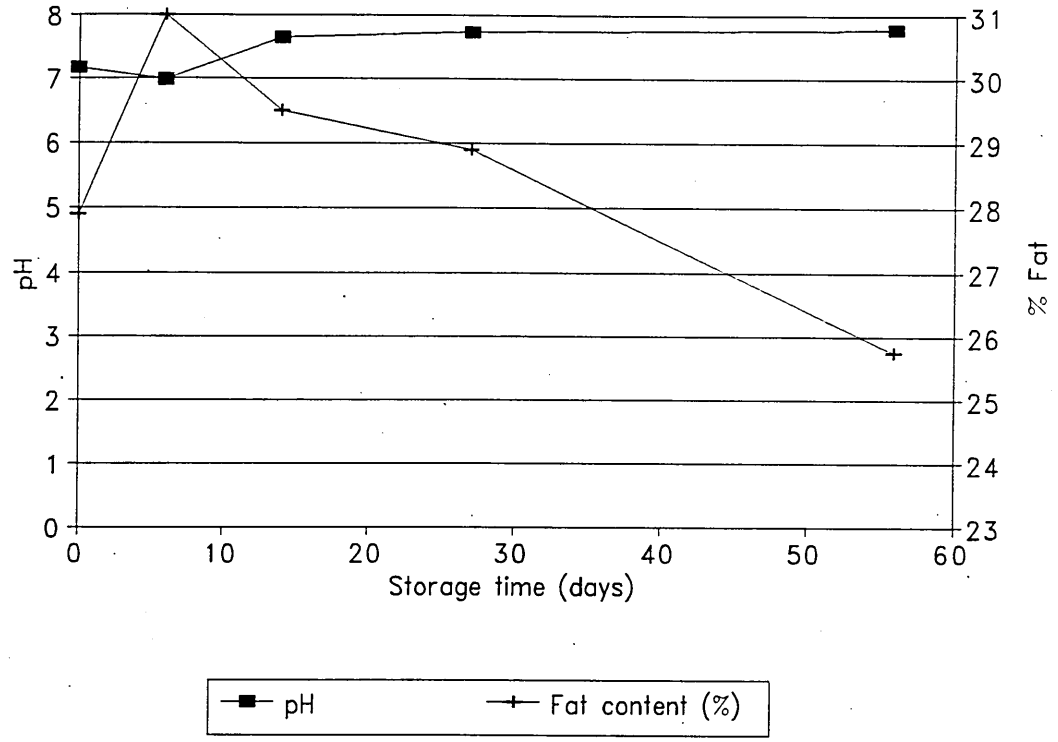


Figure 5.3 pH and Fat Content (%) at the Surface of Brie Cheese during Storage at 12 °C

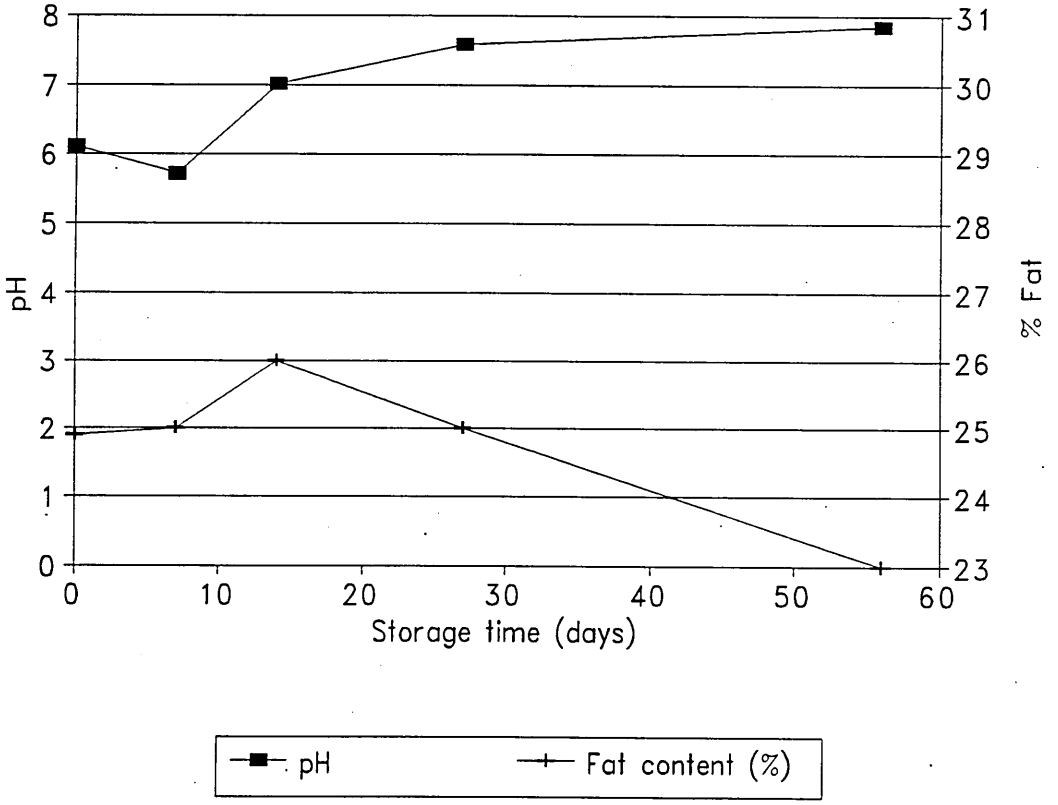


Figure 5.4 pH and Fat Content (%) in the Centre of Brie Cheese during Storage at 12 °C

5.2 ANALYSIS OF FREE MEDIUM CHAIN FATTY ACIDS EXTRACTED FROM BLEU d'AUVERGNE AND BRIE CHEESES

5.2.1 Changes in Free Medium Chain Fatty Acid Concentration in Bleu d'Auvergne Cheese during Storage at 12 °C

Tables 5.3 and 5.4 show the changes in concentration (mg fatty acid per g cheese) of free medium chain fatty acids in the blue and white regions of Bleu d'Auvergne cheese during storage at 12 °C.

Table 5.3 Free Medium Chain Fatty Acid Concentration in the Blue Region of Bleu d'Auvergne Cheese during storage at 12 ± 0.5 °C

		Storage time (days)				
FFA	Name	Purchase	6	14	27	56
C 6:0	Hexanoic acid	0.044 ± 0.02	0.066 ± <0.01	0.072 ± 0.03	0.119 ± 0.01	0.593 ± 0.10
C 8:0	Octanoic acid	0.897 ± 0.13	0.810 ± 0.07	1.543 ± 0.06	2.175 ± <0.01	2.705 ± 0.06
C 10:0	Decanoic acid	1.195 ± 0.27	0.948 ± 0.08	1.003 ± 0.04	1.297 ± 0.29	2.998 ± 0.12
C 12:0	Dodecanoic acid	2.495 ± 0.39	1.826 ± 0.11	3.453 ± 0.48	2.825 ± 0.44	4.459 ± 0.73
C 14:0	Tetradecanoic acid	6.381 ± 1.48	5.017 ± 0.51	11.059 ± 0.86	9.160 ± 0.65	16.647 ± 1.41
Total		**10.652 ± 1.31**	**8.737 ± 0.50**	**16.540 ± 0.96**	**15.990 ± 2.10**	**23.399 ± 2.19**

Table 5.4 Free Medium Chain Fatty Acid Concentration in the White Region of Bleu d'Auvergne Cheese during Storage at 12 ± 0.5 °C

		Storage time (days)				
FFA	Name	Purchase	6	14	27	56
C 6:0	Hexanoic acid	nd	0.101 ± 0.02	0.077 ± 0.02	0.117 ± 0.02	0.141 ± 0.01
C 8:0	Octanoic acid	0.893 ± 0.17	1.252 ± 0.11	1.181 ± 0.23	1.789 ± <0.01	1.982 ± 0.16
C 10:0	Decanoic acid	0.271 ± 0.07	0.532 ± 0.17	0.115 ± 0.03	0.160 ± 0.01	nd
C 12:0	Dodecanoic acid	nd	nd	nd	nd	nd
C 14:0	Tetradecanoic acid	nd	nd	nd	nd	nd
Total		**1.169 ± 0.24**	**1.884 ± 0.21**	**1.354 ± 0.21**	**2.066 ± 0.03**	**2.144 ± 0.20**

Results expressed as mg fatty acid per g cheese
Results are the mean ± standard deviation of 2 extractions and 3 GC analyses for each extraction
nd - not detected

Some significant differences in concentration were observed both within and between each region of the Bleu d'Auvergne cheese. In the blue region, all free medium chain fatty acids were detected (C6:0, C8:0, C10:0, C12:0 and C14:0), whereas in the white region, only hexanoic, octanoic and decanoic acids were found. The concentrations of the fatty acids at time 0 differ slightly to those reported in the cheese investigated in the point of purchase, where both dodecanoic and tetradecanoic acids were detected. An increase in concentration of all of the individual free medium chain fatty acids was seen in

the blue region, especially from 27 to 56 days whereas in the white region only octanoic acid appeared to increase in concentration (Figures 5.5 and 5.6). The concentration of the free fatty acids in the blue region increased as the carbon chain length increased, thus tetradecanoic acid was the dominant fatty acid there. In the white region, octanoic acid was detected in the highest concentration. The total medium chain fatty acid content in the blue region actually doubled from time 0 to 56 days.

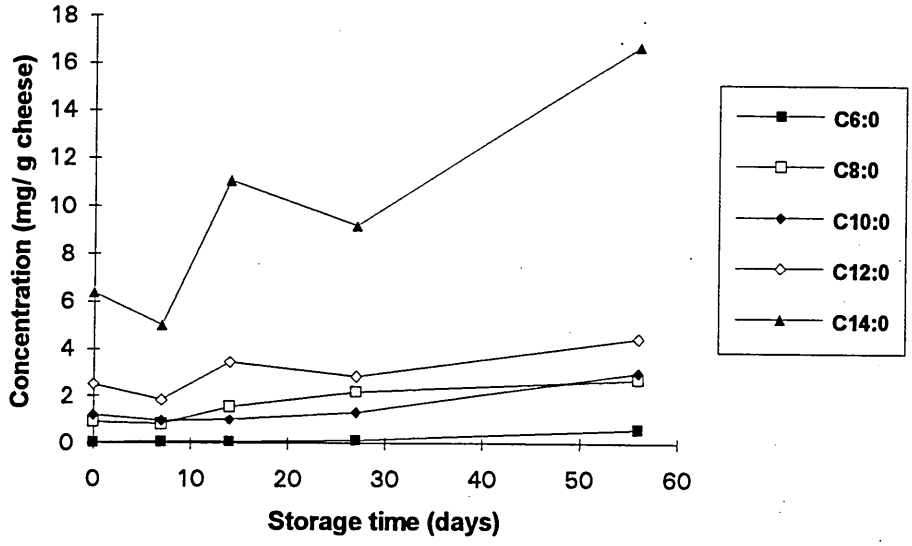


Figure 5.5 Free Medium Chain Fatty Acid Concentration (mg fatty acid /g cheese) in the Blue Region of Bleu d'Auvergne Cheese during Storage at 12 °C

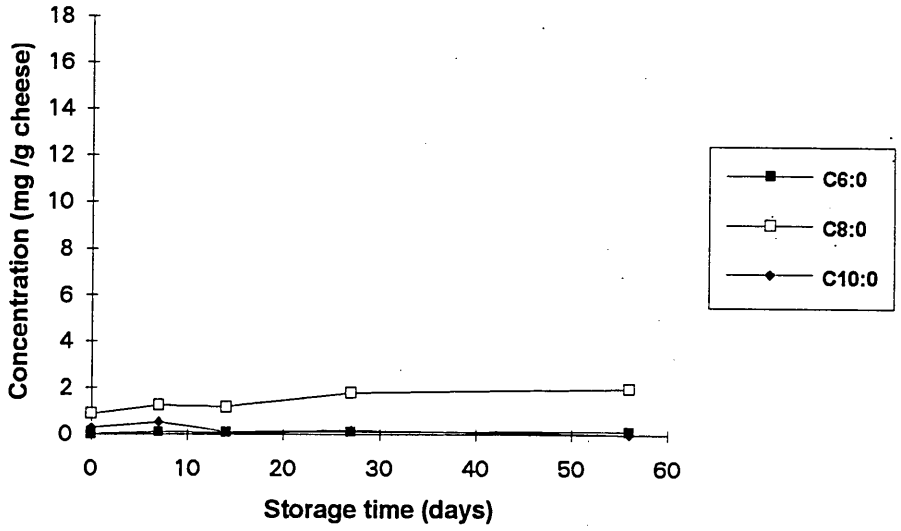


Figure 5.6 Free Medium Chain Fatty Acid Concentration (mg fatty acid /g cheese) in the White Region of Bleu d'Auvergne Cheese during Storage at 12 °C

5.2.2 Changes in Free Medium Chain Fatty Acid Concentration in Brie Cheese during Storage at 12 °C

Tables 5.5 and 5.6 show the changes in concentration (mg fatty acid per g cheese) of free medium chain fatty acids detected at the surface and centre of Brie cheese during storage at 12°C.

Table 5.5 Free Medium Chain Fatty Acid Concentration at the Surface of Brie Cheese during Storage at 12 ± 0.5 °C

		Storage time (days)				
FFA	Systematic name	0	7	14	27	56
C 6:0	Hexanoic acid	0.027 ± 0.01	0.040 ± 0.02	< 0.01 ± 0.01	nd	nd
C 8:0	Octanoic acid	0.606 ± 0.05	1.004 ± 0.07	0.844 ± 0.04	nd	nd
C 10:0	Decanoic acid	0.037 ± 0.04	0.244 ± 0.02	0.032 ± 0.05	nd	nd
C 12:0	Dodecanoic acid	nd	nd	nd	nd	nd
C 14:0	Tetradecanoic acid	nd	nd	nd	nd	nd
Total		**0.670 ± 0.08**	**1.287 ± 0.08**	**0.880 ± 0.08**	**nd**	**nd**

Table 5.6 Free Medium Chain Fatty Acid Concentration in the Centre of Brie Cheese during Storage at 12 ± 0.5 °C

		Storage time (days)				
FFA	Systematic name	0	7	14	27	56
C 6:0	Hexanoic acid	0.037 ± 0.01	0.045 ± 0.03	0.051 ± 0.01	nd	nd
C 8:0	Octanoic acid	0.525 ± 0.05	0.913 ± 0.03	0.981 ± 0.05	nd	nd
C 10:0	Decanoic acid	0.027 ± 0.03	0.056 ±<0.01	0.039 ± 0.05	nd	nd
C 12:0	Dodecanoic acid	nd	nd	nd	nd	nd
C 14:0	Tetradecanoic acid	nd	nd	nd	nd	nd
Total		**0.589 ± 0.05**	**0.972 ± 0.03**	**1.071 ± 0.08**	**nd**	**nd**

Results expressed as mg fatty acid per g cheese
Results are the mean ± standard deviation of 2 extractions and 3 GC analyses for each extraction
nd - not detected

No significant differences in free medium chain fatty acid concentration were observed between both the surface and centre during storage. Only hexanoic, octanoic and decanoic acids were detected in both regions and only up to 14 days. The acids were recovered in similar concentrations in the surface and centre of the cheese. Octanoic acid was the dominant free fatty acid in both regions and present in significantly higher concentrations than hexanoic and decanoic acids (Figures 5.7 and 5.8).

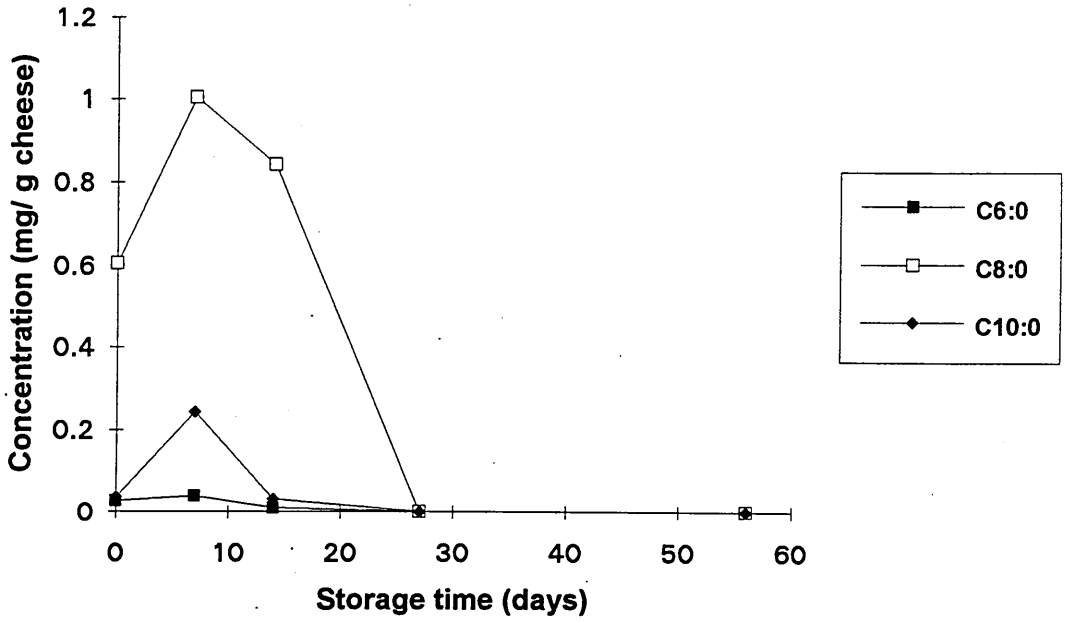


Figure 5.7 Free Medium Chain Fatty Acid Concentration (mg fatty acid /g cheese) at the Surface of Brie Cheese during Storage at 12 °C

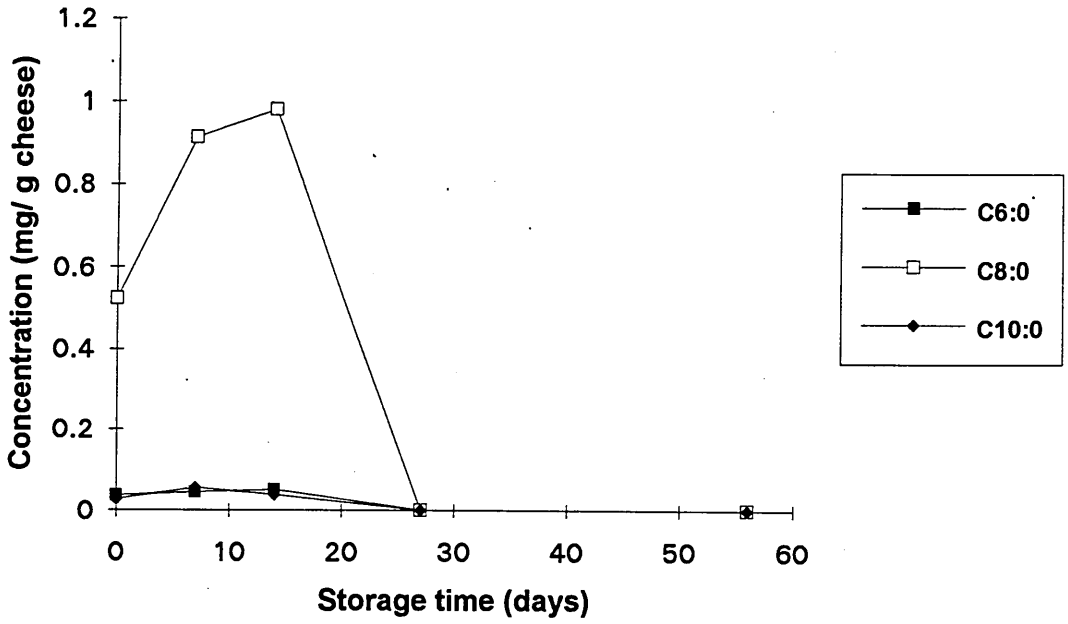


Figure 5.8 Free Medium Chain Fatty Acid Concentration (mg fatty acid /g cheese) at the Centre of Brie Cheese during Storage at 12 °C

5.2.3 Differences between the Bleu d'Auvergne and Brie Cheeses

It can be clearly seen in Figure 5.9 that in the Bleu d'Auvergne cheese, more free medium chain fatty acids were extracted from the blue than the white region. In fact, all acids C6:0, C8:0, C10:0, C12:0 and C14:0 were found in the lipid extract from the blue region whereas only hexanoic, octanoic and decanoic acids were found in the lipid extract from the white region of the cheese. The longer chain length free medium chain fatty acids increased in concentration during storage in the blue region whereas octanoic acid was the dominant acid in the white region.

In Brie cheese, the free fatty acids at both the surface and centre resembled that of the white region of Bleu d'Auvergne. The only fatty acids to be detected were hexanoic, octanoic and decanoic acids. The fatty acids were present in similar concentrations in both regions with octanoic acid the dominant acid. Figure 5.10 shows that there were no significant differences between the two regions of the Brie cheese.

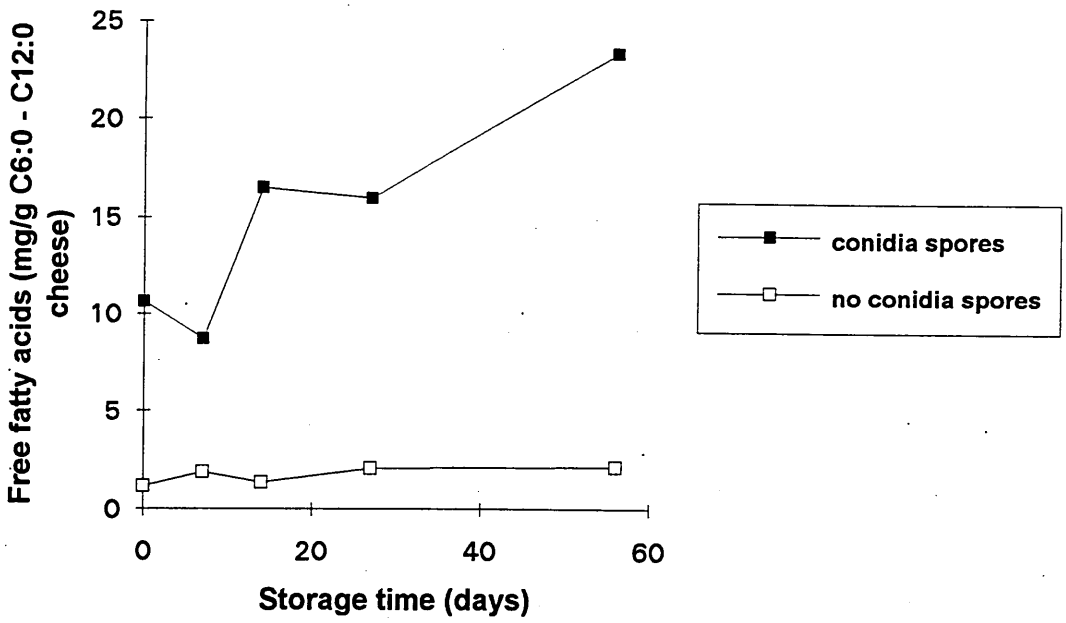


Figure 5.9 Concentration of Total Free Medium Chain Fatty Acid (C6:0, C8:0, C10:0, C12:0 and C14:0) in the Blue and White Regions of Bleu d'Auvergne Cheese during Storage at 12 °C

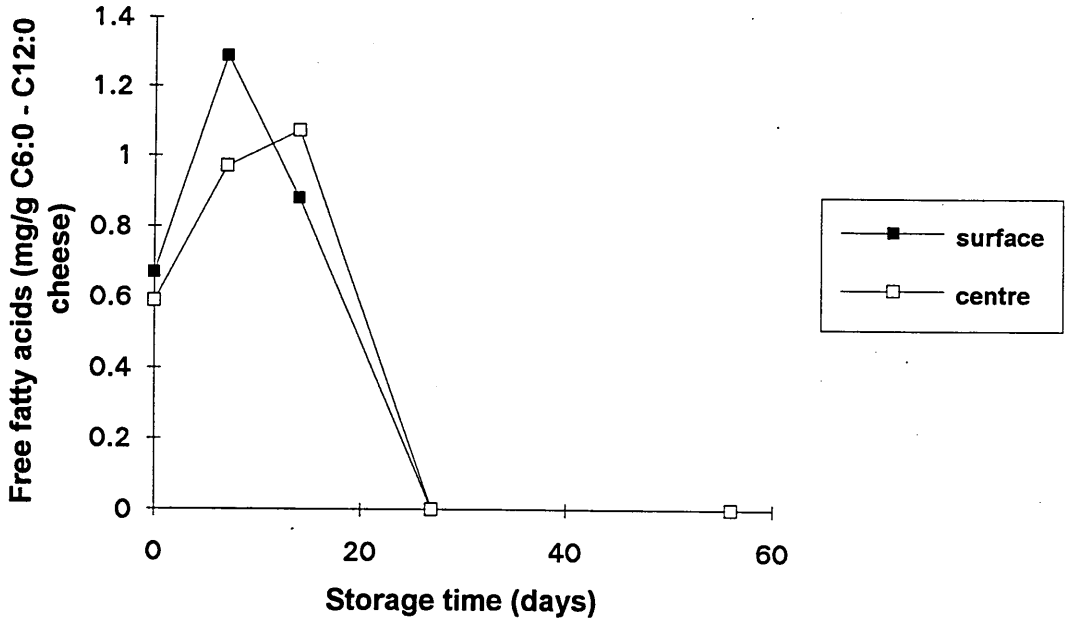


Figure 5.10 Concentration of Total Free Medium Chain Fatty Acid (C6:0, C8:0 and C10:0) at the Surface and Centre of Brie Cheese during Storage at 12 °C

5.3 ANALYSIS OF TOTAL FATTY ACID COMPOSITION OF THE ESTERIFIED LIPID FRACTION OF BLEU d'AUVERGNE AND BRIE CHEESES DURING STORAGE AT 12 °C

5.3.1 Changes in Medium and Long Chain Fatty Acid Composition of the Acylglycerols in Bleu d'Auvergne Cheese during Storage at 12 °C

The lipid fraction of the cheese was extracted (2.2.1). The data obtained from the GC was converted to weight percentage of the extracted oil by the following equation :-

$$\frac{\text{mg FAME / g oil}}{\text{mg total FAME / g oil}} \times 100\ \%$$

Tables 5.7 and 5.8 show the changes in **medium chain** fatty acids (expressed as weight percent of the extracted oil) of the acylglycerols in both the blue and white regions of Bleu d'Auvergne cheese during storage at 12 °C.

Table 5.7 Medium Chain Fatty Acid Composition of the Acylglycerols in the Blue Region of Bleu d'Auvergne Cheese during Storage at 12 ± 0.5 °C

		Storage time (days)				
FAME	Name	0	6	14	27	56
C 6:0	Hexanoic acid	2.46 ± 0.68	1.73 ± 0.24	1.71 ± 0.29	1.69 ± 0.09	1.18 ± 0.40
C 8:0	Octanoic acid	1.53 ± 0.31	1.15 ± 0.17	1.14 ± 0.16	1.22 ± 0.08	0.96 ± 0.05
C 10:0	Decanoic acid	4.74 ± 0.93	4.05 ± 0.42	5.09 ± 0.57	4.37 ± 1.45	3.06 ± 0.13
C 12:0	Dodecanoic acid	4.26 ± 0.53	3.84 ± 0.22	4.64 ± 0.35	3.92 ± 0.31	3.08 ± 0.16
Total		**12.99 ± 1.78**	**10.77 ± 0.90**	**12.58 ± 0.94**	**11.20 ± 1.68**	**8.28 ± 0.28**

Table 5.8 Medium Chain Fatty Acid Composition of the Acylglycerols in the White Region of Bleu d'Auvergne Cheese during Storage at 12 ± 0.5 °C

		Storage time (days)				
FAME	Name	0	6	14	27	56
C 6:0	Hexanoic acid	2.30 ± 0.08	2.29 ± 0.14	2.48 ± 0.21	2.03 ± 0.37	2.68 ± 0.44
C 8:0	Octanoic acid	1.35 ± 0.06	1.42 ± 0.11	1.57 ± 0.10	1.33 ± 0.22	1.65 ± 0.22
C 10:0	Decanoic acid	4.03 ± 0.68	3.99 ± 0.47	3.75 ± 0.19	5.21 ± 0.55	4.00 ± 0.63
C 12:0	Dodecanoic acid	3.88 ± 0.17	3.98 ± 0.29	3.83 ± 0.18	3.92 ± 0.53	4.14 ± 0.52
Total		**11.56 ± 0.76**	**11.68 ± 0.35**	**11.64 ± 0.54**	**12.48 ± 1.42**	**12.47 ± 1.74**

Results expressed as weight percent of the extracted oil
Results are the mean ± standard deviation of two extractions and three GC analyses for each extraction

Differences in the composition of medium chain fatty acids were observed in the blue and white regions of Bleu d'Auvergne cheese. In the blue region of this cheese, the composition (weight percent) of the medium chain fatty acids (C6:0 - C12:0) decreased over the storage time, whilst remaining relatively unchanged in the white region of Bleu d'Auvergne (Tables 5.7 and 5.8). The **long chain** fatty acids were also analysed and the results shown in Tables 5.9 and 5.10.

Table 5.9 Long Chain Fatty Acid Composition of the Acylglycerols in the Blue Region of Bleu d'Auvergne Cheese during Storage at 12 ± 0.5 °C

		Storage time (days)				
FAME	Name	Purchase	6	14	27	56
C 14:0	Tetradecanoic acid	13.71 ±2.05	12.73 ±0.67	13.14±0.57	13.83 0.60	12.70 ±2.59
C16:0	Hexadecanoic acid	34.24 ±3.21	33.69 ±1.60	36.29±0.94	34.09 ±1.54	36.55 ±1.22
C 18:0	Octadecanoic acid	10.94 ±1.05	10.95 ±0.75	10.70±0.52	11.44 ±1.08	11.05 ±0.49
C 18:1	Octadecenoic acid (9-)	26.53 ±2.26	30.14 ±1.76	25.53±1.41	27.78 ±1.34	29.66 ± 2.41
C 18:2	Octadecadienoic acid (9,12-)	1.60 ± 0.27	1.73 ± 0.24	1.77 ± 0.35	1.99 ± 0.17	2.04 ± 0.16

Table 5.10 Long Chain Fatty Acid Composition of the Acylglycerols in the White Region of Bleu d'Auvergne Cheese during Storage at 12 ± 0.5 °C

		Storage time (days)				
FAME	Systematic name	Purchase	6	14	27	56
C 14:0	Tetradecanoic acid	13.42 ±0.56	13.90 ±0.65	13.61 ±1.39	13.83 ±0.54	13.64 ±1.08
C16:0	Hexadecanoic acid	33.50 ±1.50	33.21 ±3.08	31.43 ±1.00	33.21 ±0.86	34.15 ±1.71
C 18:0	Octadecanoic acid	12.29 ±1.26	12.25 ±1.04	13.02 ±0.76	12.04 ±0.56	11.10 ±1.09
C 18:1	Octadecenoic acid (9-)	27.58 ±1.24	27.44 ±3.32	28.73 ±1.16	26.81 ±2.27	27.27 ±3.25
C 18:2	Octadecadienoic acid (9,12)	1.65 ± 0.11	1.52 ± 0.24	1.57 ± 0.13	1.65 ± 0.19	1.38 ± 0.32

Results expressed as weight percent of the extracted oil
Results are the mean ± standard deviation of two extractions and three GC analyses for each extraction

Hexadecanoic, 9- octadecenoic, tetradecanoic and octadecanoic acids were present in much higher concentrations than the medium chain fatty acids in both the blue and white regions of Bleu d'Auvergne (Figures 5.11 and 5.12). An apparent increase in hexadecanoic and octadecenoic acids was seen, probably due to the metabolism of the medium chain fatty acids.

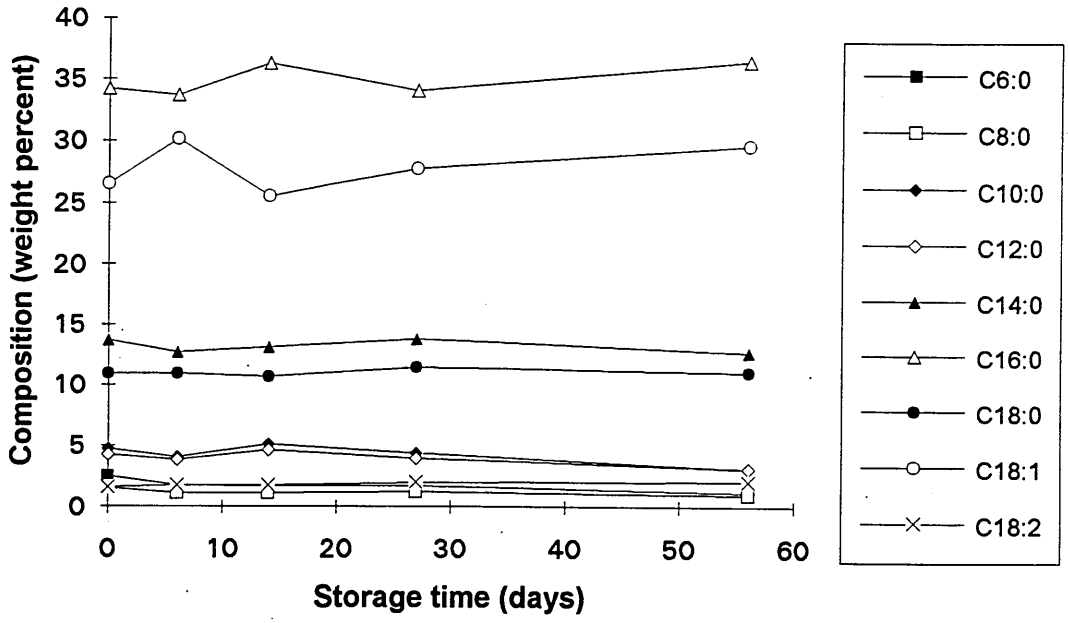


Figure 5.11 Medium and Long Chain Fatty Acid Composition (weight percent) of Acylglycerols in the Blue Region of Bleu d'Auvergne Cheese during Storage at 12 °C

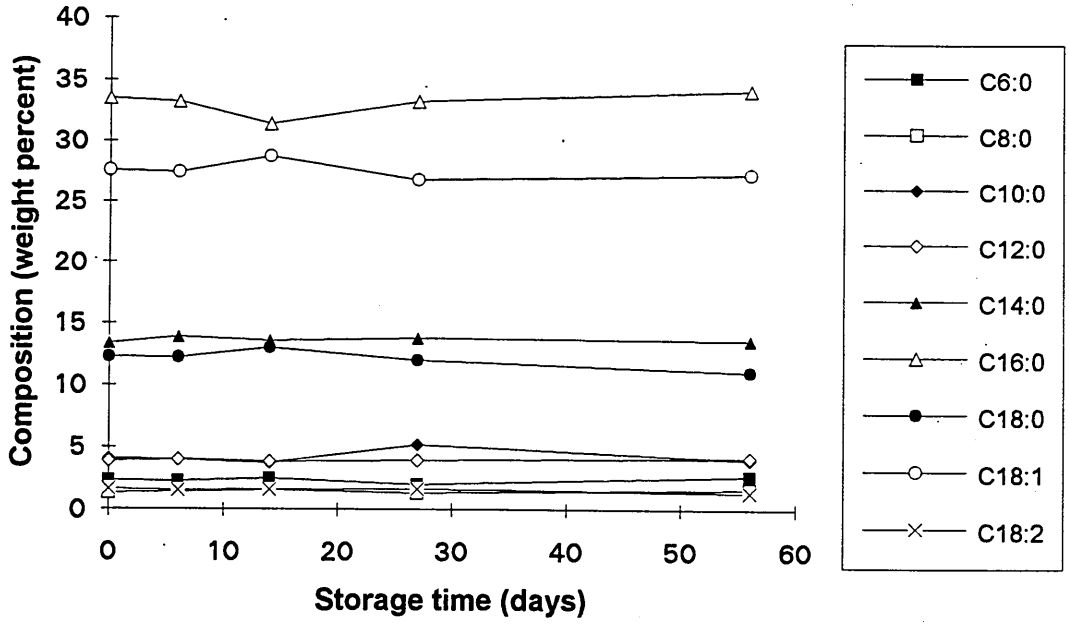


Figure 5.12 Total Fatty Acid Composition (weight percent) of Acylglycerols in the White Region of Bleu d'Auvergne Cheese during Storage at 12 °C

5.3.2 Changes in Medium and Long Chain Fatty Acid Composition of the Acylglycerols in French Brie Cheese during Storage at 12 °C

Tables 5.11 and 5.12 show the changes in **medium chain** fatty acids (expressed as weight percent of the extracted oil) of the acylglycerols at both the surface and centre of the cheese during storage at 12 °C.

Table 5.11 Medium Chain Fatty Acid Composition of the Acylglycerols at the Surface of Brie Cheese during Storage at 12 ± 0.5 °C

		Storage time (days)			
FAME	**Name**	**Purchase**	**14**	**27**	**56**
C 6:0	Hexanoic acid	1.98 ± 0.17	2.11 ± 0.16	2.04 ± 0.16	2.09 ± 0.11
C 8:0	Octanoic acid	1.26 ± 0.07	1.28 ± 0.09	1.30 ± 0.08	1.25 ± 0.10
C 10:0	Decanoic acid	3.28 ± 0.20	3.32 ± 0.26	3.39 ± 0.16	3.38 ± 0.17
C 12:0	Dodecanoic acid	3.97 ± 0.29	3.99 ± 0.26	4.06 ± 0.22	4.17 ± 0.28
Total		**10.49 ± 0.62**	**10.69 ± 0.76**	**10.78 ± 0.57**	**10.89 ± 0.53**

Table 5.12 Medium Chain Fatty Acid Composition of the Acylglycerols in the Centre of Brie Cheese during Storage at 12 ± 0.5 °C

		Storage time (days)			
FAME	**Name**	**Purchase**	**14**	**27**	**56**
C 6:0	Hexanoic acid	1.97 ± 0.07	2.20 ± 0.16	1.95 ± 0.18	2.29 ± 0.08
C 8:0	Octanoic acid	1.33 ± 0.06	1.42 ± 0.09	1.32 ± 0.12	1.49 ± 0.13
C 10:0	Decanoic acid	3.45 ± 0.18	4.08 ± 0.18	3.41 ± 0.18	3.75 ± 0.09
C 12:0	Dodecanoic acid	4.02 ± 0.56	4.59 ± 0.11	4.26 ± 0.25	4.52 ± 0.26
Total		**10.78 ± 0.45**	**12.29 ± 0.32**	**10.93 ± 0.72**	**12.05 ± 0.51**

Results expressed as weight percent of the extracted oil
Results are the mean ± standard deviation of two extractions and three GC analyses for each extraction

No significant differences in the composition of each medium chain fatty acid were observed at both the surface and centre of Brie cheese during the storage period. The **long chain** fatty acids were analysed, namely tetradecanoic, hexadecanoic, octadecanoic, octadecenoic and octadecadienoic acids. The results of this analysis at the surface and centre respectively are shown in Tables 5.13 and 5.14.

Table 5.13 Long Chain Fatty Acid Composition of the Acylglycerols at the Surface of Brie Cheese during Storage at 12 ± 0.5 °C

		Storage time (days)			
FAME	Name	Purchase	14	27	56
C 14:0	Tetradecanoic acid	14.07 ± 0.51	13.79 ± 0.60	14.12 ± 0.16	14.88 ± 0.72
C16:0	Hexadecanoic acid	36.91 ± 1.06	36.08 ± 0.64	36.74 ± 0.51	38.73 ± 1.48
C 18:0	Octadecanoic acid	14.10 ± 1.07	14.10 ± 1.21	13.56 ± 0.62	13.65 ± 1.14
C 18:1	Octadecenoic acid (9-)	22.52 ± 0.69	23.96 ± 0.58	23.10 ± 0.54	20.48 ± 1.24
C 18:2	Octadecadienoic acid (9,12)	1.92 ± 0.51	1.37 ± 0.07	1.70 ± 0.23	1.39 ± 0.21

Table 5.14 Long Chain Fatty Acid Composition of the Acylglycerols in the Centre of Brie Cheese during Storage at 12 ± 0.5 °C

		Storage time (days)			
FAME	Name	Purchase	14	27	56
C 14:0	Tetradecanoic acid	14.02 ± 0.56	14.01 ± 1.00	14.21 ± 0.70	15.03 ± 0.42
C 16:0	Hexadecanoic acid	37.33 ± 0.87	36.75 ± 1.39	37.24 ± 0.91	38.68 ± 0.86
C 18:0	Octadecanoic acid	13.00 ± 0.94	11.96 ± 0.46	12.53 ± 0.51	11.26 ± 0.27
C 18:1	Octadecenoic acid (9-)	23.19 ± 0.85	23.58 ± 1.11	23.54 ±0.54	21.57 ± 0.59
C 18:2	Octadecadienoic acid (9,12)	1.68 ± 0.22	1.41 ± 0.11	1.56 ± 0.27	1.41 ± 0.37

expressed as weight percent of the extracted oil
Results are the mean ± standard deviation of two extractions and three GC analyses for each extraction

Figures 5.13 and 5.14 clearly show that the longer chain fatty acids were present in higher concentrations than the medium chain fatty acids. Hexadecanoic, 9- octadecenoic, tetradecanoic and octadecanoic acids were the major fatty acids at both the surface and centre of the Brie cheese. Hexadecanoic acid appeared to increase during the storage period, whereas 9- octadecenoic acid appeared to decrease during storage of the cheese.

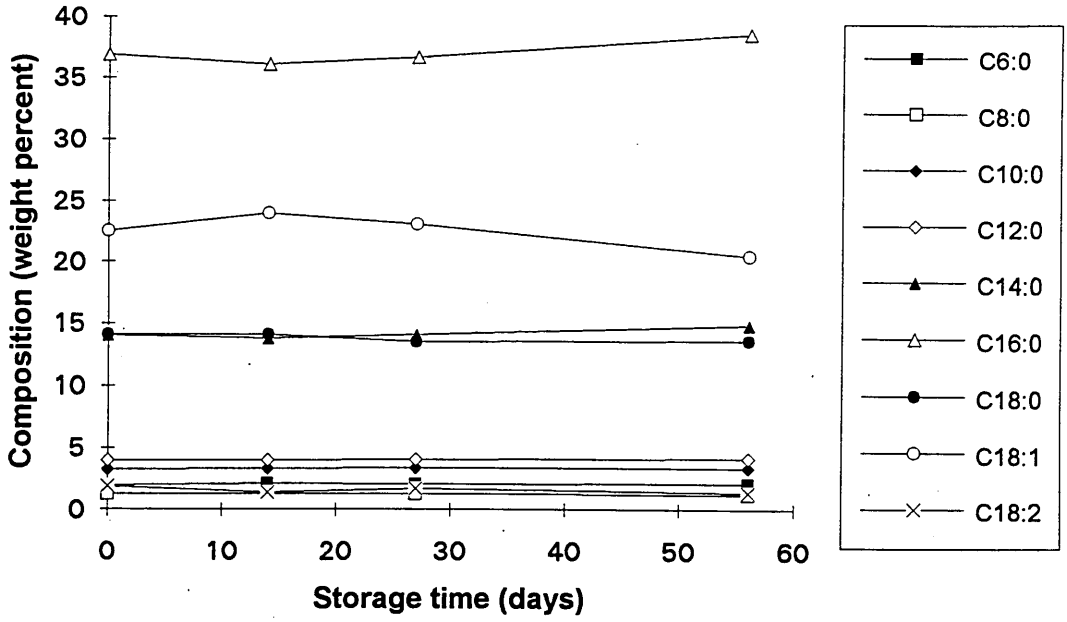


Figure 5.13 Fatty acid Composition (weight percent) of Acylglycerols at the Surface of Brie Cheese during Storage at 12 °C

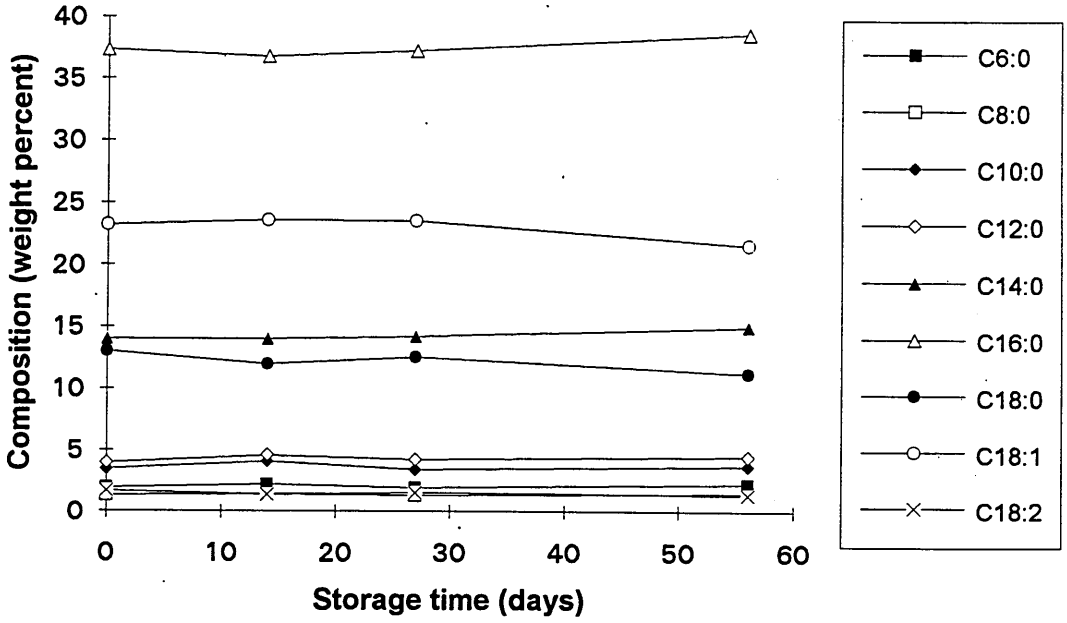


Figure 5.14 Fatty acid Composition (weight percent) of Acylglycerols in the Centre of Brie Cheese during Storage at 12 °C

A decrease in medium chain fatty acids was seen during storage in the blue region of Bleu d'Auvergne, whilst in the white region of the cheese, the fatty acid composition appeared to be more constant. In the Brie cheese no change in medium chain fatty acids was seen during storage. Two of the long chain fatty acids (C16:0 and C18:1) did not increase significantly in the blue and white regions of Bleu d'Auvergne cheese. In the Brie cheese however, an increase in hexadecanoic acid was seen at the surface and centre whilst a decrease in octadecenoic acid was observed in the two regions.

These results did not take into account the varying fat content (%) in the regions of cheese during the storage period. To compensate for this the results from the extraction of the acylglycerol fraction of the cheeses (Tables 5.7 - 5.14) were expressed as mg fatty acid per g cheese. To convert the data obtained from the gas chromatograph into this format the following calculation was used.

$$\text{mg FAME / g oil} \times \frac{\text{fat content (\%)}}{100} \times \text{1 g cheese}$$

Tables 5.15 and 5.16 and Figures 5.15 and 5.16 show the data for the concentration of total fatty acids in the blue and white regions of Bleu d'Auvergne, when expressed as mg fatty acid/ g cheese.

Table 5.15 Total Fatty Acid Concentration in the Blue Region of Bleu d'Auvergne Cheese during Storage at 12 °C

		Storage time (days)				
Fame	FAME	0	6	14	27	56
C6:0	Hexanoic acid	4.323 ± 0.41	3.497 ± 0.34	3.751 ±0. 71	4.106 ± 0.16	3.517 ± 0.43
C8:0	Octanoic acid	2.921 ± 0.10	2.301 ± 0.13	2.512 ± 0.41	2.953 ± 0.19	2.861 ± 0.12
C10:0	Decanoic acid	9.777 ± 1.89	8.171 ± 0.37	9.786 ± 0.38	10.770 ± 4.08	9.084 ± 0.04
C12:0	Dodecanoic acid	8.783 ± 077	8.452 ± < 0.01	9.996 ± 0.48	9.527 ± 1.13	9.153 ± 0.40
C14:0	Tetradecanoic acid	28.349 ± 4.11	29.131 ± 0.29	29.415 ± 1.23	33.601 ± 2.65	32.705 ± 3.26
C16:0	Hexadecanoic acid	70.382 ± 4.46	75.980 ± 5.53	79.632 ± 3.52	82.813 ± 6.08	108.715 ± 2.42
C18:0	Octadecanoic acid	22.826 ± 3.62	22.490 ± 4.28	23.478 ± 1.50	27.710 ± 2.46	33.075 ± 1.01
C18:1	Octadecenoic acid	55.363 ± 8.66	55.437 ± 3.73	56.872 ± 1.45	67.433 ± 4.51	84.876 ± 3.44
C18:2	Octadecadienoic acid	3.611 ± 0.51	3.560 ± 0.88	3.878 ± 0.81	4.819 ± 0.42	6.061 ± 0.28
Total		**218.196 ± 5.89**	**219.241 ± 1.77**	**217.532 ± 8.07**	**250.836 ± 14.45**	**297.781 ± 13.03**

Table 5.16 Total Fatty Acid Concentration in the White Region of Bleu d'Auvergne Cheese during Storage at 12 °C

		Storage time (days)				
	Fatty acid	0	7	14	27	56
C6:0	Hexanoic acid	5.144 ± 0.22	4.869 ± 0.51	5.084 ± 0.31	4.424 ± 0.64	7.058 ± 0.44
C8:0	Octanoic acid	2.986 ± 0.10	3.012 ± 0.05	3.179 ± 0.16	2.890 ± 0.35	4.354 ± 0.20
C10:0	Decanoic acid	8.950 ± 1.64	8.535 ± 1.54	7.690 ± 0.60	11.417 ± 0.78	10.575 ± 0.67
C12:0	Dodecanoic acid	8.586 ± 0038	8.451 ± 0.53	7.835 ± 0.60	8.556 ± 0.76	10.959 ± 0.25
C14:0	Tetradecanoic acid	29.725 ± 1.26	29.493 ± 0.96	27.458 ± 2.95	30.357 ± 0.89	36.360 ± 3.48
C16:0	Hexadecanoic acid	73.544 ± 4.96	70.940 ± 10.76	65.744 ± 6.88	73.011 ± 4.23	91.817 ± 14.25
C18:0	Octadecanoic acid	27.180 ± 2.44	26.056 ± 2.81	26.339 ± 1.78	26.442 ± 2.31	29.94 ± 5.87
C18:1	Octadecenoic acid	61.059 ± 2.21	58.121 ± 5.45	57.972 ± 4.04	59.205 ± 8.59	73.668 ± 16.17
C18:2	Octadecadienoic acid	3.651 ± 0.24	3.236 ± 0.06	3.181 ± 0.30	3.636 ± 0.61	3.816 ± 0.95
Total		**221.501 ± 0.57**	**204.889 ± 11.94**	**201.743 ± 10.87**	**219.995 ± 14.17**	**259.369 ± 18039**

Results expressed as mg fatty acid per g cheese
Results are the mean ± standard deviation of 2 extractions and 3 GC analyses for each extraction

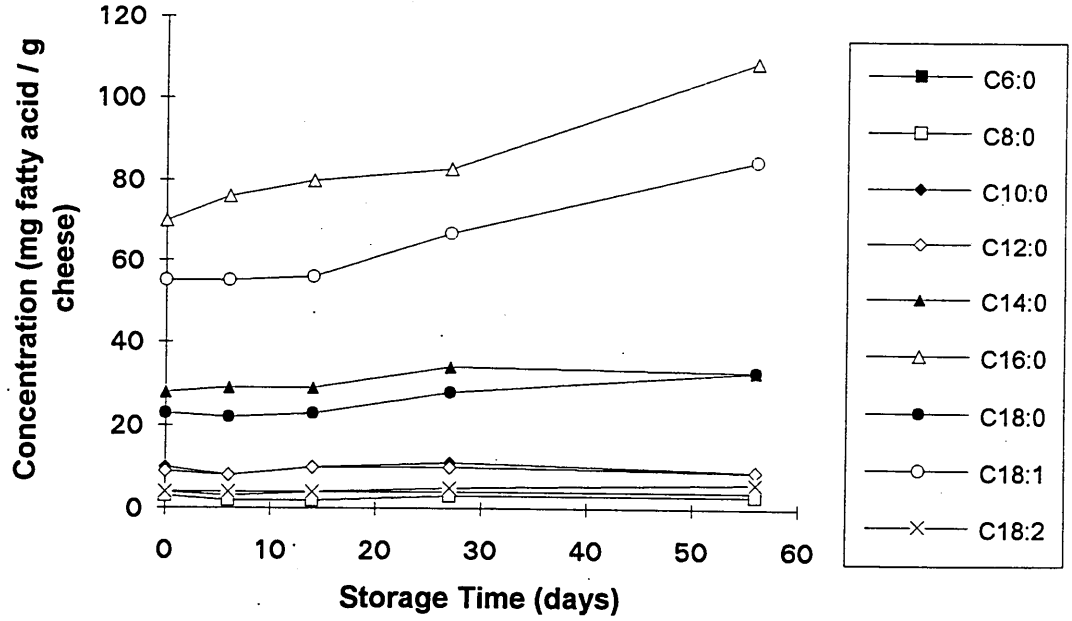


Figure 5.15 Total Fatty Acid Concentration (mg fatty acid /g cheese) in the Blue Region of Bleu d'Auvergne Cheese during Storage at 12 °C

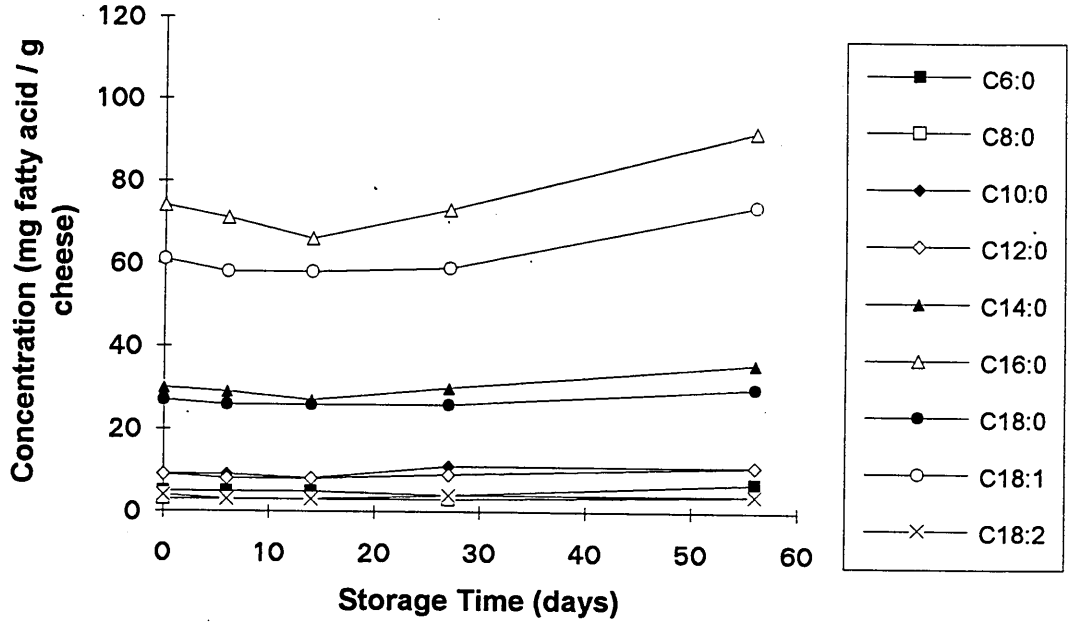


Figure 5.16 Total Fatty Acid Concentration (mg fatty acid /g cheese) in the White Region of Bleu d'Auvergne Cheese during Storage at 12 °C

5.3.5 Total Fatty Acid Concentration at the Surface and Centre of Brie Cheese during Storage at 12 °C

Tables 5.17 and 5.18 and Figures 5.17 and 5.18 show the data for the concentration of total fatty acids detected at the surface and centre of French Brie Cheese when expressed as mg fatty acid per g cheese.

Table 5.17 Changes in Total Fatty Acid Concentration at the Surface of Brie Cheese during Storage at 12 °C

		Storage time (days)			
	Fatty acid	**0**	**14**	**27**	**56**
C6:0	Hexanoic acid	4.678 ± 0.02	5.312 ± 0.08	4.873 ± 0.16	4.436 ± 0.24
C8:0	Octanoic acid	2.969 ± 0.11	3.242 ± 0.10	3.111 ± 0.05	2.650 ± 0.18
C10:0	Decanoic acid	7.762 ± 0.35	8.391 ± 0.19	8.141 ± 0.28	7.175 ± 0.17
C12:0	Dodecanoic acid	9.376 ± < 0.01	10.086 ± 0.10	9.722 ± 0.20	8.83 ± 1.85
C14:0	Tetradecanoic acid	33.279 ± 1.26	34.934 ± 1.41	33.910 ± 3.58	31.606 ± 0.26
C16:0	Hexadecanoic acid	87.285 ± 1.59	91.563 ± 5.92	88.30 ± 5.68	82.301 ± 4.02
C18:0	Octadecanoic acid	33.379 ± 2.98	35.927 ± 5.20	32.602 ± 2.65	29.097 ± 3.77
C18:1	Octadecenoic acid	53.320 ± 2.90	60.851 ± 4.74	55.546 ± 4.18	47.558 ± 1.40
C18:2	Octadecadienoic acid	4.577 ± 1.28	3.479 ± 0.13	4.08 ± 0.56	2.945 ± 0.31
Total		**236.615 ± 5.98**	**253.786 ± 16.26**	**240.267 ± 9.94**	**212.655 ± 11.33**

Table 5.18 Changes in Total Fatty Acid Concentration in the Centre of Brie Cheese during Storage at 12 °C

		Storage time (days)			
	Fatty acid	**0**	**14**	**27**	**56**
C6:0	Hexanoic acid	4.059 ± 0.13	4.488 ± 0.26	3.945 ± 0.03	4.171 ± 0.08
C8:0	Octanoic acid	2.706 ± 0.09	2.901 ± 0.17	2.260 ± 0.07	2.722 ± 0.21
C10:0	Decanoic acid	7.010 ± 0.22	8.312 ± 0.39	6.894 ± 0.05	6.834 ± 0.13
C12:0	Dodecanoic acid	8.158 ± 0.11	9.375 ± 0.34	8.629 ± 0.15	8.236 ± 0.38
C14:0	Tetradecanoic acid	28.484 ± 0.75	28.616 ± 2.47	28.817 ± 1.55	27.414 ± 0.93
C16:0	Hexadecanoic acid	75.869 ± 2.62	74.973 ± 3.34	75.688 ± 5.77	70.570 ± 2.50
C18:0	Octadecanoic acid	26.454 ± 2.44	24.404 ± 1.06	25.446 ± 1.92	20.549 ± 0.83
C18:1	Octadecenoic acid	47.174 ± 2.92	48.104 ± 2.16	47.817 ± 3.30	39.367 ± 1.55
C18:2	Octadecadienoic acid	3.405 ± 0.45	2.873 ± 0.26	3.151 ± 0.43	2.573 ± 0.68
Total		**201.970 ± 6.34**	**204.045 ± 4.83**	**203.031 ± 10.90**	**182.420 ± 4.52**

Results expressed as mg fatty acid/ g cheese
Results are the mean ± standard deviation of two extractions and three GC analyses for each extraction

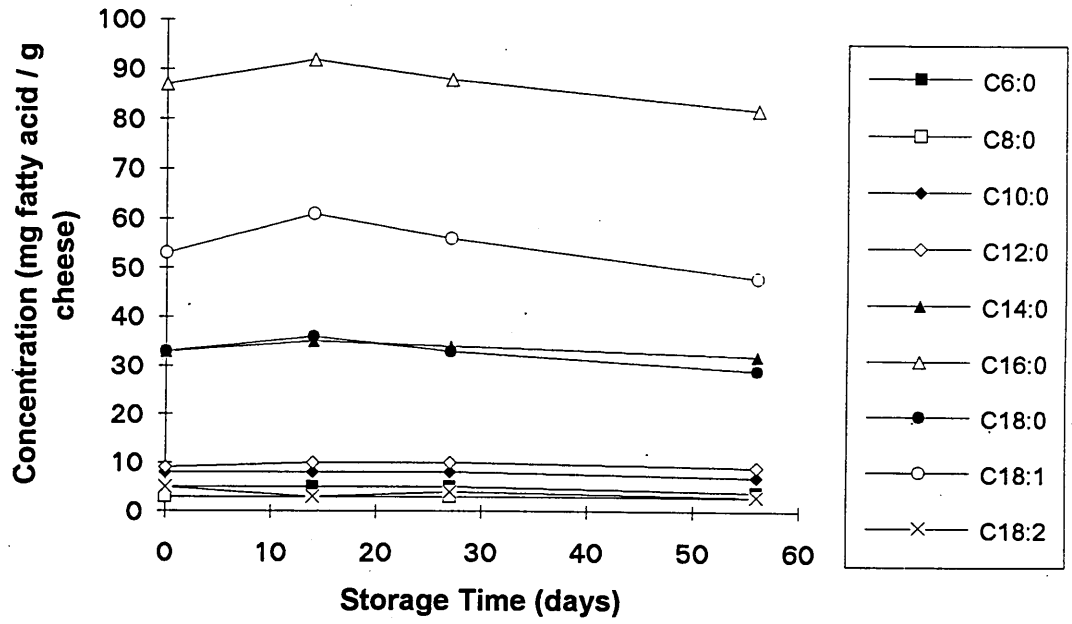


Figure 5.17 Fatty Acid Concentration (mg fatty acid /g cheese) at the Surface of Brie Cheese during Storage at 12 °C

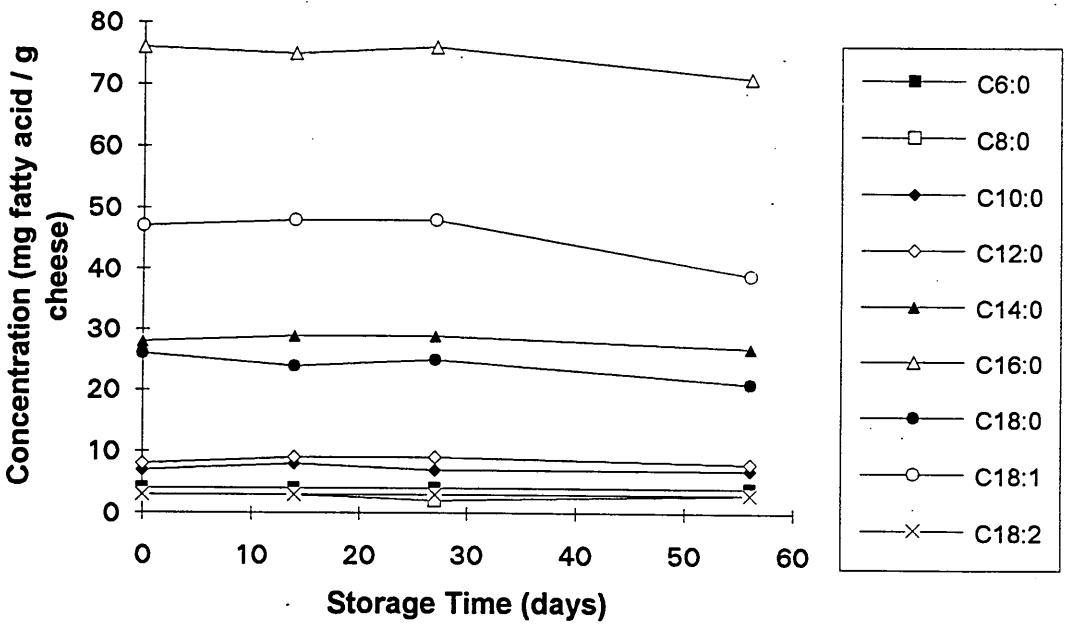


Figure 5.18 Fatty Acid Concentration (mg fatty acid /g cheese) at the Centre of Brie Cheese during Storage at 12 °C

In the Blue cheese, the total fatty acids appeared to increase in concentration during storage in both the blue and white regions of the Bleu d'Auvergne when expressed as mg fatty acid/ g cheese. This was due to a loss of water. As samples of cheese were removed during the storage experiment it was not

possible to measure the water loss accurately. The cheese therefore appeared to become more concentrated. If the concentration of fatty acids remained constant over time one would expect to see them increase. An increase was observed in both regions of the Bleu d'Auvergne cheese. The slight increase in fat content (expressed as fresh weight) over time in the blue region (Table 5.1) also reflected the loss of water.

In the Brie cheese, much less water loss was evident during storage. The medium chain fatty acids appeared to be constant over time on both the extracted oil (Tables 5.11 and 5.12) and when expressed on a fresh cheese weight basis. The concentration of long chain fatty acids hexadecanoic and octadecenoic acids appeared to decrease at the surface and centre of the Brie cheese during storage, when expressed on a fresh cheese basis. This decrease could have been due to utilisation of the fatty acids by *Penicillium camembertii*.

5.5 RELATIONSHIP OF TRIACYLGLYCEROL BREAKDOWN TO HEAT DISSIPATION WITHIN THE SELECTED REGIONS OF CHEESE

The triacylglycerols are energy rich and carbon poor. They provide relatively little carbon which can be used for anabolic reactions leading to cell synthesis. In eukaryotic organisms most fatty acids are degraded in the microbody. Relatively little ATP is produced, with excess energy dissipated as heat (Gurr and Harwood 1991[1], Hatton and Kinderlerer 1991). Therefore in the region of mould growth, one may expect to see a higher temperature than in the region of no obvious mould growth. This could account for the ability of listerias to multiply more rapidly in the outer regions of Brie and Camembert cheeses.

Both the Bleu d'Auvergne and Brie cheeses were analysed by a thermal imaging camera to see if a difference in temperature in the two regions could be found. The limitations of the camera with such small temperature differences caused difficulties with interpretation of the data. In part this was due to the radiation released from the cheese (emmisivity) and the shape of the cheese. The images produced by the camera **indicated** a possible temperature difference in the Brie cheese at the surface and centre, whilst no temperature difference in the two regions of the Blue cheese was probable.

The excess energy lost from each region of Bleu d'Auvergne and Brie cheese in the form of heat, due to the breakdown of fat was calculated on the basis :-

1g fat releases 9 kcal or 38 kJ of energy

(Gaman and Sherrington 1977)

In the blue region of Bleu d'Auvergne cheese, the loss of fat from 27 to 56 days (Table 5.1) was 2.8 %. Therefore **106 kJ** excess energy was lost as heat in the blue region of Bleu d'Auvergne. In the white region of the cheese, an apparent increase in fat content was seen.

At the surface of Brie cheese from 27 to 56 days (Table 5.2) a loss of 3.15 % fat was seen. Therefore **120 kJ** excess energy was lost as heat from the surface of Brie cheese. In the centre of Brie cheese, less fat loss was seen, only 2 % (Table 5.2). Therefore **76 kJ** energy was lost as heat from the centre of the cheese.

An apparent loss of heat was seen in the blue region of Bleu d'Auvergne cheese,due to the metabolism of the fat, but not in the white region of the cheese, probably due to water loss from the cheese during storage.

In the Brie cheese there could be a difference in the excess energy released as heat from the breakdown of fat, i.e. a difference in temperature at the surface and centre of the cheese, which could contribute to the conditions ideal for listeria growth.

5.6 DISCUSSION

The total fatty acid composition of butter oil (Table 1.3) and blue and white mould-ripened cheeses (from the results given in Tables 5.7 - 5.14), are similar. This demonstrates that the composition of fatty acids in soft-ripened cheeses does not change very much during ripening.

Free fatty acids are important flavour compounds in blue cheeses. Higher concentrations accumulate in Blue cheeses than in most other types of cheese (Woo *et al.* 1984). Madkor *et al.* (1987) found that the concentration of all individual free fatty acids in an experimental Stilton cheese increased as the ripening process progressed (Table 5.19). Free hexanoic and octadecenoic acids were not seen in the Stilton cheese until day 20 of the ripening process. The free fatty acids may have been utilised in further metabolic reactions by the mould during the early stages of ripening, or relatively little lipolysis occurred until after 20 days. Kinsella and Hwang (1976[2]) found the moulds to release lipolytic enzymes after sporylation which in turn occurred after 28 days of the ripening process. At this stage the cheese was visibly marbled with *Penicillium roquefortii.*

Table 5.19 Concentration (mg/ g cheese) of Free Fatty Acids in Stilton Cheese made with Fresh Milk and 2 Commercial Samples of Blue Cheese During Ripening

Age (days)*	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2
4	-	0.03	0.03	0.03	0.06	0.18	0.12	0.20	-
20	0.02	0.02	0.06	0.03	0.07	0.22	0.09	0.26	0.04
28	0.02	0.04	0.04	0.03	0.07	0.27	0.19	0.28	0.04
45	0.04	0.04	0.07	0.07	0.17	0.47	0.18	0.98	0.14
55	0.14	0.09	0.14	0.14	0.34	0.99	0.34	1.72	0.35
70	0.17	0.19	0.33	0.33	0.81	2.00	0.73	4.35	0.90
Stilton[a]	0.24	0.18	0.57	0.80	2.07	4.66	1.95	5.44	1.05
Danablu[b]	0.53	0.27	0.50	0.61	1.58	3.89	1.38	7.03	1.18

* - These cheeses were prepared experimentally
a - Commercial sample of Stilton cheese
b - Commercial sample of Danablu cheese

Madkor *et al.* (1987)

The literature values for the free fatty acids in the experimental Stilton cheese (Table 5.19), were lower than those found in the present study (Tables 5.3 - 5.6). The values for Bleu d'Auvergne and Brie cheeses in this study were obtained after the initial ripening period at the manufacturer's of up to 4 months, whilst those for the experimental Stilton cheese were obtained from about day 3 of the maturation stage when the cheese was placed in the ripening room. This point is illustrated by the concentration of hexadecanoic acid in a commercial sample of Stilton cheese being twice the concentration of the experimental cheese (4.66 : 2.00 mg /g cheese) at day 70 of ripening.

De la Fuente *et al.* (1993) analysed the free fatty acid and triacylglycerol fractions in representative samples of cheeses made from cow's milk. They found the composition (%) of free fatty acids to be generally greater than the acylated fatty acids in the triacylglycerol fraction. Table 5.20 illustrates that this was evident to a greater extent in the white mould-ripened Camembert cheese than the blue mould-ripened cheese Cabrales.

Table 5.20 Average Fatty Acid Composition (%) in the Free Form and in the Triacylglycerols

FAME	Cabrales*		Camembert	
	Free Fatty Acids	Triacylglycerols	Free Fatty Acids	Triacylglycerols
hexanoic acid	1	2.1	5.5	2.1
octanoic acid	1.2	1.3	3.3	0.8
decanoic acid	2.9	3.2	4.1	2.2

Results expressed as percentage fatty acid
* cheese is made with a blend of cows', ewes' and goats' milk, predominantly cow's milk

de la Fuente *et al.* (1993)

These authors only analysed the triacylglycerol fraction of the acylglycerols. The composition of medium chain fatty acids reported in this document (Tables 5.7 - 5.8 and Tables 5.11 - 5.12) for all the acylglycerols (tri-, di- and mono and free fatty acids) in Bleu d'Auvergne and Brie cheeses do not differ greatly to the sum of the free acids and triacylglycerol data of de la Fuente *et al* (Table 5.20).

CHAPTER 6

CONCLUSIONS

To analyse the fatty acid composition of the **acylglycerols** (tri- di- and mono-), an esterification was carried out. To verify that the peaks of interest were definitely fatty acids, the samples were analysed by GC/MS and the resulting peaks identified by comparison to standard data.

Odd and even saturated straight chain aliphatic fatty acid methyl esters (C6:0 to C20:0) were identified in both the Bleu d'Auvergne and Brie cheeses. Monoenoic fatty acid methyl esters were also detected. In both cheeses, tetradecenoic, hexadecenoic and 9- octadecenoic acids were identified whilst in the Brie cheese, 4- decenoic acid was also detected.

Methyl branched saturated fatty acid methyl esters tetradecanoic acid-12-methyl methyl esters and hexadecanoic acid-14-methyl methyl ester were detected in both the Bleu d'Auvergne and Brie cheeses. Pentadecanoic acid-14-methyl methyl ester and hexadecanoic acid-15-methyl methyl ester were also detected in the Bleu d'Auvergne cheese.

The **free** medium chain fatty acids hexanoic, octanoic, decanoic and dodecanoic acids and the long chain fatty acids tetradecanoic and hexadecanoic acids were detected in the lipid extract of both Bleu d'Auvergne and Brie cheeses. Hexanoic acid was not detected as the peak was probably masked by the solvent front.

Two ketones were detected in the Brie cheese (2 - nonanone and 8 - nonen - 2 one). There was no significant difference in the fatty acid composition of Bleu d'Auvergne and Brie cheeses.

The major fatty acids in cheeses made from cow's milk are hexadecanoic acid (palmitic acid) and octadecenoic acid (oleic acid) with lower concentrations of tetradecanoic acid (myristic acid) being found.

Of the total fatty acids in cheese, medium chain fatty acids (C6:0 - C12:0) represent roughly ten percent. In the surface-ripened cheeses such as Brie and Vacherin Mont d'Or, there was relatively little difference in the concentration of the free medium chain fatty acids detected at the surface and centre. In Brie, only hexanoic, octanoic and decanoic acids were found with octanoic acid present in the highest concentration, $\approx$ 0.4 - 0.7 mg/g cheese. In Vacherin Mont d'Or cheese, all the fatty acids were detected (C6:0 - C14:0), with tetradecanoic and octanoic acids present in the highest concentrations (1.01mg/g and 0.70 mg/g cheese respectively).

However in the blue veined cheeses, higher concentrations of free medium chain fatty acids were detected in the blue region particularly the longer chain acids. The concentration of the medium chain fatty acids in the white region of the cheese was similar to the surface-ripened cheeses. This result would suggest that the conidia in the blue cheeses are more metabolically active than those on the surface of the surface-ripened cheeses.

This result demonstrated that fungal metabolism is different in different regions of the cheese. It may be that the increased metabolism in the blue region of the cheese provides a barrier which could inhibit the growth of potential pathogens. Unfortunately, the pH in the blue region of Bleu d'Auvergne was higher than in the white region of the cheese, which could promote microbial growth. However, the higher fat content in the blue region would reduce the water content, and hence contribute to inhibit multiplication of potential pathogens.

In cheeses which were stored under simulated ripening conditions there was relatively little change in the free medium chain fatty acid concentration in surface-ripened cheese whereas changes in these acids were observed in the blue veined cheese. In the blue region of the Bleu d'Auvergne cheese the concentration of the longer length medium chain fatty acids (dodecanoic and tetradecanoic acids) increased considerably during storage. The concentration of these acids remained relatively constant in the white region of the cheese. Again, this result demonstrates that considerable fungal metabolic activity occurs in the blue region of the cheese presumably due to the large numbers of blue-green conidia spores found in this region.

Differences in the total fatty acid concentration of the free fatty acids and the acylglycerols were observed in the blue cheese. The medium chain acids C6:0, C8:0, C10:0, C12:0 and C14:0 appeared to decrease in the blue region of the cheese. Some of this loss could be due to the conversion of the fatty acid to the methyl ketone (one carbon atom less than the parent fatty acid). The long chain fatty acids (C16:0 and C18:1) appear to increase in the blue region whilst all detected fatty acids in the white region of the cheese did not change significantly with time. The increase in concentration on storage could be accounted for by the loss of water. Oleic acid (C18:1) appeared to increase (based on a fresh cheese weight) in the blue region of the cheese suggesting that this acid is not metabolised by the fungus unlike in white surface-ripened cheeses.

No significant difference was evident in the free and acylated medium chain fatty acids at both the surface and centre of the Brie cheese. In the Brie cheese, the long chain fatty acid hexadecanoic acid appeared to increase slightly, whilst tetradecanoic, octadecanoic and octadecenoic acids appeared to decrease in concentration during the storage period. This was probably due to fungal metabolic activity. The more significant decrease in oleic acid (C18:1)

could have been due to it being metabolised by enzymes produced by *Penicillium camembertii*, as the mould can utilise oleic acid as an energy source. Hexadecanoic and octadecenoic acids were present in the highest concentration in both cheeses (≈ 38 % and ≈ 24 % respectively).

The pH was relatively constant in the blue cheese during storage. In the surface-ripened cheese the pH was initially higher at the surface, but during storage increased in the centre until both were similar at the end of the ripening period. The pH increase in the centre of the cheese was, probably, due to the fungal mycelium spreading from the outside to the middle of the cheese. Both cheeses had been purchased after the initial ripening period at the manufacturer's so the breakdown of the triacylglycerols would have already commenced causing little change in the pH to occur during the simulated ripening experiment, especially in the Bleu d'Auvergne cheese.

The fat content in the blue region of the blue cheese decreased during storage probably due to fungal activity. In the white region on the other hand, an apparent increase in fat content was seen, probably due to the loss of water from the cheese during storage. In the Brie cheese, the fat content was significantly higher at the surface than the centre of the cheese. A decrease in fat content in both regions of the Brie cheese was seen, again probably due to fungal metabolic activity. This decrease in fat content would not contribute to the inhibition of potential pathogens in Brie cheese.

6.3.6 Suggestions for Further Work

1) The medium chain free fatty acids in mould-ripened cheeses were detected in concentrations too low to have a listericidal effect. Kinderlerer J.L. (1994) found differences in the concentration of triacylglycerols in butter oil, Bleu d'Auvergne and Brie cheeses. Monolaurin has also been found to be

listericidal. The concentrations of tri-, di- and mono- acylglycerols should therefore be analysed in the blue and white mould-ripened cheeses.

2) The lipid extraction method would have to be modified to enable the extraction of tri-, di- and monoacylglycerols separately.

3) For all future GC analysis, it would be advised to use capillary columns. The improved resolution of peaks using these columns would give better efficiency. The higher temperatures may also allow tri- di- and mono-acylglycerols to be separated and analysed.

4) Water lost from the cheese should be measured, especially for Bleu d'Auvergne cheese, to account for the apparent increase in both free and total fatty acids and fat content (%) during storage.

5) Water activity and salt content of the cheese should be calculated.

CHAPTER 7

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