Advanced Dairy Chemistry

Advanced Dairy Chemistry Volume 2 Lipids

Third Edition

Edited by

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Preface to the Third Edition

Advanced Dairy Chemistry 2: Lipids is the second volume of the third edition of the series on advanced topics in Dairy Chemistry, which started in 1982 with the publication of *Developments in Dairy Chemistry*. The first volume, on milk proteins, of the third edition of *Advanced Dairy Chemistry* was published in 2003. This series of volumes is intended to be a coordinated and authoritative treatise on Dairy Chemistry. In the decade since the second edition of this volume was published (1995), there have been considerable advances in the study of milk lipids, which are reflected in changes to this book.

Most topics included in the second edition are retained in the current edition, which has been updated and considerably expanded from 10 to 22 chapters. For various reasons, the authors of many chapters have been changed and hence, in effect, are new chapters, at least the topic is viewed from a different perspective.

The new chapters cover the following subjects: Biosynthesis and nutritional significance of conjugated linoleic acid, which has assumed major significance during the past decade; Formation and biological significance of oxysterols; The milk fat globule membrane as a source of nutritionally and technologically significant products; Physical, chemical and enzymatic modification of milk fat; Significance of fat in dairy products: creams, cheese, ice cream, milk powders and infant formulae; Analytical methods: chromatographic, spectroscopic, ultrasound and physical methods.

Like its predecessor, this book is intended for academics, researchers at universities and industry, and senior students; each chapter is referenced extensively.

We wish to thank sincerely the 37 contributors to the 22 chapters of this volume, whose cooperation made our task as editors a pleasure. The generous assistance of Ms. Anne Cahalane is gratefully acknowledged.

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Preface to the Second Edition

Advanced Dairy Chemistry can be regarded as the second edition of *Developments in Dairy Chemistry*. The first volume in the series, on Milk Proteins, was published in 1992; this, the second volume, is devoted to Milk Lipids. Considerable progress has been made in several aspects of milk lipids during the past 11 years which is reflected in revised versions of seven of the eight chapters included in *Developments in Dairy Chemistry –2*, most of them by the same authors. The theme of one chapter has been changed from physical properties and modification of milk fat to the crystallization of milk fat. Two new chapters have been added, i.e. chemistry and technology aspects of low-fat spreads and the significance of fat in consumer perception of food quality, which reflect the continuing consumer awareness of a healthy diet. Low-fat spreads have become increasingly significant during the past decade and are now the major type of spread in many countries. However, reducing the fat content of foods generally results in a concomitant decrease in the organoleptic quality of the food; consumer attitudes to reduced-fat dairy products are discussed in one of the new chapters.

Like its predecessor, the book is intended for lecturers, senior students and research personnel and each chapter is extensively referenced.

I would like to thank all the authors who contributed to this book and whose cooperation made my task as editor a pleasure.

P. F. Fox

Preface to the First Edition

Many of the desirable flavour and textural attributes of dairy products are due to their lipid components; consequently, milk lipids have, traditionally, been highly valued, in fact to the exclusion of other milk components in many cases. Today, milk is a major source of dietary lipids in western diets and although consumption of milk fat in the form of butter has declined in some countries, this has been offset in many cases by increasing consumption of cheese and fermented liquid dairy products.

This text on milk lipids is the second in a series entitled *Developments in Dairy Chemistry*, the first being devoted to milk proteins. The series is produced as a co-ordinated treatise on dairy chemistry with the objective of providing an authoritative reference source for lecturers, researchers and advanced students. The biosynthesis, chemical, physical and nutritional properties of milk lipids have been reviewed in eight chapters by world experts. However, space does not permit consideration of the more product-related aspects of milk lipids which play major functional roles in several dairy products, especially cheese, dehydrated milks and butter.

Arising from the mechanism of fatty acid biosynthesis and export of fat globules from the secretory cells, the fat of ruminant milks is particularly complex, containing members of all the major lipid classes and as many as 400 distinct fatty acids. The composition and structure of the lipids of bovine milk are described in Chapter 1, with limited comparison with non-bovine milk fats. Since the fatty acid profile of milk fat, especially in monogastric animals, may be modified by diet and other environmental factors, the biosynthesis of milk lipids is reviewed in Chapter 2 with the objective of indicating means by which the fatty acid profile, and hence the functional properties of the lipids, might be modified. Lipids in foods are normally present as an emulsion, stabilized by a layer of protein adsorbed at the oil-water interface. The fat in milk and cream exists as an oil-in-water emulsion with a unique stabilizing lipoprotein membrane, referred to as the milk fat globule membrane (MFGM). The inner layers of the MFGM are formed within the secretory cell and are relatively stable; however, the outer layers, which are acquired as the fat globule is exported through the apical membrane of the secretory cells, are unstable. Damage to the MFGM leads to chemical and physical instability of the fat phase in milk and hence the

structure of the membrane has been the subject of considerable research, the results of which are reviewed in Chapter 3.

Lipids strongly influence, for good or evil, the flavour and texture of foods, especially high-fat products such as butter. The influence of various colloidal features of milk fat on the properties of milk and cream is considered in Chapter 4, while the crystallization of milk fat and how this may be controlled, modified and measured are reviewed in Chapter 5. Unfortunately, lipids are subject to chemical and enzymatic alterations which can cause flavour defects referred to as oxidative and hydrolytic rancidity, respectively. The storage stability of high-fat foods, especially mildly flavoured foods like milk, cream and butter, is strongly influenced by these changes which have been reviewed in Chapters 6 and 7.

Dietary lipids play many diverse nutritional roles, some of which are essential. However, dietary lipids, especially saturated lipids of animal origin, have been the subject of much controversy in recent years, particularly in regard to their possible role in atherosclerosis. Various aspects of the nutritional significance of lipids are discussed in Chapter 8.

Finally, I wish to thank sincerely the 14 authors who have contributed to this text and whose co-operation has made my task as editor a pleasure.

P. F. Fox

Contents

1. Composition and Structure of Bovine Milk Lipids

A.K.H. MacGibbon and M.W. Taylor

2. Milk Fat: Origin of Fatty Acids and Influence of Nutritional Factors Thereon

D.L. Palmquist

3. Conjugated Linoleic Acid: Biosynthesis and Nutritional Significance

D.E. Bauman and A.L. Lock

6. Composition, Applications, Fractionation, Technological and Nutritional Significance of Milk Fat Globule Membrane Material

R.E. Ward, J.B. German and M. Corredig

7. Crystallization and Rheological Properties of Milk Fat

A.J. Wright and A.G. Marangoni

8. Milk Fat: Physical, Chemical and Enzymatic Modification

M.A. Augustin and C. Versteeg

9. Chemistry and Technology of Butter and Milk Fat Spreads

M.K. Keogh

10. Significance of Milk Fat in Cream Products

W. Hoffmann and W. Buchheim

11. Significance of Milk Fat in Cheese

T.P. Guinee and P.L.H. McSweeney

12. Ice Cream

H.D. Goff

13. Significance of Milk Fat in Milk Powder

N.Y. Farkye

14. Significance of Milk Fat in Infant Formulae

G. Hendricks and M. Guo

15. Lipolytic Enzymes and Hydrolytic Rancidity

H.C. Deeth and C.H. Fitz-Gerald

16. Lipid Oxidation

T.P. O'Connor and N.M. O'Brien

17. Nutritional Significance of Milk Lipids

P.W. Parodi

18. Oxysterols: Formation and Biological Function

P.A. Morrissey and M. Kiely

19. High Performance Liquid Chromatography and Gas Chromatography Methods for Lipid Analysis

K.N. Kilcawley

20. Spectroscopic Techniques (NMR, Infrared and Fluorescence) for the Determination of Lipid Composition and Structure in Dairy Products

É. Dufour

21. Applications of Ultrasound to Analysis/Quantitation of Dairy Lipids

M.J.W. Povey and R.E. Challis

22. Physical Characterization of Milk Fat and Milk Fat-Based Products

O.J. McCarthy

1

Composition and Structure of Bovine Milk Lipids

A.K.H. MacGibbon and M.W. Taylor

1.1. Introduction

The lipids in bovine milk are present in microscopic globules as an oil-in-water emulsion. The primary purpose of these lipids is to provide a source of energy to the newborn calf. Both the fat content of the milk and the fatty acid composition of the lipids can vary considerably as a result of changes in factors like breed of cow, diet and stage of lactation. The fat content can vary from about 3.0 to 6.0%, but typically is in the range 3.5 to 4.7%. Changes in the composition of the fatty acids (e.g., 16:0 and 18:1) can be quite marked and can lead to changes in physical properties of the fat. These changes make comparison difficult between different samples of milk fat, and ideally comparisons should be made between cows in mid-lactation and fed on similar diets. From a practical viewpoint, milk lipids are very important as they confer distinctive nutritional, textural and organoleptic properties on dairy products, such as cream, butter, whole milk powder and cheese.

The composition and structure of bovine milk fat have been reviewed extensively. There are early reviews by Morrison (1970), Christie (1978, 1995), Jensen and Clark (1988), and Jensen and Newberg (1995); recent articles include a comprehensive review of recent research by Jensen (2002) and two book chapters by Vanhoutte and Huyghebaert (2003), and Zegarska (2003). Bovine milk lipids are similar to the milk lipids of other species as they are largely composed of triacylglycerols; however, there are also minor amounts of diacylglycerols, monoacylglycerols, free (unesterified) fatty acids, phospholipids and

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Table 1.1. Main classes of lipids in milk[a]

Lipid class	Amount (%, w/w)
Triacylglycerols	98.3
Diacylglycerols	0.3
Monoacylglycerols	0.03
Free fatty acids	0.1
Phospholipids	0.8
Sterols	0.3
Carotenoids	trace
Fat-soluble vitamins	trace
Flavour compounds	trace

[a] Walstra and Jenness (1984)

sterols. Trace amounts of fat-soluble vitamins, β-carotene and fat-soluble flavouring compounds are also present in the bovine milk lipids (Table 1.1).

Because the triacylglycerols account for about 98% of the total fat, they have a major and direct effect on the properties of milk fat, for example hydrophobicity, density and melting characteristics. These triacylglycerols are a complex mixture, and vary considerably in molecular weight and degree of unsaturation. After milking, fresh milk contains only small amounts of diacylglycerols and monoacylglycerols and free fatty acids. The small proportion of diacylglycerols are largely *sn*-1,2 diacylglycerols and are, therefore, probably intermediates in the biosynthesis of triacylglycerols rather than the products of lipolysis (Lok, 1979). The profile of free fatty acids in freshly-drawn milk differs somewhat from the profile of the fatty acids esterified to the triacylglycerols (e.g., there appears to be very little free butanoic acid), also indicating that they are unlikely to be the result of lipase action (Walstra and Jenness, 1984).

Phospholipids account for only 0.8% of milk lipids. However, they play a major role in milk due to their amphiphilic properties. About 65% of them are found in the milk fat globule membrane (MFGM), whereas the rest remain in the aqueous phase. Phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin are the major phospholipids of milk, which together comprise about 90% of the total. Sterols are also a minor component, comprising about 0.3% of the fat; cholesterol, being the principal sterol, accounts for over 95% of the total sterols.

Milk fat is present in spherical droplets, which range from about 0.2 to 15.0 μm in diameter, with the bulk of the fat being in globules 1.0 to 8.0 μm diameter. The MFGM, which envelopes the fat globule, consists largely of proteins and lipids. The protein of the membrane has a complex composition and over 40 polypeptides have been identified. Xanthine oxidoreductase,

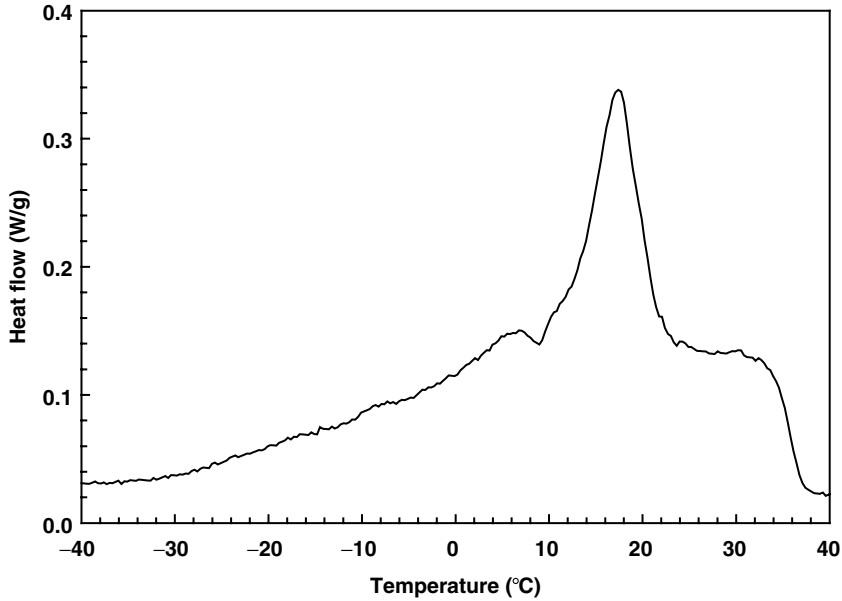

Figure 1.1. Melting profile of New Zealand milk fat, determined by differential scanning calorimetry (MacGibbon, 1988).

butyrophilin, PAS 6 and PAS 7 are found to be the major proteins. The lipids in the membrane are largely phospholipids and triacylglycerols. In contrast to the MFGM, the fat globule core almost exclusively consists of triacylglycerols (Keenan and Dylewski, 1995; see Chapter 4, Keenan and Mather).

The chemical properties of milk lipids can have a considerable influence on the melting characteristics of milk fat, which in turn can have a marked effect on the functional properties of a number of dairy products, such as cheese and butter (Chen *et al.*, 2004). Milk fat melts over a wide range, from about −35°C to 38°C (Figure 1.1). There is a small broad peak centred at about 7°C, a major melting peak at about 17°C, and a plateau from 22°C to 36°C. It can be seen that a substantial proportion of milk fat melts between 10°C and 20°C. This broad melting range is directly attributable to the large number of different types of triacylglycerols present in the milk fat.

1.2. Fatty Acids

Bovine milk fat is regarded as one of the most complex naturally-occurring fats and oils, because of the large number of fatty acids with a variety of structures. Using a combination of chromatographic and spectroscopic

techniques, researchers have identified approximately 400 fatty acids in milk fat. A listing of the various types of fatty acids has been compiled by Jensen (2002). The vast majority of these acids are present in extremely small quantities (<0.01%). However, there are about 15 fatty acids that are present at or above 1.0% concentration. The quantities of these "major" fatty acids are determined relatively easily by capillary gas chromatography (GC) (IDF, 2002). Percentages for these fatty acids in milk fat are shown in Table 1.2. The typical values are for cows in mid-lactation, grazing on mature pasture. The range of values is for the dairying season in New Zealand, where cows graze on pasture throughout the year.

1.2.1. Origins of the Fatty Acids

The fatty acids of bovine milk fat arise from two sources: synthesis *de novo* in the mammary glands and the plasma lipids originating from the feed. The fatty acids from these two sources differ in their structure. The fatty acids that are synthesised *de novo* are short-chain and medium-chain length acids, from 4:0 to 14:0 and also some 16:0, while the C_{18} fatty acids and some 16:0 arise from the plasma lipids. *De novo* fatty acid synthesis accounts for approximately 45% (w/w) of the total fatty acids in milk fat, while lipids of dietary origin account for the rest (Moore and Christie, 1979).

The *de novo* synthesis of fatty acids in the mammary gland utilizes mainly acetate and some β-hydroxybutyrate. These precursors arise from the microbial fermentation of cellulose and related materials in the rumen. Once in the mammary gland, acetate is activated to acetyl-CoA. The mechanism of fatty acid synthesis essentially involves the carboxylation of acetyl-CoA to malonyl-CoA, which is then used in a step-wise chain elongation process. This leads to a series of short-chain and medium-chain length fatty acids, which differ by two CH_2 groups (e.g., 4:0, 6:0, 8:0, etc.) (Hawke and Taylor, 1995). These are straight-chain, even-numbered carbon fatty acids. However, if a precursor such as propionate, valerate or isobutyrate, rather than acetate, is used, branched-chain or odd-numbered carbon fatty acids are synthesised (Jenkins, 1993; see Chapter 2).

Other fatty acids originate mainly from the source of diet, although these include fatty acids that can also be released from adipose tissues. Dietary lipids consist largely of glycolipids, phospholipids and triacylglycerols, and the major fatty acids are linoleic acid (9*c*, 12*c*-18:2) and linolenic acid (9*c*, 12*c*, 15*c*-18:3). In the rumen, these lipids are hydrolyzed initially to produce non-esterified fatty acids, which are then subjected to extensive biohydrogenation by micro-organisms (Jenkins, 1993). The biohydrogenation sequence for linoleic acid begins with an isomerisation step, which produces conjugated linoleic acid (9*c*, 11*t*-18:2). This is followed by a

reduction reaction to give vaccenic acid (11*t*-18:1), and then a further reduction to 18:0. Biohydrogenation of linolenic acid follows a similar pathway (Bauman *et al*., 1999; see Chapter 3, Bauman & Lock). The mix of fatty acids that results from biohydrogenation is esterified to triacylglycerols, which then circulate in the bloodstream within chylomicrons. These triacylglycerols are taken up by the mammary gland and cleaved to give non-esterified fatty acids. The mammary gland contains a desaturase system, which converts substantial quantities of 18:0 to oleic acid (9*c*-18:1).

The net result of these processes is that the fatty acids in the mammary gland, which originate from the dietary lipids, consist of substantial quantities of 16:0, 18:0 and oleic acid, small amounts of linoleic and linolenic acids, and limited quantities of other monoenoic and dienoic fatty acids such as 11*t*-18:1 and 9*c*, 11*t*-18:2.

1.2.2. Saturated Fatty Acids

The saturated fatty acids that are present in significant quantities in milk fat are molecules with un-branched hydrocarbon chains, which vary in length from 4 to 18 carbon atoms. These fatty acids account for approximately 70 to 75% of the total fatty acids. The most important saturated fatty acid from a quantitative viewpoint is 16:0, which accounts for about 25 to 30% of the total, while two other fatty acids, 14:0 and 18:0 have values in the region 10 to 13% (Table 1.2). The amounts of the short-chain fatty acids, 4:0 and 6:0, are reasonably high when their proportions are expressed as molar percentages (approximately 10 and 5%, respectively—Table 1.2); appreciable amounts of medium-chain length fatty acids (C_8 to C_{12}) are also present.

Short-chain and medium-chain fatty acids in milk fat have certain interesting characteristics, which may explain some of the reasons for their presence. Unlike long-chain fatty acids, short-chain and medium-chain fatty acids are absorbed as non-esterified fatty acids into the portal bloodstream and are metabolised rapidly in the liver (Noble, 1978). Hence, they are able to make a direct and rapid contribution to the energy metabolism of the new-born calf. Furthermore, short-chain fatty acids and, to a lesser extent, medium-chain fatty acids lower the melting point of triacylglycerols and, thus, their presence helps keep milk fat liquid at physiological temperatures. This helps in compensating the relatively low concentration of low melting point, unsaturated fatty acids in milk fat.

1.2.3. *Cis*-unsaturated Fatty Acids

The *cis*-monoenoic acid content of bovine milk fat is about 18 to 24% (Table 1.2). Oleic acid (9*c*-18:1) is the principal *cis*-monounsaturated fatty acid, accounting for around 15–21% of the total. There is

Table 1.2. Major fatty acids in bovine milk fat

		Composition		
		Typical[a]		Range[b,c]
	Common Name	%(w/w)	mol %	%(w/w)
4:0	Butyric	3.9	10.1	3.1–4.4
6:0	Caproic	2.5	4.9	1.8–2.7
8:0	Caprylic	1.5	2.4	1.0–1.7
10:0	Capric	3.2	4.3	2.2–3.8
12:0	Lauric	3.6	4.1	2.6–4.2
14:0	Myristic	11.1	11.1	9.1–11.9
14:1	Myristoleic	0.8	0.8	0.5–1.1
15:0	–	1.2	1.1	0.9–1.4
16:0	Palmitic	27.9	24.9	23.6–31.4
16:1	Palmitoleic	1.5	1.4	1.4–2.0
18:0	Stearic	12.2	9.8	10.4–14.6
18:1 *cis*	Oleic	17.2	13.9	14.9–22.0
18:1 *trans*		3.9	3.2	
18:2	Linoleic	1.4	1.1	1.2–1.7
18:2 conj	Conjugated Linoleic acid	1.1	0.9	0.8–1.5
18:3	α Linolenic	1.0	0.8	0.9–1.2
	Minor acids	6.0	5.1	4.8–7.5

[a] Creamer and MacGibbon (1996).
[b] MacGibbon (unpublished).
[c] Range of values for dairying season.

about 0.5% of 11*c*-18:1, while the proportions of other *cis*-18:1 isomers are small. There are also relatively small but significant contributions from other *cis*- monounsaturated acids, namely 14:1 (about 1.0%) and 16:1 (about 1.5%).

Cis-polyenoic acids are present at low concentrations in milk fat, because of the biohydrogenation reactions that take place in the rumen. These acids are comprised almost exclusively of linoleic acid (9*c*, 12*c*-18:2), about 1.2 to 1.7% and α-linolenic acid (9*c*, 12*c*, 15*c*-18:3), about 0.9 to 1.2% (Table 1.2). These two fatty acids are essential fatty acids; they cannot be synthesised within the body and must be supplied by the diet. In recent times, the usage of the term ''essential'' has been extended to include derivatives of these fatty acids, which are not synthesised in significant quantities (e.g., eicosapentaenoic acid, 20:5 and docosahexaenoic acid, 22:6). The proportion of α-linolenic acid appears to be affected by the cow's diet; the concentration is higher in milk from pasture-fed cows than in milk from barn-fed cows (Hebeisen *et al.*, 1993; Wolff *et al.*,1995). In the case of linoleic

Table 1.3. Concentration of *trans*-octadecenoic acids in bovine milk fat[a]

	Trans-octadecenoic acid isomers										
	Composition (% (w/w) of total fatty acids)										
	Δ4	Δ5	Δ6–8	Δ9	Δ10	Δ11	Δ12	Δ13/14	Δ15	Δ16	Total
Mean value	0.05	0.05	0.17	0.24	0.17	1.75	0.21	0.48	0.28	0.34	3.74
Max value	0.13	0.12	0.30	0.31	0.26	4.00	0.31	0.73	0.47	0.51	6.34
Min value	0.02	0.02	0.03	0.16	0.00	0.52	0.11	0.25	0.10	0.16	1.91

[a] Precht and Molkentin (1996); 100 samples were analyzed.

acid, the picture is less clear with differing trends being reported by the two research groups.

1.2.4. *Trans*-unsaturated Fatty Acids

The presence of C_{18} *trans*-fatty acids in milk fat is the result of incomplete biohydrogenation of the unsaturated dietary lipids in the rumen. These fatty acids have attracted attention because of their adverse nutritional affects. Clinical trials have shown that *trans*-octadecenoic acids, relative to the *cis* isomer, can increase the LDL-cholesterol and decrease the HDL-cholesterol, thus, producing an unfavourable affect on the LDL:HDL ratio (Mensink and Katan, 1993).

The quantitative determination of individual isomers of *trans*-18:1 fatty acids in milk fat is not straightforward. It involves a multi-stage analytical procedure (i.e., transesterification of milk fat, argentation TLC of the fatty acid esters to separate the *cis*-isomers and *trans*-isomers, followed by capillary GC). This method gives an almost complete separation of the 13 individual *trans*-18:1 isomers, from Δ4 to Δ16 (Precht and Molkentin, 1996).

Vaccenic acid (11*t*-18:1) is the most important *trans* isomer with values ranging from about 30 to 60% of the total *trans*-18:1 (Table 1.3). The concentration of *trans*-18:1 varies considerably from about 2.0 to 6.0%, with mean values for milk fats from several European countries in the range 3.3 to 4.4% (Precht and Molkentin, 2000). The higher values are for milk fat samples that were obtained from cows fed on summer pasture, whereas the lower values were associated with the feeding of concentrates and silage to cows in the winter. The feeding of fresh grass to cows appears to reduce the efficiency of the biohydrogenation reactions in the rumen, which leads to higher amounts of *trans* fatty acids.