A STUDY OF THE STRUCTURE OF TRIACYLGLYCEROLS OF BOVINE MILK FAT

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SUMMARY

Samples of milk fat obtained at different stages of the dairying season were investigated to determine the influence of the observed seasonal changes in the fatty acid (FA) compositions of New Zealand milk fats on the structure of milk triacylglycerols (TG's). Of the three milk fats studied the January and March samples had FA compositions typical of hard summer milk fat, while the September sample had a FA composition typical of soft spring milk fat.

Each of the three selected samples of milk fat was effectively separated into TG fractions of high, medium and low molecular weight which had distinctly different FA and TG compositions. Stereospecific analyses of these fractions showed that in TG fractions of similar average molecular weight the arrangement of FA's within the TG's was similar and that in all TG fractions the FA's were arranged within positions 1, 2 and 3 of the TG's in a highly selective manner.

The TG fractions were separated into TG classes of different degrees of unsaturation. Corresponding TG classes of the three samples of milk fat had generally comparable FA compositions. However an important distinguishing feature was that each TG fraction of the September sample contained a higher proportion of unsaturated TG's than the corresponding TG fractions of the January and March samples.

Thus in New Zealand milk fats of differing FA composition, the nature of the TG species is similar but there exist differences in the relative proportions of the TG species. The variation in the proportions of the constituent TG species of New Zealand milk fat would appear to be the overriding factor which influences the seasonal variation in its physical characteristics.

The thermal properties of TG fractions of milk fat were examined with a view to determining the influence of TG structure on the physical characteristics of milk fat. It was found that the unsaturated TG's of low molecular weight, were largely responsible for the considerable proportion of milk fat which melted below $0^{o}C$ and consequently for the wide melting range which is characteristic of milk fat. The structural difference between these TG's and the remaining TG's of milk fat was found to be sufficiently large to prevent significant formation of solid solutions. Consequently the wide melting range of milk fat is due to both the large number of different TG species and to the large structural difference between the various TG species.

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NOMENCLATURE OF LIPIDS

For lipids containing glycerol the nomenclature suggested by the IUPAC-IUB Commission on Biochemical Nomenclature (Biochem J. (1967). 105, 897) is followed. However, for sake of brevity abbreviations are used, e.g. 2,3-diacyl-sn-glycerol 1-phosphoryl phenol is referred to as 2,3-PL's.

Fatty acids are designated by the shorthand notation of number of carbon atoms: number of double bonds, e.g. 16:0 refers to 1-hexadecanoic acid.

For sake of brevity bovine milk fat is referred to as milk fat.

Chapter 1.

INTRODUCTION

Section 1.1. Triacylglycerol analysis of milk fat.

1.1.1. Historical account of studies of the triacylglycerols of fats.

As pointed out by Hilditch (Hilditch and Williams, 1964), in 1823 Chevreul made one of the most important discoveries in lipid chemistry when he established that natural fats were comprised of glycerol esters of long-chain fatty acids (FA's). However for almost one century after this discovery few attempts were made to investigate the triacylglycerol (TG) structure of natural fats. The early investigations of the TG's of fats which were carried out around 1900 generally employed crystallization from solvents to separate TG fractions (Hilditch and Williams, 1964). Although these studies were qualitative they did establish that fats are largely mixtures of mixed TG's. This was a significant discovery since up until this time it had been generally assumed that natural fats were necessarily mixtures of simple TG's (the monoacid theory).

More detailed investigations of mixtures of TG's were carried out following the development by Hilditch and Lea (1927) of the permanganate oxidation method which enabled the proportion of saturated TG's in fats

to be determined. Using this method in conjunction with fractionation of TG's by crystallization from solvents and ester fractionation Hilditch and co-workers carried out, over a number of years, an extensive series of investigations of the TG composition of natural fats. These studies generally produced information relating to the composition of broad classes of TG's of fats (Hilditch and Williams, 1964).

The development of a number of new methods in recent years has made possible more detailed investigations of the TG composition and structure of fats. These methods include:

(i) Fractionation of mixtures of TG's by counter-current distribution between two immissible solvents (Dutton et al. 1950).

(ii) Gas-liquid chromatography (G.L.C.) of FA's which made possible the rapid and quantitative analysis of small quantities of FA's thereby greatly facilitating the investigations of mixtures of TG's (James and Martin, 1952, 1956).

(iii) The modification of the oxidation method of Hilditch and Lea (1927) by Kartha (1953a) to permit direct determination of the proportion of monoene TG's in fats.

(iv) Hydrolysis of fats with pancreatic lipase which made possible the determination of the distribution of FA's between the secondary hydroxyl and the primary hydroxyls of the glycerol molecule (Mattson and Beck, 1956; Savary and Desnuelle, 1956).

(v) Separation of mixtures of TG's on the basis of their degree of unsaturation by silver ion chromatography (Barrett et al. 1962; Morris, 1962).

(vi) G.L.C. of intact TG's which has been developed as both an analytical and a preparative technique (Fryer et al. 1960; Kuksis and McCarthy, 1962; Kuksis and Ludwig, 1966).

(vii) Stereospecific analysis of fats which made it possible to determine the overall stereospecific placement of FA's in TG's. (Brockerhoff, 1965, 1967; Lands et al. 1966).

During recent years numerous workers have used the above methods to study the TG structure of natural fats in varying detail. In certain cases the constituent TG species of fats have been determined (e.g. Sampugna and Jensen, 1969).

Theories of TG structure which were postulated prior to studies which investigated the positioning of FA's in TG's, i.e. the theory of even distribution (Collin and Hilditch, 1929) and the random and restricted random theories (Kartha 1953b), assumed that FA's were distributed randomly within the TG molecules. However, analyses with pancreatic lipase showed that the distribution of FA's between position 2 and positions 1 and 3 was non-random. The 1,3-random-2-random theory, which accounted for this non-random distribution, was then propounded (Vander Wal , 1964). However, subsequent stereospecific analyses of natural fats have shown that the arrangement of FA's within

TG's is invariably selective with positions 1,2 and 3 each having a different FA composition. This finding led to the introduction of the 1-random-2-random-3-random theory which states that FA's are selective as to positions on the glycerol molecule but are, within these positions, evenly distributed over all TG molecules. Although this theory appears to hold for the majority of fats which have so far been subjected to detailed structural analyses it cannot be accepted as a general rule (Brockerhoff, 1971).

1.1.2. Early investigations of the triacylglycerols of milk fat.

Hilditch and co-workers were largely responsible for the early investigations of the composition of TG's of milk fat (Hilditch and Williams, 1964). Using the oxidation technique developed by Hilditch and Lea (1927) Hilditch and Sleightholme (1931) found that the proportions of saturated TG's in five samples of milk fat varied between 24.3 and 38.5 wt. %. In a more extensive study of the TG's of milk fat Hilditch and Paul (1940) separated milk fat from English cows into three fractions by crystallization from acetone and then examined in detail each fraction using oxidation, hydrogenation and crystallization methods. From the FA compositions of the resultant TG fractions the authors were able to estimate the composition and proportions of a number of the constituent TG's of milk fat. The more abundant of these are listed in Table 1. It can be seen that difficulty was experienced in making accurate

estimates of the amounts of certain of the TG types. However, the experimental data was remarkably detailed in view of the limited experimental techniques available.

Table 1. The probable composition and proportions of the more abundant constituent triacylglycerols of milk fat of English cows (Hilditch and Paul, 1940).

Triacylglycerols	Estimated proportions (mole %)
16:0, (4:0 to 14:0), (4:0 to 14:0)	7
18:0, 16:0, (4:0 to 14:0)	9
18:1, 16:0, (4:0 to 14:0)	22-30
18:1, 18:0, (4:0 to 14:0)	6-12
18:1, 18:0, 16:0	8-17
18:1, 18:1, (4:0 to 14:0)	0-10
18:1, 18:1, 16:0	4-18

Achaya and Hilditch (1950) used a similar approach, involving low-temperature crystallization and oxidation methods, to determine the probable composition of the quantitatively more important TG's of milk fats of Indian buffaloes and cows. The authors found the same major component TG's as Hilditch and Paul (1940).

More recently Haab et al. (1959) used fractional crystallization in combination with counter-current distribution to establish the presence of tripalmitoyl-glycerol, dipalmitoyl-stearoyl-glycerol and dipalmitoyl-

myristoyl-glycerol in milk fat.

1.1.3. Studies of the triacylglycerol structure of milk fat using pancreatic lipase.

As a result of the discovery by Mattson and Beck (1956), and Savary and Desnuelle (1956) that pancreatic lipase is highly specific for primary esters of acylglycerols it became possible to determine the distribution of FA's between the secondary hydroxyl and the primary hydroxyls of the glycerol molecule.

When Patton and co-workers (Patton *et al*. 1960; McCarthy *et al*. 1960) applied the method of pancreatic lipase digestion to milk fat they found that the FA's at the secondary carbon were enriched in 10:0, 12:0, 14:0 and 16:0 while 18:0 and 18:1 were preferentially esterified at the primary carbons of the glycerol molecules. Proportions of 4:0 and 6:0 were not determined. Similar results were obtained by Ast and Vander Wal (1961) who found that 14:0 and 16:0 were preferentially esterified at position 2 and 18:0 and 18:1 were preferentially esterified at positions 1 and 3. Again data for 4:0 and 6:0 were not presented. Despite the observed non-random distributions these authors concluded that the saturated and unsaturated FA's became associated in an approximately random manner. Unlike the above studies Kumar *et al*. (1960) obtained data for 4:0 in a parallel investigation which indicated that 4:0 was esterified almost exclusively at the primary hydroxyls of the glycerol molecule.

In these early experiments it was assumed that the specificity for primary esters of acylglycerols was the only specificity exhibited by pancreatic lipase. However, Entressangles et al. (1961) observed that short-chain FA's were hydrolysed by pancreatic lipase more rapidly than long-chain FA's in both simple and mixed TG's. They concluded that pancreatic lipase possessed a short-chain FA specificity and could not be used to study the structure of TG's containing short-chain FA's.

In view of the findings of Entressangles et al. (1961), Jack et al. (1963) investigated the action of pancreatic lipase on milk fat to determine whether there existed experimental conditions under which valid results could be obtained. It was found that if the reaction was stopped after one-third of the ester bonds had been hydrolysed preferential hydrolysis of a particular TG species was slight. Using these conditions they found that the majority of FA's were distributed uniformly within the TG molecules except for 4:0 and 6:0 which were preferentially esterified at the primary hydroxyls of the glycerol molecule and 16:0 which was preferentially esterified at the secondary hydroxyl.

Subsequently Jensen and co-workers (Jensen et al. 1964; Sampugna et al. 1966; and Sampugna et al. 1967) examined the specificity of pancreatic lipase more thoroughly using synthetic TG's and milk fat. Synthetic mixed TG's containing 4:0 were each digested with pancreatic lipase in the presence of equimolar quantities of trioleoylglycerol (Sampugna et al. 1967). The results

obtained showed that TG's containing 4:0 were hydrolysed more rapidly than trioleoylglycerol. However, the digestion of TG's containing 4:0 was a complicated phenomenon. For example, analysis of the reaction products after the digestion of rac-1,2-dipalmitoyl-3-butyroyl-sn-glycerol with pancreatic lipase showed that rac-1-butyroyl-2-palmitoyl-sn-glycerol was present in greater amounts than rac-1,2-dipalmitoyl-sn-glycerol and that slightly more 16:0 than 4:0 was released. These results were consistent with the faster digestion rate of rac-1,2-dipalmitoyl-sn-glycerol. This study indicated that pancreatic lipase preferentially digests certain diacylglycerols (DG's) and TG's but that it does not exhibit short-chain FA specificity as earlier postulated by Entressangles et al. (1961). In their investigation of the lipolysis of milk fat TG's Jensen and co-workers (Jensen et al. 1964; Sampugna et al. 1966) obtained data which indicated that pancreatic lipase preferentially digests TG's containing short-chain FA's. Their results relating to the arrangement of individual FA's within the constituent TG's of milk fat were comparable with those obtained by previous workers; 10:0, 12:0, 14:0 and 16:0 were preferentially esterified at position 2 while 4:0, 6:0, 18:0 and 18:1 were preferentially esterified at positions 1 and 3.

A further investigation of the digestion of milk fat with pancreatic lipase was carried out by Boudeau and de Man (1966). Unlike Jack et al. (1963) and Sampugna et al. (1966) they found a greater proportion of short-chain FA's

in the DG products of the lipolysis reaction than in the original TG's. The authors assumed that pancreatic lipase possessed a short-chain FA specificity and as a result concluded that substantial amounts of short-chain FA's must be esterified at position 2. The absence of short-chain FA's in the monoacylglycerol (MG) products was therefore interpreted as evidence that MG's containing 4:0 and 6:0 at position 2 were hydrolysed by pancreatic lipase. However, as shown later by Sampugna et al. (1967), synthetic TG's with 4:0 esterified at the primary carbons of the glycerol molecule give DG products which are enriched with 4:0 when digested with pancreatic lipase.

The data obtained by Jensen and co-workers (Jensen et al. 1964; Sampugna et al. 1966) indicate that when pancreatic lipase is incubated with milk fat it preferentially digests TG's containing short-chain FA's. Despite this complication investigations of the structure of milk TG's by hydrolysis of milk fat with pancreatic lipase have been useful in establishing general relationships concerning the distribution of FA's in the TG's of milk fat. Especially noteworthy was the establishment of a non-random distribution of FA's in milk TG's between position 2 on the one hand and positions 1 and 3 on the other.

1.1.4. Studies of the triacylglycerol structure of fractions of milk fat using pancreatic lipase.

Once it became evident that hydrolysis of milk fat

with pancreatic lipase would not give quantitative results because of the complications arising from the presence of TG's containing short-chain FA's efforts were directed towards the study of the action of pancreatic lipase on fractions of milk fat.

Clement et al. (1963) separated milk fat by silicic acid column chromatography into three fractions, one containing high mol. wt. TG's and the other two containing low mol. wt. TG's. Blank and Privett (1964) combined silicic acid column chromatography and silver ion thin-layer chromatography (silver ion-T.L.C.) to prepare fractions of high mol. wt. TG's of differing degrees of unsaturation. Smith et al. (1965) combined fractional crystallization and counter-current distribution to prepare milk fat fractions of differing mol. wt. and Jensen et al. (1967) fractionated milk fat by successive crystallizations from acetone at -25 and -70^{o}C.

Hydrolysis of the above TG fractions with pancreatic lipase yielded results which may be summarized as follows. Clement et al. (1963) noted that the FA composition of TG's remaining after the digestion differed from the FA composition of the original TG's and concluded that the results could only be interpreted in terms of preferential attack by pancreatic lipase on TG's containing short-chain FA's. In contrast Smith et al. (1965) found that if the lipolysis reaction was terminated after one-third of the ester bonds had been hydrolysed the FA composition of TG's remaining after the digestion closely resembled the FA composition of the original TG's thereby indicating that

pancreatic lipase exhibited no appreciable specificity. This finding was in accord with the conclusions obtained previously by the same group of workers (Jack et al. 1963) concerning the action of pancreatic lipase on total milk fat. Jensen et al. (1967) found that when short digestion times were employed e.g. 2.5 minutes, the preferential digestion of TG's containing 4:0 was slight. Longer lipolysis times, however, produced data which indicated considerable preferential hydrolysis.

The above results indicate that there exist experimental conditions under which fractions of milk fat can be hydrolysed by pancreatic lipase with little preferential digestion of TG's containing 4:0. However these conditions will not necessarily produce valid results since it has been shown by Sampugna et al. (1967) that certain DG species produced during the lipolysis of TG's containing 4:0 are preferentially hydrolysed by pancreatic lipase.

Although the results obtained may not be strictly quantitative these investigations have provided valuable information concerning the positioning of FA's in the component TG's of milk fat fractions. The different fractionation procedures used by the above workers meant that the fractions of milk TG's subjected to hydrolysis by pancreatic lipase were generally not of comparable TG composition. Despite this it was found that the arrangement of individual FA's within the constituent TG's of different TG fractions was similar. In all fractions which contained low mol. wt. TG's, 4:0 and 6:0 were largely

esterified at the primary carbons of the glycerol molecules. Clement *et al.* (1963) estimated that at least 75% of 4:0 present in milk fat is esterified at the primary carbons. 12:0 and 14:0 were preferentially esterified at the secondary carbon while 18:0 and 18:1 were, in the main, concentrated at the primary carbons. The above workers found that 16:0 was preferentially esterified at the secondary carbon. However Dimick *et al.* (1965) found that in high mol. wt. fractions of milk fat 16:0 was preferentially esterified to the secondary hydroxyl of the glycerol molecule while in milk fat fractions of low mol. wt. 16:0 was preferentially esterified to the primary hydroxyl groups.

1.1.5. Detailed investigations of the composition and structure of triacylglycerols of milk fat.

The detailed investigations of the structure of the TG's of milk fat which have been carried out in recent years are characterized by the extensive use of chromatographic methods. In nearly all these studies a combination of chromatographic methods was employed to fractionate milk TG's into classes on the basis of their constituent FA's and G.L.C. was used to analyse the TG classes obtained.

Blank and Privett (1964) fractionated the TG's of milk fat into fractions of high and low mol. wt. using silicic acid column chromatography and examined the fractions in detail in the following ways:

(i) The high and low mol. wt. fractions were resolved by silver ion-T.L.C. and by ozonolysis-T.L.C. into TG classes of differing degrees of unsaturation, the proportions of which are given in Table 2. The positional arrangement of FA's in each of the TG classes of the high mol. wt. fraction was investigated using pancreatic lipase and from the data obtained some 35 TG types of milk fat were determined.

(ii) The low mol. wt. fraction, after interesterification, was resolved by T.L.C. into three groups of TG's which were shown to contain 0, 1, and 2 short-chain FA's per TG molecule. Since the original fraction was found to consist of a single spot which had the same R_F value as TG's containing 1 short-chain FA per TG molecule it was concluded that the component TG's of the low mol. wt. fraction consist of one short-chain FA in combination with two long-chain FA's. On the basis of this finding it was calculated that 78% of the TG's of the low mol. wt. fraction contain 4:0 in combination with two long-chain FA's.

It is clear from the above results that FA's are distributed in a non-random manner in both high and low mol. wt. TG's of milk fat.

A detailed study of the structure and composition of milk TG's of low mol. wt. was carried out by Nutter and Privett (1967). TG's of low mol. wt. were obtained from milk fat by column chromatography on Florisil and then separated into TG classes of differing levels of unsaturation by silver ion-T.L.C. The proportions of the different TG classes are given in Table 2. Further resolution of

these TG classes on the basis of mol. wt. by liquid-liquid partition chromatography made possible the identification of 168 different molecular species of TG's. The more abundant molecular species of TG's identified in this low mol. wt. fraction of milk fat are listed in Table 3. The short-chain FA's were found to be widely distributed among the constituent TG's with practically all TG's containing one short-chain FA in combination with two medium- and long-chain FA's. Nevertheless small but significant amounts of TG's containing two short-chain FA's were found. There was no one principal TG species.

In a subsequent investigation carried out by Breckenridge and Kuksis (1968a, 1969) T.L.C. was used to separate milk TG's into fractions of high, medium and low mol. wt. Each of these TG fractions was separated by silver ion-T.L.C. into TG classes of differing degrees of unsaturation, the proportions of which are presented in Table 2. The saturated TG's and monoene TG's obtained from the fraction of low mol. wt. were further resolved on the basis of mol. wt. by preparative G.L.C. of intact TG's. Analysis of the resultant mixtures of small numbers of TG's enabled some 38 molecular species of TG's representing 28% of total TG's, to be identified and their relative proportions to be estimated. The quantitatively more important molecular species of TG's are given in Table 3. Results again showed the non-random distribution of FA's in milk TG's of low mol. wt. with 4:0 and 6:0 being found almost exclusively in combination with medium- and long-chain FA's. This

investigation appears to be one of the most detailed studies of the constituent TG's of milk fat and as a result it is a useful source of basic data about the detailed chemical composition of milk fat and its fractions.

A detailed examination of milk TG's of high mol. wt. was recently carried out by Shehata *et al*. (1971, 1972). Fractions of milk fat of high mol. wt., which were prepared from milk fat by column chromatography on silicic acid, were separated into TG classes of differing levels of unsaturation by silver ion-T.L.C. The proportions of the classes of TG's obtained are shown in Table 2. From the analyses of these TG classes the identity and proportions of the component molecular species of TG's in these high mol. wt. fractions of milk fat were deduced. The quantitatively more important molecular species of TG's, which consist of combinations of 14:0, 16:0, 18:0 and 18:1, are given in Table 3.

In each of the above detailed investigations of the TG's of milk fat, TG fractions of differing mol. wt. prepared from milk fat by adsorption chromatography were separated according to their degree of unsaturation by preparative silver ion-T.L.C. The proportions of the TG classes prepared in this manner are presented in Table 2. It can be seen that in the main different sets of workers found comparable proportions of corresponding TG classes in fractions of similar mol. wt. The only exception occurs in the data obtained for milk fat fractions of high mol. wt. where results obtained by

Table 2. Proportions of triacylglycerol classes of differing degrees of unsaturation prepared from milk fat by a combination of adsorption chromatography and silver ion - T.L.C.

Triacylglycerol class	Proportions in triacylglycerol fractions of differing molecular weight								Proportions in milk fat	
	High				Medium	Low				
	Ref.1[a] (wt.%)	Ref.3[c] (mole %)	Ref.4[d] (wt.%)		Ref.3[c] (mole %)	Ref.1[a] (wt.%)	Ref.2[b] (wt.%)	Ref.3[c] (mole %)	Ref.1[a] (wt.%)	Ref.3[c] (mole %)
Saturated TG's	19.6	16.5	16.4	18.0	38.7	43.7	47.2	45.0	28.1	33.0
Monoene TG's	58.3	36.7	38.5	43.9	38.3	40.8	33.5	38.1	50.4	37.6
Diene TG's	22.1	27.7	30.4	29.1	14.4	9.8	15.2	11.7	16.9	18.4
Triene TG's	-	12.9	10.5	5.8	8.7	5.6	4.1	5.2	2.1	8.8
Polyene TG's	-	6.2	4.7	4.2	-	-	-	-	-	2.5
	100.0	100.0	100.5	101.0	100.1	99.9	100.0	100.0	97.5	100.3

[a]Blank and Privett (1964)

[b]Nutter and Privett (1967)

[c]Breckenridge and Kuksis (1968a, 1969)

[d]Shehata et al. (1971, 1972)

Blank and Privett (1964) differ somewhat from those obtained by other workers. In going from high to low mol. wt. fractions of milk fat the proportion of saturated TG's increases, the proportion of diene, triene, and polyene TG's decreases and the proportion of monoene TG's remains approximately constant. One result of this trend is that the saturated TG's comprise 16-20% and 44-47% respectively of the high and low mol. wt. fractions.

The more abundant molecular species of TG's determined in fractions of milk TG's of differing mol. wt. are presented in Table 3. The same major molecular species of TG's are present in the two samples of milk TG's of low mol. wt. which were analysed (Nutter and Privett, 1967; Breckenridge and Kuksis, 1968a, 1969). In each case these molecular species consist of 4:0 in combination with two of the following long-chain FA's; 14:0, 16:0, 18:0 and 18:1. The quantitatively important molecular species of milk TG's of high mol. wt. (Shehata et al. 1971, 1972) consist of combinations of the major FA's in the high mol. wt. fraction of milk fat i.e. 14:0, 16:0, 18:0 and 18:1.

It is noteworthy that the molecular species of TG's listed in Table 3 resemble the more abundant constituent TG's of milk fat determined by Hilditch and Paul (1940).

Table 3. Composition of the more abundant molecular species of triacylglycerols present in triacylglycerol fractions of milk fat.

Triacylglycerol class	Triacylglycerol fractions of differing molecular weight		
	High Ref. 3[c]	Low Ref. 1[a]	Low Ref. 2[b]
Saturated TG's	18:0, 18:0, 14:0	18:0, 16:0, 4:0	18:0, 16:0, 4:0
	18:0, 16:0, 16:0	18:0, 14:0, 4:0	
	18:0, 16:0, 14:0	16:0, 16:0, 4:0	16:0, 16:0, 4:0
	16:0, 16:0, 16:0	16:0, 14:0, 4:0	16:0, 14:0, 4:0
	18:0, 14:0, 14:0		
	16:0, 16:0, 14:0		
Monoene TG's	18:1, 18:0, 16:0	18:1, 18:0, 4:0	18:1, 18:0, 4:0
	18:1, 18:0, 14:0	18:1, 16:0, 4:0	18:1, 16:0, 4:0
	18:1, 16:0, 16:0	18:1, 14:0, 4:0	
	18:1 16:0, 14:0		
Diene TG's	18:1, 18:1, 16:0	18:1, 18:1, 4:0	ND[d]

[a]Nutter and Privett (1967)

[b]Breckenridge and Kuksis (1968a, 1969)

[c]Shehata et al. (1971, 1972)

[d]Not determined

1.1.6. Stereospecific analyses of triacylglycerols of milk fat.

To date two sets of workers, Pitas et al. (1967) and Breckenridge and Kuksis (1968b, 1969), have studied the stereospecific positioning of individual FA's in TG's of milk fat using the procedure of Brockerhoff (1965).

Results obtained from the stereospecific analysis of milk fat by Pitas et al. (1967) show that FA's are distributed within the TG's of milk fat in a highly selective manner. The short-chain FA's 4:0 and 6:0 are esterified almost entirely at position 3. In contrast the greater proportions of 10:0, 12:0 and 14:0 are esterified at carbon 2 of the glycerol molecules. 16:0 is preferentially esterified at positions 1 and 2 while 18:0 and 18:1 are concentrated at position 1. Stereospecific analysis of TG's using the procedure of Brockerhoff (1965) requires 1,2(2,3)-DG's as intermediates. Pitas et al. (1967) found that the 1,2(2,3)-DG's prepared for this study by the action of pancreatic lipase on milk fat, were not representative of the FA's of the original TG's. This was the expected finding since studies of the hydrolysis of milk fat by pancreatic lipase (section 1.1.3.) have shown that pancreatic lipase preferentially digests certain milk TG's. The results obtained in this investigation are therefore subject to some error.

Two molecular distillates of butteroil, one representative of the high mol. wt. TG's of milk fat and the other representative of the low mol. wt. TG's of milk fat were

each subjected to stereospecific analysis by Breckenridge and Kuksis (1968b, 1969). When the molecular distillate containing low mol. wt. TG's was digested with pancreatic lipase to prepare 1,2(2,3)-DG intermediates the results obtained indicated that there was no selective hydrolysis of any specific TG type. On the basis of this finding the authors concluded that the results of the analysis were quantitatively correct but data obtained by Sampugna *et al.* (1967), which has been discussed in Section 1.1.3., suggests that the 1,2(2,3)-DG's formed would be non-representative of the original TG's. The data obtained in this study showed a stereospecific placement of FA's in the TG's of milk fat which was similar to that determined by Pitas *et al.* (1967).

It is evident from the data obtained by the two sets of workers that none of the major FA's in milk fat is randomly distributed and, furthermore, that the FA's do not fit a 1,3-random-2-random distribution pattern.

Section 1.2. Seasonal variations in the properties of New Zealand milk fat.

1.2.1. Seasonal fluctuations in the general properties of New Zealand milk fat.

A seasonal survey of the general properties of New Zealand milk fat was carried out by Cox and McDowall (1948) who determined iodine values, Reichert values, saponification values and softening points on monthly samples obtained from nine factories over a period of four years. Each of the four properties measured showed marked and regularly occurring seasonal fluctuations. Reichert values rose from low levels at the beginning of the season (July) to a maximum in Spring (August-September) and then declined gradually until a minimum value was reached in May. Saponification values exhibited a trend which was comparable to that shown by Reichert values. On the other hand iodine values fell sharply, from high spring values, during late spring-early summer (October) and remained at a low level during summer after which they increased. Softening points were relatively low during spring and relatively high during summer and autumn.

In more recent studies of the seasonal variation of properties of New Zealand milk fat, McDowell and Creamer (1970) and Norris *et al*. (1973) noted the same trends in these properties of milk fat.

1.2.2. Influence of feed conditions and stage of lactation on the general properties of New Zealand milk fat.

In New Zealand, cows are grazed on pasture throughout the year and lactation normally commences in early spring. Hence there occur changes in stage of lactation and in feed conditions with the progress of the dairying season which are, more or less, consistent for successive dairying seasons. It can therefore be theorised that properties of milk fat which vary on a regularly recurring seasonal basis are influenced by either or both of these factors. Consequently studies have been carried out on the influence of feed and stage of lactation on the properties of milk fat which vary in this manner (McDowall *et al.* 1961; McDowall, 1962; McDowall and McGillivray, 1963a, 1963b; Hawke, 1963). Extensive use was made of monozygotic twin cows in these investigations to facilitate experimental design.

In a study designed to determine the effect of stage of lactation on the characteristics of milk fat early and late calving cows of monozygotic twin pairs were grazed on the same pasture over the period when both cows of the twin pairs were in lactation (McDowall, 1962). Seasonal fluctuations in the properties of milk fat; namely iodine value, refractive index, saponification value, Reichert value, softening point, and carotene and vitamin A contents, were found to be closely similar for the two groups of cows

which indicated that the seasonal changes in these properties are not the result of changes in the state of lactation of cows.

This negative correlation led to an investigation of the influence of seasonal changes in diet on the characteristics of milk fat. In this connection it seemed significant that the emergence of clover in late spring-early summer as the dominant botanical species in dairy pastures coincides with a marked decrease in the iodine values and vitamin A and carotene contents of milk fat. However trials carried out by McDowall and McGillivray (1963a) have shown that although the properties of milk fat can be influenced by the botanical composition of pasture the seasonal changes in the characteristics of New Zealand milk fat were not directly attributable to the changes which occur in the proportion of clover present in pastures.

At the same time that clover emerges as the dominant species in pasture in late spring-early summer ryegrass changes from soft, lush, immature foliage to a harder, more mature grass. An investigation of the lipids of ryegrass by Hawke (1963) showed that the lipid content of new ryegrass was greater than that of mature ryegrass and that the lipid from the new growth was more unsaturated than mature ryegrass containing a greater proportion of 18:3 and lesser proportions of 18:2 and 16:0. In addition the milk fat from cows grazed on the new growth was found to contain greater proportions of 4:0, 18:0 and 18:1 and lesser proportions of 6:0, 8:0, 14:0 and 16:0. The

findings concerning milk fat were supported by McDowall and McGillivray (1963b) who noted that milk fat from cows grazing immature ryegrass was more unsaturated and had higher carotene and vitamin A contents than that from cows grazing mature ryegrass. Hawke (1963) theorised that the increased unsaturation of milk lipids from cows fed immature ryegrass could arise from the greater total intake of dietary lipid which could result in a decrease in the overall hydrogenation in the rumen.

It would appear from the above investigations that the seasonal fluctuations in the general characteristics of milk fat are mainly attributable to the variation in the stage of maturity of the ryegrass in the pastures (McDowall et al. 1961; McDowall and McGillivray, 1963b). In particular, the marked fall in the iodine value and in carotene and vitamin A contents of milk fat in late spring-early summer occurred as ryegrass became mature while the subsequent rise in these properties in autumn coincided with the appearance of new growth due to autumn rains.

1.2.3. Seasonal fluctuations in the fatty acid composition of New Zealand milk fat.

Hansen and Shorland (1952) used ester fractionation techniques to determine the FA composition of samples of New Zealand milk fat which were collected at two-monthly intervals throughout the dairying season. The following seasonal fluctuations in the proportions of FA's were

found:

(i) 4:0 showed a general decline throughout the season.

(ii) Each of 6:0, 8:0, 10:0, 12:0 and 14:0 were found to increase from a low value at the beginning of the season (July) to a maximum value during early summer (November) and then to decline throughout the rest of the season.

(iii) Values for 16:0 rose slightly during late spring (October) and then decreased slightly in autumn (April).

(iv) Proportions of 18:0 remained constant for most of the season but increased in autumn (April-May).

(v) 18:1 fell from high values early in the season (July-September) to low values during summer and then increased again to maximum values in autumn.

In a more recent survey Gray (1973) used G.L.C. to study the seasonal variation in the FA composition of New Zealand milk fat. Samples were collected at two-weekly intervals throughout the dairying season from herds consisting of cows which calved in spring. In general, the results obtained were comparable with those mentioned above. However values for 16:0 were found to vary considerably throughout the season. 16:0 rose from low values in spring (August-September) to high values during summer and then decreased to low values during autumn. The seasonal trend shown by 16:0 was the inverse of that exhibited by 18:1.

Hansen and Shorland (1952) and Norris et al. (1973) found the predicted relationships between the seasonal changes in the proportion of FA's and the seasonal

fluctuations in the chemical constants, iodine value and Reichert value. Pooled data for short-chain FA's exhibited a trend similar to that noted for seasonal fluctuations in the Reichert value while data for total unsaturated FA's followed the same trend as iodine value.

1.2.4. Influence of stage of lactation, diet and plane of nutrition on the fatty acid composition of New Zealand milk fat.

It can be seen from the above results that maximum proportions of FA's 6:0 to 14:0 were attained during early summer (November), some 10-14 weeks after calving. Gray (1973) suggested that this pattern may be caused by lactational effects since it has been reported by Decaen and Adda (1966) that, in experiments in which cows were fed a uniform diet, the total amounts of FA's 6:0 to 14:0 synthesised rose from low levels at the beginning of lactation to a maximum 6-12 weeks after calving and then decreased as lactation progressed. In the same study Decaen and Adda (1966) showed that the amounts of 18:0 and 18:1 produced declined rapidly, from high initial levels, during the first eight weeks of lactation and thereafter decreased at a less rapid rate. The amounts of 16:0 synthesised during lactation were found to decline slowly as lactation progressed. These findings suggest that seasonal changes in the proportions of 16:0, 18:0 and 18:1 reported above for New Zealand milk fat are not significantly influenced by changes in the state of lactation of cows.

As noted previously (Section 1.2.2.) Hawke (1963) showed that milk fat from cows grazed on mature ryegrass contained lesser proportions of 18:0 and 18:1 and a greater proportion of 16:0 than milk fat from cows grazed on immature ryegrass. It is therefore significant that the decrease in the proportions of 18:0 and 18:1 and the increase in the proportion of 16:0 which occur in New Zealand milk fat in late spring - early summer coincides with ryegrass becoming more mature. Similarly the inverse changes in the proportions of these FA's which occur in autumn is coincident with the appearance of new growth caused by autumn rains. It would appear that the marked seasonal fluctuations in 16:0, 18:0 and 18:1 which occur in late spring - early summer and during autumn can be largely attributed to changes in diet, namely changes in the maturity of ryegrass in pastures.

The above findings are not unexpected since it is now generally accepted that in milk fat FA's 4:0 to 14:0 are synthesised within the mammary gland, FA's containing 18 carbon atoms are derived from the blood plasma TG's and 16:0 originates from both sources (Jones, 1969).

Near the end of the season (late autumn - early winter) there occurred an increase in the proportions of 18:0 and 18:1 and a decrease in the proportions of FA's 4:0 to 14:0. These fluctuations may be the result of a lowering of the plane of nutrition since in late autumn - early winter there is little growth of pasture and consequently there occurs a reduction in the amount of feed available to the cow unless supplementary feeding

is undertaken. In this connection it has been shown that a sub-optimum plane of nutrition reduces the supply of short-chain FA's from the rumen and increases the utilization of FA's from body fat by the mammary gland thereby decreasing the proportions of short-chain FA's and increasing the proportions of FA's containing 18 carbons in the resultant milk fat (Smith and Dastur, 1938; Luick and Smith, 1963; Munford et al. 1964).

1.2.5. Seasonal variations in the fatty acid composition of milk fat from other countries.

In an investigation of the seasonal variation in the FA composition of Australian milk fat, Parodi (1970) obtained results comparable with those reported by Hansen and Shorland (1952) and by Gray (1973) for New Zealand milk fat. The similarity between the results is not surprising because conditions for milk production in Australia are similar to those which exist in New Zealand, i.e. cows are maintained on pasture throughout the year and calving occurs in late winter - early spring to coincide with new spring pasture.

On the other hand, in Northern Hemisphere countries, where cows are housed in winter and where calving is not confined to early spring the proportions of 18:0 and 18:1 in milk fat are lower in winter (November-March) than in summer (July-September) and the proportion of 16:0 shows the reverse trend. (Jensen et al. 1962; Hutton et al. 1969; Hall, 1970). This pattern may be

attributed to the change from indoor feeding of cows during winter to outdoor pasture grazing during summer since it has been shown by Reiter et al. (1969) that a change from a diet of hay and concentrate to a diet of fresh grass causes an increase in the proportions of FA's containing 18 carbons and a decrease in the proportion of 16:0. It would appear therefore that different feeding conditions are responsible for the difference between the seasonal trends of 16:0, 18:0 and 18:1 in milk fats from Northern Hemisphere countries and the seasonal trends of 16:0, 18:0 and 18:1 in milk fats from Australia and New Zealand.

Section 1.3. Physical characteristics of milk fat.

1.3.1. Factors influencing the physical characteristics of fats.

Most of the investigations into both the physical and chemical properties of fats have for commercial reasons been related to factors which influence the consistency of fat and fat products. The main physical processes involved in determining the consistency of fat are polymorphism and solid solution formation which are both influenced by temperature treatment of fat (tempering). The chemical composition of fat i.e. FA composition and TG structure, are known to markedly affect the physical properties of fat, in particular the consistency of fat. Considerable work has been carried out on the correlation between chemical composition and physical properties of fats.

Polymorphism and solid solution formation invariably occur together in fats. Polymorphism is most evident in sharp melting fats which consist predominantly of a small number of TG's (e.g. cocoa butter) and is the major factor influencing the consistency of fats of this type. For example, during chocolate manufacture the cocoa butter mix is tempered to produce a particular polymorphic form which has the desirable characteristics of snap and narrow melting range (Wille and Lutton, 1966). On the other hand in more complex fats with a broad melting range (e.g. milk fat) solid solution

formation is the predominant factor influencing the consistency of fats and fat products. Generally polymorphism is not a factor which significantly affects the consistency of this type of fat (Bailey, 1950). The term solid solution formation used here refers to multi-component phase behaviour which for mixtures of TG's normally involves limited solid solution formation i.e. formation of mixed crystals between different TG species.

Partial hydrogenation, which is used extensively in the margarine industry, demonstrates clearly how a change in FA composition alone affects the physical properties and consistency of fat (Mattil, 1964). The influence of TG structure on the physical properties of fats is shown by a comparison between two natural fats, cocoa butter and tallow. These two fats have similar FA compositions but widely different physical properties. Cocoa butter is brittle, non-greasy and has a very narrow melting range while tallow is soft, greasy and has a considerably broader melting range. The difference in physical properties of these fats is due to their differing TG compositions and structures. Cocoa butter consists predominantly of three monoene TG's: 1,3-dipalmitoyl-2-oleoyl-glycerol; 1,3-distearoyl-2-oleoyl-glycerol; and rac-1-palmitoyl-2-oleoyl-3-stearoyl-sn-glycerol (Sampugna and Jensen, 1969). On the other hand tallow contains a greater number of quantitatively important TG species and a more diverse structural range of TG's (Hilditch and Williams, 1964).

1.3.2. Polymorphism and solid solution formation in milk fat.

(a) Polymorphism

Mulder (1953) was among the first to note polymorphism in milk fat. He observed a double melting point when heating milk fat which had been rapidly cooled to 0°C. Subsequently a number of investigations, using techniques such as X-ray diffraction and infrared spectroscopy have conclusively demonstrated the occurrence of various polymorphic forms in milk fat. Woodrow and de Man (1968) have shown the occurrence of three polymorphic forms i.e. alpha, beta-prime and beta, in milk fat. Results obtained for slowly cooled milk fat indicated the presence of crystals in the beta-prime and beta modifications. Rapid cooling of milk fat resulted in the formation of crystals which were predominantly in the alpha form. On holding at 5°C these crystals slowly transformed to the beta-prime and beta forms. These findings differ somewhat from earlier results obtained by de Man (1961b) who established that slow cooling of milk fat produced crystals in both beta-prime and beta modifications while rapid cooling resulted only in the beta-prime form. However, in this study the temperature of the samples used for the X-ray examinations was not as precisely controlled as in the later investigation. In a recent study van Beresteyn (1972) obtained data which again showed the three polymorphic forms in milk fat obtained by Woodrow and de Man (1968).

The above findings indicate that polymorphism is unlikely to affect the physical properties of butter made from cream by the conventional method because the processing conditions result in the formation of the most stable polymorphic form in the butter. In this connection Wood and Dolby (1965) observed an abrupt break in the setting curve of butter made from cream given abnormal treatment i.e. rapid cooling followed by churning after only 30 min. They speculated that this phenomenon may indicate a change in the polymorphic form of milk fat crystals.

(b) Solid solution formation

In a review article Mulder (1953) noted that the available experimental data relating to the melting and solidification of milk fat could be explained if it was assumed that milk TG's could crystallize as mixed crystals i.e. that milk fat exhibited solid solution formation. Using this hypothesis Mulder was able to put forward a number of general conclusions concerning the melting and solidification properties of milk fat:

(i) On cooling, the lower the temperature at which liquid milk fat begins to crystallize the lower the final melting point of the solid formed.

(ii) A larger proportion of milk fat solidifies with rapid cooling than with either slow cooling or stepwise cooling.

(iii) Recrystallization can take place if semi-solid milk fat is tempered, the rate of recrystallization depending on the amount of liquid phase present.

(iv) If milk fat is cooled rapidly below the solidus line the effect of the solidification temperature will be removed.

Evidence supporting Mulder's hypothesis has subsequently been reported by a number of investigators.

De Man and Wood (1959) obtained dilatometric measurements which showed that rapid cooling produced a higher solid fat content than slow cooling. The larger amount of solid fat obtained by rapid cooling results from the inclusion of lower melting TG's in the solid phase which would have remained in the liquid phase in the case of slow cooling. The liquid phase of rapidly cooled fat is in turn depleted in these TG's and consequently it has a greater proportion of very low melting TG's. Since the very low melting TG's of milk fat contain high levels of short-chain and unsaturated FA's it would be expected that rapid cooling would produce a liquid phase higher in short-chain and unsaturated FA's than slow cooling. A later investigation by Vasic and de Man (1966) showed this to be the case.

Knoop and Samhammer (1962) interpreted X-ray diffraction patterns as indicating the presence of two solid solutions in milk fat, one consisting of saturated TG's and the other consisting of monoene TG's. Using a combination of X-ray diffraction and adiabatic calorimetric techniques Sherbon and Coulter (1966) showed that solid solution formation occurred between high and low melting fractions of milk fat. The presence of two

solutions was noted, the solid phase consisting of a solid solution of a low melting fat fraction in a high melting fat fraction and the liquid phase consisting of a solution of a high melting fat fraction in a low melting fat fraction.

1.3.3. Influence of chemical composition on the physical characteristics of milk fat.

(a) Fatty acid composition

The marked influence of unsaturated FA's on the thermal properties of milk fat was demonstrated by Yoncoskie *et al.* (1969) who showed, using differential thermal analysis, that the proportion of low melting TG's gradually decreased as milk fat was progressively hydrogenated. Original milk fat melted over the range -30 to 40°C while completely hydrogenated milk fat melted between 0 and 60°C.

(b) Triacylglycerol structure

Interesterification of milk fat, which changes a highly selective arrangement of FA'S (Pitas *et al.* 1967) into a random FA distribution, demonstrates how a change in TG structure alone affects the physical properties of milk fat. de Man (1961a) has shown that interesterification markedly increased hardness, solid fat content and the proportion of high melting TG's of milk fat.

(c) Seasonal variation in chemical composition

In New Zealand a number of investigations of the

seasonal variations in the characteristics of milk fat have been carried out with a view to relating the chemical composition to the physical properties of milk fat.

Dolby (1949) examined the seasonal changes in the characteristics of milk fat and butter obtained from a number of commercial dairies and noted that hardness of butter samples was significantly correlated with iodine value and with softening point. In addition he found that only a small part of the seasonal variation in butter hardness was due to variations in the manufacturing process and that at least 80% of this seasonal change could be attributed to differences in the chemical composition of milk fat. His findings were supported by Russell (1970) who showed that the hardness of butter, manufactured under constant processing conditions, varies considerably throughout the season and exhibits a seasonal trend similar to that shown for iodine value.

Recently Norris *et al.* (1973) measured the seasonal changes in the liquid fat content of milk fat at various temperatures using differential scanning calorimetry (D.S.C.) and related them to seasonal fluctuations in FA composition. Data obtained showed that seasonal variations in liquid fat content at 12 and 22°C were significantly correlated with seasonal variations in the sum of short-chain and *cis*-unsaturated FA's. Short-chain FA's were shown to account for a greater proportion of the variance. Liquid fat content at 12 and 22°C exhibited a seasonal trend comparable to that found by Russell (1970) for hardness of butter manufactured under

constant processing conditions.

1.3.4. The consistency of butter.

Numerous workers have, for commercial reasons, investigated factors influencing the consistency of butter in an attempt to find practical methods of controlling butter consistency. These studies, which in recent years have been directed towards producing a more spreadable butter, have shown that the following procedures can be used to produce a soft butter: thermal treatment of cream, mechanical treatment of butter, alteration of the chemical composition of milk fat.

The Alnarp process, in which cream is cooled to 5-7°C and then heated and held at 16-19°C for 2 to 6 h before being cooled to churning temperature, is widely used in European countries to produce a soft butter (Samuelsson and Peterson, 1937).

The hardness of butter increases rapidly during the first few hours after manufacture and thereafter at a decreasing rate. This phenomenon is known as setting and has been attributed to changes of a thixotropic nature. (Mulder, 1949). It has been shown that mechanical working of butter after manufacture produces a softening effect, the reduction in hardness increasing with the extent of setting which occurs before reworking (Taylor *et al.* 1971).

The above two processes soften butter by altering its physical state and as a result the softening effect

produced may be destroyed if significant recrystallization occurs during storage. Methods which produce a soft butter by changing the chemical composition of milk fat do not suffer from this disadvantage. One such method is to separate milk fat into fractions of different melting ranges by fractional crystallization. The recent development of a commercial process by the Alfa Laval Co. for separating milk fat into high and low melting fractions has made it possible to make hard and soft butters (Norris et al. 1971). The low melting fraction produced by this process is currently being used in New Zealand to manufacture soft butter on a commercial scale.

Scott et al. (1970) have developed an alternative method of changing the chemical composition of milk fat. The procedure involves the feeding to cows of lipids protected against biohydrogenation in the rumen which results in milk fat containing considerable proportions of the polyunsaturated FA's, 18:2 and 18:3. Butter produced from this milk fat is very soft.

Section 1.4. The aim of the present work.

The present study was undertaken to investigate changes in the structure of milk TG's which occur as a result of the marked and regular seasonal fluctuations in the characteristics of New Zealand milk fat. Consequently a detailed study of the constituent TG's of samples of milk fat, representative of different stages of the dairying season, was carried out. In addition the thermal properties of TG fractions of milk fat were examined with a view to determining the influence of TG structure on the physical characteristics of milk fat.

Chapter 2.

MATERIALS AND METHODS

Section 2.1. Materials.

2.1.1. Reagents and Solvents.

All the chemicals used in this work were supplied by British Drug Houses Ltd. (Poole, England) and by May and Baker Ltd. (Dageham, England) except for the following:

Chromosorb W, 60-80 mesh, acid-washed and treated with dichlorodimethyloilane (Varian-Aerograph, California, USA).

Thermally stabilized diethylene glycol adipate polyester (Analabs, Connecticut, USA).

3% (w/w) JXR on Gas-Chrom Q, 100-120 mesh; and methanol containing 14% (w/v) boron trifluoride (Applied Science Laboratories, California, USA).

Standard triacylglycerols, 99% pure; pancreatic lipase, Steapsin; and Ophiophagus hannah snake venom (Sigma Chemical Co., St. Louis, USA).

Kieselgel G, according to Stahl (E. Merck A.G., Darmstadt, Germany).

Phenyldichlorophosphate (Aldrich Chemical Co., Milwaukee, USA).

Mr R. Norris generously provided a number of pure standard triacylglycerols and diacylglycerols.

All solvents used were of analytical grade and were distilled before use.

2.1.2. Preparation of samples of milk fat.

At monthly intervals during the 1969/70 dairying season one day samples of milk were obtained from the Massey University No. 2 herd, which is typical of dairy units operating in New Zealand. The herd consisted of 29 pairs of monozygotic twin cows; the majority of which were Jersey, and the remainder Friesian, Friesian-Jersey cross and Ayrshire-Jersey cross. The calving period for the herd was spread over a six-seven week period beginning about the middle of July and finishing near the end of August. The herd was grazed on pasture throughout the dairying season which is normal practice in New Zealand. The milk from a few twin cows involved in special feeding experiments was excluded from the bulked sample of milk collected.

After collection the milk was passed through a cream separator, the cream held overnight at 5°C and then churned in the laboratory. The butter produced was melted in a water-bath at 60°C, centrifuged, and the supernatant fat filtered in an oven at 60°C. Portions of the melted filtered fat were weighed, dissolved in hexane, and stored in a glass-stoppered flask at -10°C until required.

Section 2.2. Analytical methods.

2.2.1. Thin-layer chromatography.

(a) Preparation of thin-layers

For preparative T.L.C. organic impurities were removed from 200 g batches of Kieselgel G (silica gel G) by slurrying with 500 ml $CHCl_3$, transferring to sintered glass filter funnels and washing with 5 vol. $CHCl_3$: CH_3OH (2:1, v/v) followed by 5 vol. $CHCl_3$: CH_3OH (1:1, v/v). The washed adsorbent was air-dried, dried overnight at 100°C and stored in glass-stoppered reagent bottles until required.

Silica gel G was slurried with water in the proportions 1:2, w/v and spread on to glass plates (20 x 20 cm) to a thickness of either 0.25 mm (analytical) or 0.5 mm (preparative). After air-drying for 30 min the thin-layers of silica gel G were activated by heating at 110°C for 2 h.

Thin-layers of silica gel G impregnated with boric acid were prepared in a similar manner, the slurry consisting of silica gel G and 3% aqueous boric acid solution (1:2, w/v).

Thin-layers impregnated with $AgNO_3$ were prepared by spreading a slurry, consisting of 30 g silica gel G and 65 ml 10% $AgNO_3$ solution, on to glass plates with a perspex spreader. The thin-layers were allowed to air-dry in the dark for 2 h and then activated at 105°C for 1 h.

After activation, thin-layer plates were allowed to

cool in a dessicator for 2 h before use. Thin-layers of silica gel G impregnated with $AgNO_3$ were prepared and used the same day while other thin-layers were stored for up to one week in a dessicator.

(b) Conditions for thin-layer chromatography

Samples were applied as small bands approximately 1 cm long using capillary pipettes for analytical work and as one continuous band across the thin-layer with a 50 µl syringe for preparative separations. During application of samples thin-layers were covered with clear glass plates to retard adsorption of water by silica gel G. Thin-layer plates were developed by the ascending method. Chromatographic tanks (Desaga Co., Germany) were lined with filter paper to enhance vapour saturation of the atmosphere within the tank. Developing solvent was added to the tank to a depth of approximately 0.5 cm 30 min before thin-layer plates were chromatographed. Plates were developed at room temperature (about 20°C) to 12-14 cm above the origin which was set 2 cm from the bottom of the plate. After developing, thin-layer chromatograms were allowed to dry before spraying.

(c) Detection of lipids on thin-layer chromatograms

General lipid spray. Lipids were normally detected by spraying thin-layers with a 0.1% (w/v) solution of 2:7-dichlorofluorscein in CH_3OH and viewing under ultraviolet light. Lipid components showed as yellow fluorescent spots on an orange background.

Non-specific spray. Organic compounds were detected by

spraying thin-layer chromatograms with a solution of 1% $K_2Cr_2O_7$ in 9 M sulphuric acid and heating plates at 140°C for approximately 1 h. Under these conditions organic compounds appeared as charred black spots on a white background. Acylglycerols containing double bonds charred more readily than saturated acylglycerols. This spray, although more sensitive than dichlorofluorescein, is destructive and consequently unsuitable for preparative T.L.C.

Phosphate ester spray. A spray reagent, specific for compounds containing a phosphate ester group, was prepared according to the method of Vaskovsky and Kostetsky (1968). Upon spraying on thin-layer chromatograms it reacted immediately with phospholipids to give blue spots on a white background. The colour gradually disappeared over 2 h.

2.2.2. Analysis of fatty acids.

(a) Preparation of methyl esters by method A

(Adapted from the procedure of Van Wijngaarden, 1967).

To a 5 ml round bottomed flask a 5 mg sample of TG's and 0.5 ml 0.5 M methanolic NaOH were added. The flask was connected to a double surface condenser and the mixture refluxed for 3 min in a sand bath. 0.5 ml of CH_3OH containing 14% (w/v) boron trifluoride was then added through the condenser and refluxing was continued for a further 2 min. 1 ml of pentane was added and after refluxing for a further 30 s the flask was cooled in water

at 0°C. The condenser was removed and sufficient saturated NaCl solution added to raise the pentane layer into the neck of the flask from where it was transferred to a glass-stoppered centrifuge tube. A small amount of anhydrous Na_2SO_4 was added and 2-5 µl of the solution was analysed by G.L.C.

(b) Preparation of methyl esters by method B

(The method of Shehata et al. 1970).

50 µl of transesterifying reagent was added to a 1-4 mg sample of acylglycerols in a 0.3 ml reaction vial (Kontes Glass Co., USA). The vial was capped and rotated gently for 2-3 min. At this stage transesterification was complete. The vial was opened, 50 µl of pentane added, the vial rapidly re-capped and then shaken gently. 2-5 µl of the pentane layer was injected directly into the gas-liquid chromatograph.

The transesterifying reagent consisted of 0.5 ml 0.5 M $NaOCH_3$ in CH_3OH (prepared by dissolving Na in CH_3OH), 2 ml pentane, and 0.85 ml diethyl ether. Although consisting of two phases at 5°C the reagent formed one phase upon mixing at room temperature. Fresh reagent was prepared every two or three days from a stock solution of 0.5 M $NaOCH_3$ in CH_3OH which was stored in a dessicator at 5°C in tubes fitted with teflon-lined, screw caps.

The completeness of the conversion to methyl esters was checked by T.L.C. Reaction products together with marker standards were applied to silica gel G thin-layer plates impregnated with boric acid, which were developed

in hexane: diethyl ether (4:1, v/v). Both methods were found to completely convert TG's, DG's and MG's to methyl esters. Solvents used in the above methods were distilled before use and analysed by G.L.C. to ensure that no impurities were present.

(c) Comparison of methods used to prepare methyl esters with respect to quantitative recoveries of methyl esters

Using the two methods described above, aliquots of a standard mixture of high purity TG's were converted to methyl esters and injected into the gas-liquid chromatograph. Weight response factors ($F_w = \frac{\text{weight}}{\text{area}}$) were calculated for individual methyl esters from the weight of each methyl ester in the standard mixture and its peak area. For each method a set of mean F_w values was determined. Theoretical F_w values were determined using the calculation of Ackman and Sipos (1964), which assumes that the carbonyl carbon atom gives no response in hydrogen flame ionization detectors and that methyl esters of FA's are completely recovered from the column. The two sets of F_w values determined from experimental data and the set of theoretical F_w values are given in Table 4. For each set methyl caprate was assigned a F_w value of 1.00.

It can be seen that F_w values which were calculated from results obtained by method B correspond reasonably closely with the set of F_w values determined from theoretical considerations. On the other hand, F_w values for methyl esters of short-chain FA's calculated from data obtained by Method A, were considerably greater than

the corresponding theoretical F_w values and the corresponding F_w values determined for Method B, indicating that losses of methyl esters of short-chain FA's occurred when Method A was used. On the basis of this finding it was decided to use Method B for all subsequent FA analyses.

Table 4. Weight response factors (F_w) for methyl esters of fatty acids, which were calculated from experimental data and determined from theoretical considerations. (Methyl caprate assigned a F_w value of 1.00).

	F_w values		
	Experimental		Theoretical[c]
Methyl esters of fatty acids	Method A[a]	Method B[b]	
methyl butyrate	2.67	1.35	1.38
methyl caproate	1.45	1.11	1.17
methyl caprylate	1.12	1.03	1.07
methyl caprate	1.00	1.00	1.00
methyl myristate	0.99	0.96	0.96
methyl palmitate	0.97	0.95	0.91
methyl stearate	1.00	0.99	0.89

[a] F_w values, each a mean of three determinations, calculated from results obtained by Method A.

[b] F_w values, each a mean of six determinations, calculated from results obtained by Method B.

[c] F_w values determined using the calculation of Ackman and Sipos (1964).

(d) Gas-liquid chromatography

Methyl esters of FA's were analysed by G.L.C. using a Varian Aerograph Series 1520 Chromatograph equipped with a hydrogen flame ionization detector and a linear temperature programmer. The chromatographic separations were made on a glass column (8 ft x $\frac{1}{8}$ in. o.d.) packed with 18% diethylene glycol adipate polyester on Chromosorb W (60-80 mesh). The column packing was prepared by the "funnel coating method" (McNair and Bonelli, 1968) and the column was packed to permit on-column injection using the procedure outlined by Kuksis and Breckenridge (1966). Prior to use, the column was conditioned overnight at 220^{o}C with 20 ml/min nitrogen gas flow.

For normal operation the flow rate of the carrier gas, nitrogen, was 25 ml/min while the flow of air and hydrogen to the detector was 300 ml/min and 20 ml/min respectively. The injector was maintained at 180^{o}C and the detector base at 240^{o}C. After injection of the sample at an initial column temperature of 60^{o}C, the chromatograph oven was temperature programmed at 4^{o}C/min to 180^{o}C and held at this temperature until all methyl esters were eluted. Under these conditions methyl butyrate was completely separated from CH_3OH.

The identity of methyl esters was established by comparison with the retention times and elution temperatures of standard methyl esters. Peaks were triangulated and their vertical heights and base-widths measured. The relative proportions of FA's in a sample were calculated

using the formula below. Results were expressed as mole %.

$$\text{mole } \%_i = \frac{\frac{h_i \; b_i \; F_{w_i}}{M_i}}{\sum \frac{h_i \; b_i \; F_{w_i}}{M_i}} \times 100$$

where: h = peak height.

b = peak width at base.

M = molecular weight of methyl ester.

F_w = experimentally determined weight response factor.

Data obtained for duplicate samples showed a relative error of less than 5% for any peak comprising more than 5% of the sample.

2.2.3. Analysis of triacylglycerols.

(a) Gas-liquid chromatography

TG's were analysed by G.L.C. using a Varian Aerograph Series 1520 Chromatograph fitted with a hydrogen flame ionization detector and a linear temperature programmer. The chromatographic separations were made with a glass column (2 ft x $\frac{1}{8}$ in. o.d.), fitted with glass to metal Kovar seals, and packed with 3% (w/w) JXR on Gas-Chrom Q (100-120 mesh) to permit on-column injection according to the technique of Kuksis and Breckenridge (1966). Prior to use the column was conditioned for 4 h at 350°C with 100 ml/min nitrogen gas flow. The first ten runs on the

newly conditioned column were not used for quantitative analyses.

For normal operation the injector was maintained at 320°C and the detector base at 350°C. After injection of the sample (3-5 µl of a 1% solution of TG's) at an initial column temperature of 220°C the chromatograph oven was temperature programmed to 350°C at 4°C/min. Nitrogen was used as the carrier gas at a flow rate of 100 ml/min at room temperature. After passing through the column the carrier gas was split in the proportions 1:5 between detector and collector. The flow of air and hydrogen to the detector was 300 ml/min and 15 ml/min respectively.

TG peaks were designated by their carbon number i.e. the number of acyl carbon atoms per TG molecule. Identification of peaks was accomplished by comparison with the retention times and elution temperatures of standard TG's. Peaks were triangulated and their vertical heights and base-widths were measured. Odd-carbon number TG's in the samples were estimated by completing the elution curves between the adjacent even-carbon number TG's. The relative proportions of TG peaks (TG species with the same carbon number) in a sample were calculated using the formula below. Results were expressed as mole %.

$$\text{mole \%}_{i} = \frac{\dfrac{h_i \; b_i \; F_{w_i}}{M_i}}{\sum \dfrac{h_i \; b_i \; F_{w_i}}{M_i}} \times 100$$

where: h = peak height.

b = peak width at base.

M = molecular weight of TG.

F_w = experimentally determined weight response factor.

The gas-liquid chromatograph was calibrated by injecting a standard mixture of trilauroyl-glycerol, trimyristoyl-glycerol, tripalmitoyl-glycerol and tristearoyl-glycerol, and calculating weight response factors ($F_w = \frac{\text{weight}}{\text{area}}$). With trilauroyl-glycerol assigned a F_w value of 1.00 the F_w values for trimyristoyl-glycerol, tripalmitoyl-glycerol and tristearoyl-glycerol were 1.06, 1.10 and 1.09 respectively.

(b) Thermal analysis

Thermal analyses of TG fractions of milk fat were carried out using a Perkin-Elmer differential scanning calorimeter (D.S.C.-1B). The D.S.C. was calibrated for power and temperature readout according to the method of Norris et al. (1973).

A solution containing a 2 - 5 mg sample of TG's was transferred to a 0.3 ml reaction vial and evaporated to dryness under nitrogen. Traces of solvent were removed by placing the sample under vacuum in a dessicator overnight. The solvent-free sample was melted and transferred as completely as possible to a sample pan, pre-weighed with lid, using a glass capillary. (This operation was carried out while the sample was completely liquid). The sample

pan was then sealed and loaded into the instrument. After holding the sample at 60°C for 30 min to erase previous thermal history a cooling thermogram was recorded down to -60°C at a rate of 8°C/min. The sample was then held at this temperature for 5 min prior to recording a heating thermogram up to 60°C at a rate of 8°C/min.

Some samples when rapidly cooled in this way showed an exothermic transition in the heating thermogram which is a direct result of a polymorphic transition from a less stable to a more stable crystalline form. To obtain a heating thermogram for the most stable polymorphic form of a sample the following tempering procedure was adopted. From the heating thermogram of the rapidly cooled sample the last integral value of the temperature before the sample was completely melted was obtained (temperature T). The sample was cooled to -60°C at a rate of 8°C/min, held 5 min, heated to temperature T at a rate of 8°C/min, held 2-3 min and cooled to -60°C at a rate of 8°C/min. After holding for 5 min a thermogram was recorded up to 60°C at a rate of 8°C/min.

Normally tempering is carried out with a hold temperature near the middle of the melting range. Such tempering of TG fractions of milk fat allows considerable recrystallization to take place which results in a dip at the hold temperature in the resultant heating thermogram. In the tempering process used in this work the very small amount of solid present at temperature T precludes the possibility of significant recrystallization occurring.

The thermograms obtained were corrected for temperature calibration, thermal-lag and heat-capacity effects using a computer programme. The programme (Fortran II, for the I.B.M. 1620 with plotter) was developed by Sherbon (1971) and is based on the work of Heuvel and Lind (1970).

Section 2.3. Fractionation of triacylglycerols of milk fat.

2.3.1. Chromatography on columns of silicic acid.

(a) Preparation of adsorbent

1,000 ml 5 M HCl was added to 100 g silicic acid (100 mesh, Mallinckrodt) and the resultant slurry stirred for 60 min. After allowing the silicic acid to settle for 30 min the supernatant was decanted. The silicic acid was then mixed with 5 vols. of distilled water and, after allowing the slurry to settle for 30 min, the supernatant was decanted. This washing process was repeated three or four times in order to remove a large proportion of fine particles of silicic acid. The silicic acid was placed in sintered glass filter funnels and washed with distilled water until washings were neutral after which it was dried overnight at 100°C. Removal of fine particles of silicic acid increased the flow rate of eluting solvent through the column.

(b) Column preparation and elution

45 g of treated silicic acid was activated at 110°C overnight and then slurried with hexane. The slurry was placed under vacuum to remove air bubbles and then transferred slowly to a glass column (40 cm x 1.8 cm i.d., Quickfit and Quartz Ltd., England). The silicic acid produced a column 33-35 cm in height.

5 ml hexane containing 650 mg milk fat was placed on the column. The lipids were eluted using hexane, in which the proportion of diethyl ether was linearly increased

from 0 to 9% over 1,500 ml. The gradual increase in the polarity of the eluting solvent caused milk TG's to be eluted in order of decreasing mol. wt. i.e. increasing polarity. A micro metering pump (F.A. Hughes and Co., England) was employed to pump eluting solvent through the column at a rate of 90-100 ml/h. It was necessary to wash the column tip at intervals with small amounts of hexane to stop eluted TG's coating the outside of the column tip. 90 fractions, each containing 15 ml of eluant, were collected. H_2O was rigidly excluded from apparatus and solvents in order to obtain satisfactory and reproducible fractionations of milk TG's.

(c) Analysis of eluant

Selected eluant fractions were evaporated to dryness using a stream of nitrogen and redissolved in 2 ml hexane. 5-10 µl of each solution was then applied, as a 1 cm band, to silica gel G thin-layer plates. A sample of milk fat was used as a marker. The thin-layers were developed in hexane : diethyl ether (4:1, v/v) and sprayed with either dichlorofluorescein or sulphuric acid spray reagents. R_F values of eluant fractions were measured. Under these chromatographic conditions R_F values of standard TG's were shown to be proportional to their mol. wt; high mol. wt. TG's having greater R_F values than low mol. wt. TG's. The measured R_F values of eluant fractions were used to select eluant fractions for combination into three TG fractions of differing mol. wt. (designated high, medium and low mol. wt.). After bulking the eluant fractions, each mol. wt. fraction was evaporated to dryness, weighed, redissolved in

20-30 ml hexane and stored at -10°C in glass-stoppered flasks.

The following amounts of TG fractions of differing mol. wt. were prepared from milk fat using a column of silicic acid:

milk fat placed on column	640 mg
TG fraction of high mol. wt. (eluant fractions 38-50)	275 mg
TG fraction of medium mol. wt. (eluant fractions 51-55)	95 mg
TG fraction of low mol. wt. (eluant fractions (56-82)	230 mg
Recovered TG's	600 mg

A thin-layer chromatogram of TG fractions of high, medium and low mol. wt. is shown in Figure 1. It is evident that the three TG fractions gave distinctly different R_F values in hexane: diethyl ether (4:1, v/v).

FA compositions of milk fat samples and TG fractions of high, medium and low mol. wt. were determined. In addition the March samples of milk fat and its TG fractions of differing mol. wt. were subjected to analysis of intact TG's by G.L.C. and to thermal analysis.

2.3.2. Chromatography on thin-layers of silica gel G impregnated with silver nitrate.

(The method of Breckenridge and Kuksis, 1968a).

TG fractions, which were prepared from milk fat by silicic acid column chromatography, were separated into TG

high medium low milk fat

Figure 1. Thin-layer chromatogram of the March sample of milk fat and its triacylglycerol fractions of high, medium and low molecular weight. Thin-layer plate developed in hexane : diethyl ether (4:1, v/v).

classes of differing levels of unsaturation by preparative silver ion-T.L.C. Thin-layers impregnated with $AgNO_3$ were prepared and activated as described previously (section 2.2.1.). 6-8 mg of a TG fraction was dissolved in 0.2 ml of $CHCl_3$ and applied to a silica gel G thin-layer plate impregnated with $AgNO_3$. A standard mixture of TG's consisting of tripalmitoyl-glycerol; rac-1-palmitoyl-2-oleoyl-3-stearoyl-sn-glycerol; and trioleoyl-glycerol was used as a marker. After developing the plate in ethanol free - $CHCl_3$: CH_3OH (100 : 0.6, v/v) the TG bands were visualized by spraying with the dichlorofluorscein reagent and viewing under ultraviolet light. In general TG samples were separated into seven TG bands which were tentatively identified by reference to the mixture of standard TG's. Each TG band was scraped into a 50 ml centrifuge tube and extracted three times with 20 ml aliquots of diethyl ether: CH_3OH : H_2O (95:5:1, v/v/v). The combined extracts of each band were evaporated to dryness under vacuum, redissolved in 30 ml hexane and washed three times with 2 ml portions of H_2O to remove traces of dichlorofluorscein and silver ions. Each hexane extract was then evaporated to dryness, and the TG's were redissolved in 5-10 ml hexane and stored at $-10^{o}C$ in tubes fitted with teflon-lined screw caps.

The FA composition of each TG band was determined using a measured aliquot of each hexane extract. Prior to transesterification 100 µl of a standard solution of methyl heptadecanoate was added as an internal standard. This enabled the moles of FA's present in each TG band to be

determined by the following formula:

$$\text{moles of FA's in TG band} = \text{moles of internal standard} \times \frac{\sum \frac{h_i\, b_i\, F_{w_i}}{M_i}}{\frac{h_s\, b_s\, F_{w_s}}{M_s}}$$

where: h = peak height.

b = peak width at base.

F_w = experimentally determined weight response factor.

M = molecular weight of methyl ester.

s = internal standard.

The relative proportions of the respective TG bands were calculated from the moles of FA's present in each band.

From the FA compositions of each TG band the average number of double bonds per TG molecule was calculated. On the basis of this information and the initial tentative identification it was determined that the seven TG bands (in decreasing order of R_F values) contained saturated TG's, monoene TG's, monoene TG's, diene TG's, diene TG's, triene TG's and triene TG's. T.L.C. analysis of the methyl esters prepared from the constituent TG's of each band showed that, of the two bands containing monoene TG's, the one with the higher R_F value contained only trans-unsaturated FA's while the one with the lower R_F value contained only cis-unsaturated FA's. For ease of presentation FA compositions for diene TG's and for triene TG's were pooled. Consequently data obtained from FA analyses of the above TG bands are

presented in the chapter on Results (Section 3.3.) as saturated TG's, *trans*-monoene TG's, *cis*-monoene TG's, diene TG's and triene TG's.

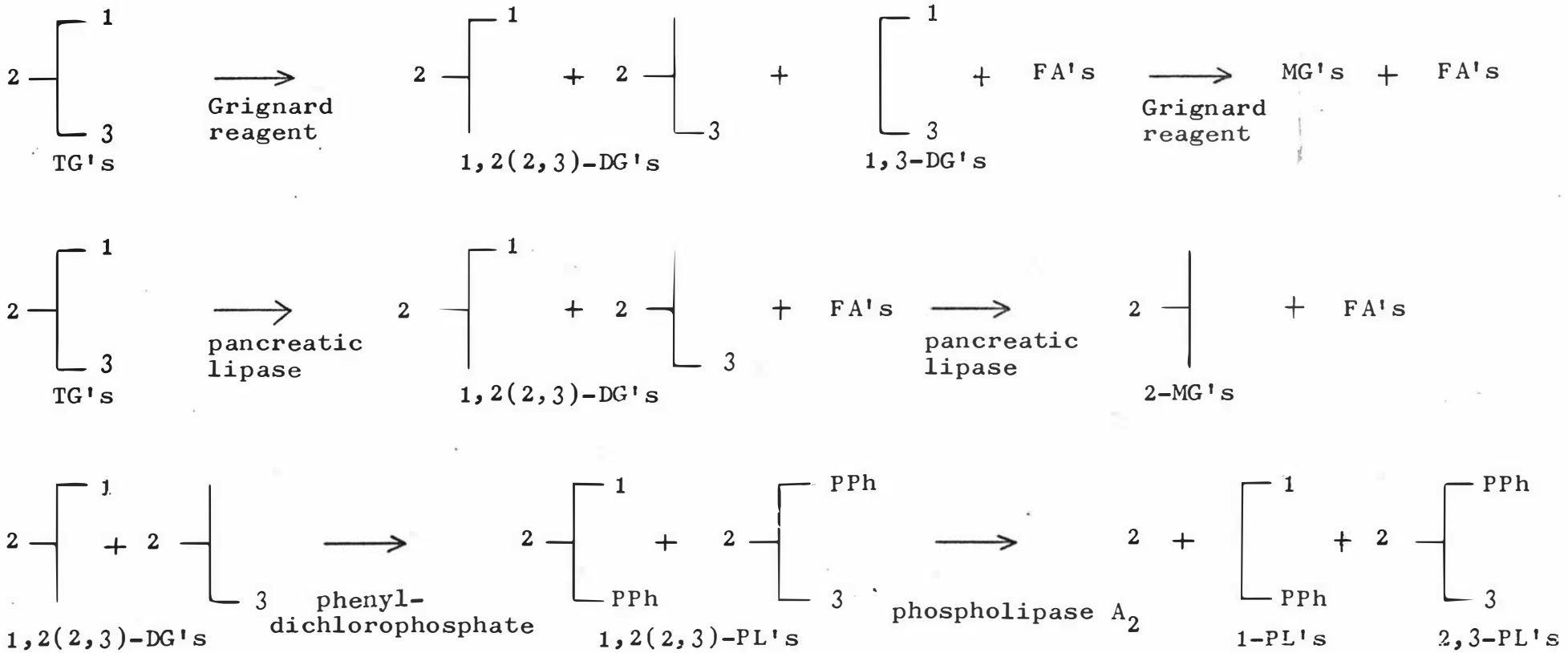
Section 2.4. Stereospecific analysis of triacylglycerols of milk fat.

2.4.1. General outline of method.

Stereospecific analyses of TG fractions of milk fat were carried out using the procedure of Brockerhoff (1965) as modified by Christie and Moore (1969). The procedure for the analysis is given in Figure 2. TG fractions of high, medium and low mol. wt., which were prepared from milk fat, were degraded to yield DG's, MG's and FA's either by deacylation with a Grignard reagent or by digestion with pancreatic lipase (EC 3.1.1.3). The mixture of 1,2-diacyl-sn-glycerols and 2,3-diacyl-sn-glycerols [1,2(2,3)-DG's] were isolated from the reaction products and converted to a mixture of 1,2-diacyl-sn-glycerol 3-phosphoryl phenols and 2,3-diacyl-sn-glycerol 1-phosphoryl phenols [1,2(2,3)-PL's], which were then digested with phospholipase A_2 (EC 3.1.1.4) from snake venom. Phospholipase A_2 hydrolysed only the 1,2-diacyl-sn-glycerol 3-phosphoryl phenols (1,2-PL's) to give the 1-acyl-sn-glycerol 3-phosphoryl phenols (1-PL's). Thus the FA composition of the 1-PL's represented that of position 1 in the original TG's. The FA composition of position 2 was determined from the 2-MG's formed during pancreatic lipase hydrolysis. The FA composition of position 3 was determined using the two calculations below:

position 3 = 2x(2,3-PL's)-(2-MG's)

position 3 = 3x(TG's)-(1-PL's)-(2-MG's)



1,2,3 = Fatty acids in these positions. (positions relative to sn-glycerol 3-phosphate).
PPh = Phosphoryl phenol.

Figure 2. The procedure for the stereospecific analysis of triacylglycerols.

The conversion of 1,2(2,3)-DG's to 1,2(2,3)-PL's and the subsequent hydrolysis with phospholipase A_2 are essentially quantitative reactions but the 1,2(2,3)-DG's are formed only as intermediates in the initial deacylation reactions. Consequently the stereospecific analysis is only valid if the 1,2(2,3)-DG's formed are representative of the FA's of the original TG's. It is therefore necessary to determine experimentally the FA composition of each 1,2(2,3)-DG preparation and to compare it with the FA composition calculated for representative 1,2(2,3)-DG's by the formula below (the calculation of Yurkowski and Brockerhoff, 1966):

$$1,2(2,3)\text{-DG's}_{\text{calc}} = \frac{3x(\text{TG's}) + (2\text{-MG's})}{4}$$

This theoretical calculation assumes that the 2-MG's formed during pancreatic lipase hydrolysis are representative of the FA's at position 2 in the original TG's.

In a similar manner the FA composition of 1,3-DG's, formed during the deacylation of the original TG's with a Grignard reagent, can be compared with the FA composition calculated for representative 1,3-DG's.

$$1,3\text{-DG's}_{\text{calc}} = \frac{3x(\text{TG's}) - (2\text{-MG's})}{2}$$

2.4.2. Hydrolysis of triacylglycerols by pancreatic lipase.

(Adapted from the method of Luddy et al. 1964).

Prior to use the crude preparation of pancreatic lipase

was extracted with diethyl ether according to the procedure of Sampugna *et al*. (1964). The extracted preparation was dried and stored at 5°C.

TG fractions, which were prepared from milk fat by silicic acid column chromatography, were digested with pancreatic lipase in a room regulated at 35-37°C. A suspension consisting of 1 ml 0.5 M tris buffer (pH 8.0), 0.1 ml 2 M $CaCl_2$, 0.2 ml 0.2% (w/v) sodium cholate, and 15 mg pancreatic lipase, was prepared and added to a 10 ml tube containing 60 mg TG's dissolved in 0.1 ml hexane. The tube was promptly capped and shaken for 75 s at maximum speed in a flask shaker. At the end of the reaction time 1.5 ml C_2H_5OH was added to stop the digestion. The reaction mixture was adjusted to pH 4 with 1 M HCl and extracted three times with 20 ml aliquots of diethyl ether. The diethyl ether extract was washed with 2 ml portions of distilled water until the washings were neutral and then evaporated to dryness under vacuum. After dissolving the residue in a small quantity of $CHCl_3$ the liberated DG's and MG's were isolated by preparative T.L.C. The $CHCl_3$ solution was applied to two silica gel G thin-layer plates impregnated with boric acid which were developed in hexane : diethyl ether (1:1, v/v). Lipid bands were visualized by spraying with the dichlorofluorescein reagent and viewing under ultraviolet light and identified by reference to marker standards run at the sides of the plates. After extraction from the thin-layers and removal of traces of dichlorofluorescein and boric acid as described previously (Section 2.3.2) the 2-MG's and 20%

of the 1,2(2,3)-DG's were subjected to FA analysis. The remaining 1,2(2,3)-DG's were subsequently used for stereospecific analysis.

When TG's were digested with pancreatic lipase under the above experimental conditions 18-25% of ester bonds were hydrolysed. Short reaction times were employed to minimize acyl migration.

In order to determine the effect of the addition of hexane to the reaction mixture on the hydrolysis of TG's by pancreatic lipase two 60 mg samples of the TG fraction of low mol. wt. prepared from the March sample of milk fat were subjected to hydrolysis by pancreatic lipase. One sample was digested as outlined above while the other sample was digested under the same conditions except that no hexane was added. 1,2(2,3)-DG's and 2-MG's were isolated from the reaction products of each digestion and subjected to FA analysis. Results obtained are shown in Table 5. The FA compositions of the respective 1,2(2,3)-DG's and 2-MG's were similar indicating that the addition of hexane to the reaction mixture did not significantly affect the specificity of pancreatic lipase under the above experimental conditions. A second experiment gave similar results. On the basis of these findings it was decided to add hexane to the reaction mixture of subsequent digestions (i.e. follow the experimental described above) because hexane should ensure a homogenous dispersion of TG's in the reaction mixture and would eliminate any possibility of TG's existing in crystalline form.

Table 5. Effect of the addition of hexane (0.1 ml) to the reaction mixture (1.3 ml) on the hydrolysis of the low molecular weight triacylglycerol fraction of the March milk fat sample by pancreatic lipase.

	Fatty acid composition (mole %) of reaction products isolated following pancreatic lipase hydrolysis.			
	Hexane added		No hexane added	
FA	1,2(2,3)-DG's	2-MG's	1,2(2,3)-DG's	2-MG's
4:0	20.9	-	21.8	-
6:0	6.1	-	6.3	-
8:0	1.4	1.7	1.7	1.8
10:0	3.5	8.1	3.7	8.3
10:1	-	0.6	0.3	0.3
12:0	4.1	8.8	4.1	8.4
14:0	13.9	23.7	13.2	23.5
14:1	0.9	2.1	0.9	2.0
15:0	1.7	2.8	1.4	2.4
16:0	27.8	33.3	26.1	32.7
16:1	1.1	2.2	1.8	2.1
18:0	6.0	3.9	5.8	4.2
18:1	12.1	12.3	12.8	13.6
18:2	0.5	0.6	-	0.8
	100.0	100.1	99.9	100.1

2.4.3. Deacylation of triacylglycerols by a Grignard reagent.

(a) Triacylglycerol fractions of high molecular weight

Initially a solution of C_2H_5MgBr was prepared. 0.4 ml C_2H_5Br was added to a 25 ml glass-stoppered flask containing 180 mg Mg turnings, 5 ml dry diethyl ether, and a small I_2 crystal. A condenser and drying tube were promptly attached to the flask. Approximately 30 s after the addition of C_2H_5Br the iodine colour disappeared and a spontaneous reaction occurred which resulted in the formation of a 1 M solution of C_2H_5MgBr.

60 mg of a high mol. wt. fraction of milk TG's dissolved in 3 ml dry diethyl ether was shaken rapidly with a flask shaker. 0.5 ml of the freshly prepared C_2H_5MgBr solution was added to the shaking flask. The deacylation of TG's was stopped after 40 s by the addition of 0.06 ml glacial acetic acid followed by 2 ml H_2O. The reaction mixture was extracted three times with 20 ml portions of diethyl ether. The diethyl ether extract was washed successively with 3 ml H_2O, 3 ml 2% $NaHCO_3$, and 3 ml H_2O and then evaporated to dryness under vacuum. After dissolving the residue in a small quantity of $CHCl_3$ the 1,2(2,3)-DG's and 1,3-DG's were isolated by preparative T.L.C. The $CHCl_3$ solution was applied to two silica gel G thin-layer plates impregnated with boric acid which were developed in hexane : diethyl ether (1:1, v/v). After visualization with the dichlorofluorescein spray reagent DG bands were identified by reference to 1,3-dipalmitoyl-

glycerol and rac-1,2-dipalmitoyl-sn-glycerol run as markers. Under these conditions the 1,3-DG band (R_F 0.3) ran slightly ahead of the 1,2(2,3)-DG band (R_F 0.25). After extraction from the thin-layers and removal of traces of dichlorofluorscein and boric acid as described previously (Section 2.3.2) the 1,3-DG's and 20% of the 1,2(2,3)-DG's were subjected to FA analysis. The remaining 1,2(2,3)-DG's were subsequently used for stereospecific analysis.

Results of FA analyses which are presented in the chapter on Results (Section 3.2.3.) show that for each high mol. wt. fraction of milk TG's the FA composition of 1,2(2,3)-DG's isolated from the reaction products of the deacylation reaction closely resembled the FA composition calculated for representative 1,2(2,3)-DG's. This indicates that the deacylation of high mol. wt. fractions of milk fat by C_2H_5MgBr produced 1,2(2,3)-DG's which were representative, within experimental errors, of the FA's present in the original TG's.

(b) Triacylglycerol fractions of low molecular weight

The TG fraction of low mol. wt., prepared from the March sample of milk fat, was deacylated with C_2H_5MgBr and the reaction products were separated by T.L.C. using the procedure outlined above. Two DG bands were obtained. Reference to pure DG standards run as markers showed that the faster-running DG band corresponded to the R_F's of 1,3-DG's containing one short-chain and one long-chain FA and 1,2(2,3)-DG's containing two long-chain FA's while the slower-running DG band corresponded to the R_F of

1,2(2,3)-DG's containing one short-chain and one long-chain FA. Consequently the 1,2(2,3)-DG's formed by the deacylation reaction were not separated from the 1,3-DG's by these chromatographic conditions.

In an attempt to overcome this problem of separation continuous development of thin-layers was carried out according to the procedure of Bennett and Heftman (1963). An aliquot of the products of the above deacylation reaction was applied to a silica gel G thin-layer plate impregnated with boric acid. A trough of aluminium foil was attached to the top of the plate and filled with silica gel G. The plate was then developed at 20°C for $3\frac{1}{2}$ h in benzene : dry diethyl ether (94:6, v/v). Under these conditions three DG bands, which overlapped slightly, were obtained. By reference to pure DG standards run as markers the bands were identified in decreasing order of R_F values as; 1,2(2,3)-DG's containing two long-chain FA's, 1,3-DG's containing one short chain and one long-chain FA, and 1,2(2,3)-DG's containing one short-chain and one long-chain FA. The three DG bands were recovered as described previously (Section 2.3.2.), the two 1,2(2,3)-DG bands pooled, and the FA compositions of the 1,2(2,3)-DG's and 1,3-DG's determined. The results are given in Table 6 together with FA compositions of 1,2(2,3)-DG and 2-MG products of pancreatic lipase hydrolysis (see Table 5) and FA compositions calculated for representative 1,2(2,3)-DG's and 1,3-DG's. A comparison of the FA compositions of the respective 1,2(2,3)-DG's showed that the 1,2(2,3)-DG's isolated from the Grignard reaction contained lower

Table 6. Fatty acid compositions of the reaction products isolated after the deacylation of the low molecular weight triacylglycerol fraction of the March milk fat sample by two different methods.

FA	Fatty acid composition (mole %)						
	TG's	2-MG's	1,3-DG's		1,2(2,3)-DG's		
	Orig.	Exp.[a]	Calc.	Exp.[b]	Calc.	Exp.[a]	Exp.[b]
4:0	23.5	-	35.2	28.1	17.6	20.9	20.7
6:0	6.7	-	10.1	6.8	5.0	6.1	5.7
8:0	2.0	1.7	2.2	3.3	1.9	1.4	0.8
10:0	2.9	8.1	0.3	6.2	4.2	3.5	1.6
10:1	0.4	0.6	0.3	0.5	0.5	-	-
12:0	3.6	8.8	1.0	5.6	4.9	4.1	2.9
14:0	10.3	23.7	3.6	9.3	13.8	13.9	12.9
14:1	1.0	2.1	0.5	0.7	1.3	0.9	1.0
15:0	1.4	2.8	0.7	1.5	1.8	1.7	1.6
16:0	24.0	33.3	19.3	19.4	26.2	27.8	28.4
16:1	1.7	2.2	1.5	0.8	1.8	1.1	1.6
17:0	0.6	-	0.9	-	0.5	-	-
18:0	7.4	3.9	9.2	7.1	6.5	6.0	7.2
18:1	13.5	12.3	14.1	10.3	13.2	12.1	15.1
18:2	0.6	0.6	0.6	0.5	0.6	0.5	0.6
18:3	0.5	-	0.8	-	0.4	-	-
	100.1	100.1	100.3	100.1	100.2	100.0	100.1

[a] reaction products isolated after hydrolysis of the triacylglycerol sample by pancreatic lipase.

[b] reaction products isolated after deacylation of the triacylglycerol sample by a Grignard reagent.

proportions of 10:0 and 12:0 than either the 1,2(2,3)-DG's formed by pancreatic lipase hydrolysis or the calculated 1,2(2,3)-DG's. On the other hand the FA composition of 1,3-DG's isolated from the Grignard reaction deviated from that calculated for representative 1,3-DG's in that it contained lesser proportions of 4:0, 6:0 and 18:1 and greater proportions of 10:0, 12:0 and 14:0. It is clear from the above results that, although representative 1,2(2,3)-DG's may be produced by the Grignard deacylation, the 1,3-DG band was not quantitatively separated from the 1,2(2,3)-DG bands under the above chromatographic conditions. Consequently it was decided to prepare the 1,2(2,3)-DG's for the stereospecific analyses of TG fractions of low and medium mol. wt. by pancreatic lipase hydrolysis even though the results in Table 6 showed that the FA composition of 1,2(2,3)-DG's prepared in this manner from the low mol. wt. TG fraction of the March milk fat sample deviated slightly from that calculated for representative 1,2(2,3)-DG's.

2.4.4. Preparation of phospholipids

6-8 mg of 1,2(2,3)-DG's dissolved in 0.5 ml ethanol-free $CHCl_3$ were added slowly at 0^oC to a 5 ml round-bottomed flask containing 0.5 ml ethanol-free $CHCl_3$, 0.5 ml pyridine and 0.1 ml phenyldichlorophosphate. The reaction mixture was stood at room temperature (about 20^oC) in a stoppered flask for 2 h during which time the flask was occasionally swirled. At the end of the reaction time the flask was placed in ice and 2 ml pyridine followed by

0.5 ml H_2O were added dropwise. 15 ml CH_3OH, 12.5 ml H_2O, 15 ml $CHCl_3$ and 0.5 ml triethylamine were then added. The mixture was shaken and the chloroform layer recovered and evaporated to dryness, under vacuum, below 35°C. Analysis of the reaction products by T.L.C. showed that 1,2(2,3)-PL's had been formed in good yield and only traces of unreacted 1,2(2,3)-DG's were present.

2.4.5. Hydrolysis of phospholipids by phospholipase A_2.

1 ml diethyl ether, 0.1 ml 0.2 M $CaCl_2$, 10 ml 0.1 M triethylammonium bicarbonate (prepared by bubbling CO_2 through 0.1 M triethylamine until a pH of 7.0 was obtained) and 1 mg snake venom (Ophiophagus hannah) were added to a 20 ml glass-stoppered conical flask containing the 1,2(2,3)-PL's. After the reaction mixture had been gently stirred overnight 10 ml isobutanol was added and the mixture evaporated to dryness, under vacuum, below 35°C, with the aid of small volumes of absolute ethanol: benzene (1:1, v/v). The residue was dissolved in a small quantity of $CHCl_3$:CH_3OH (2:1, v/v), a drop of acetic acid added, and the reaction products were isolated by preparative T.L.C. The solution was applied to a silica gel G thin-layer plate impregnated with boric acid which was developed in hexane:diethyl ether (1:1, v/v). After visualization with dichlorofluorescein spray reagent the FA band was extracted as described previously (Section 2.3.2.). The band at the origin, which contained a mixture of 1-PL's and 2,3-PL's was extracted four times,

twice with 30 ml portions of $CHCl_3$:CH_3OH:H_2O (20:10:1, v/v/v) and twice with 30 ml portions of $CHCl_3$:CH_3OH:H_2O (15:15:1, v/v/v). After evaporation to dryness under vacuum the mixture of 1-PL's and 2,3-PL's was redissolved in a small quantity of $CHCl_3$:CH_3OH (2:1 v/v) and reapplied to a silica gel G thin-layer plate, which was developed in $CHCl_3$:CH_3OH:14 M NH_3 (80:20:2, v/v/v). The phospholipid bands were identified by spraying the outside inch on both sides of the thin-layer with the phosphate ester spray. Under the above chromatographic conditions bands containing 1-PL's and 2,3-PL's had R_F values of 0.1 and 0.5 respectively. After detection the 1-PL's and 2,3-PL's were recovered as described above for the origin band and their respective FA compositions were determined. The FA's of the recovered FA band were converted to methyl esters, by refluxing with methanol containing 14% (w/v) boron trifluoride for 2-3 min, and analysed by G.L.C. The resultant FA composition was not strictly representative of FA's at position 2 in the original TG's because short-chain FA's were lost during the extraction procedure. However the analysis was useful for comparison with the FA composition of 2-MG's formed during pancreatic lipase hydrolysis.

Chapter 3.

RESULTS

Section 3.1. Selection and fractionation of samples of milk fat.

3.1.1. Selection of samples of milk fat for detailed study of triacylglycerol structure.

As discussed in the introduction (Section 1.2.3.) there occur marked and regular seasonal fluctuations in the constituent FA's of New Zealand milk fat. It was considered that the effect of this seasonal variation of the FA's on the structure of TG's of milk fat could be best studied by:

(i) selecting samples of milk fat which exhibited large differences in their respective FA compositions,

(ii) selecting samples of milk fat with similar FA compositions which were produced at different stages of the dairying season.

Accordingly the FA compositions of the milk fat samples collected from the Massey University No. 2 herd were determined. With the availability of this data three samples were selected for detailed study of TG structure. The two milk fat samples which showed the greatest difference in their respective FA compositions were selected, one from the month of September (spring)

Table 7. Fatty acid compositions of the three samples of milk fat selected for detailed analysis of triacylglycerol structure.

	Fatty acid composition (mole %)		
FA	September (4-9-69)	January (14-1-70)	March (12-3-70)
4:0	12.0	9.7	10.0
6:0	4.5	4.5	4.6
8:0	2.3	2.2	2.3
10:0	4.2	4.2	4.2
10:1	0.3	0.4	0.3
12:0	4.0	4.1	3.9
14:0	10.9	11.5	10.9
14:1	0.8	1.2	1.1
15:0	1.4	1.7	1.6
16:0	22.1	27.6	26.4
16:1	1.4	2.1	1.9
17:0	0.6	0.8	0.9
18:0	13.1	10.1	11.5
18:1	21.5	17.8	18.4
18:2	0.7	1.4	1.1
18:3	0.3	0.8	0.8
20:2	0.2	-	-
	100.3	100.1	99.9

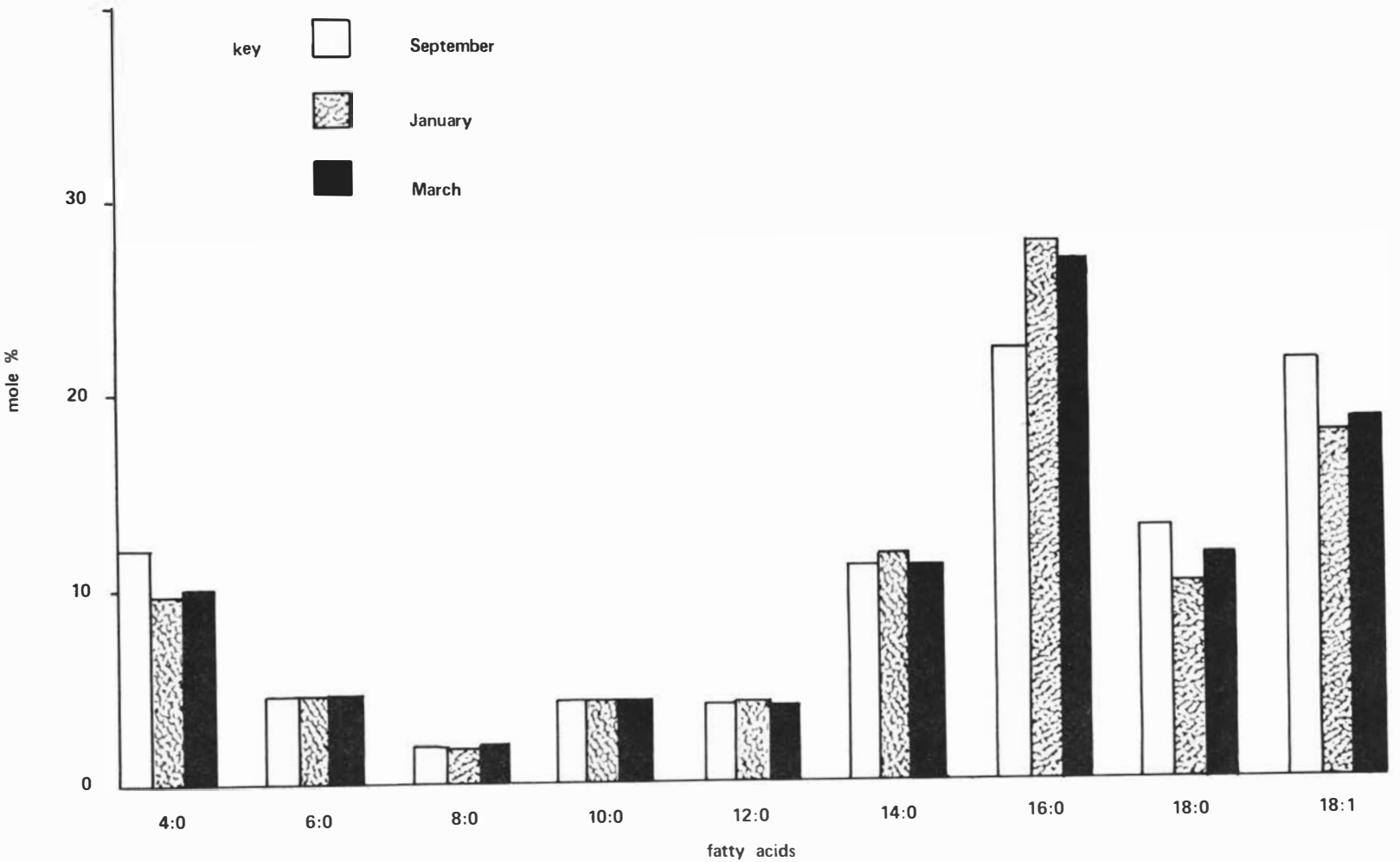


Figure 3. Fatty acid compositions of the September, January and March samples of milk fat.

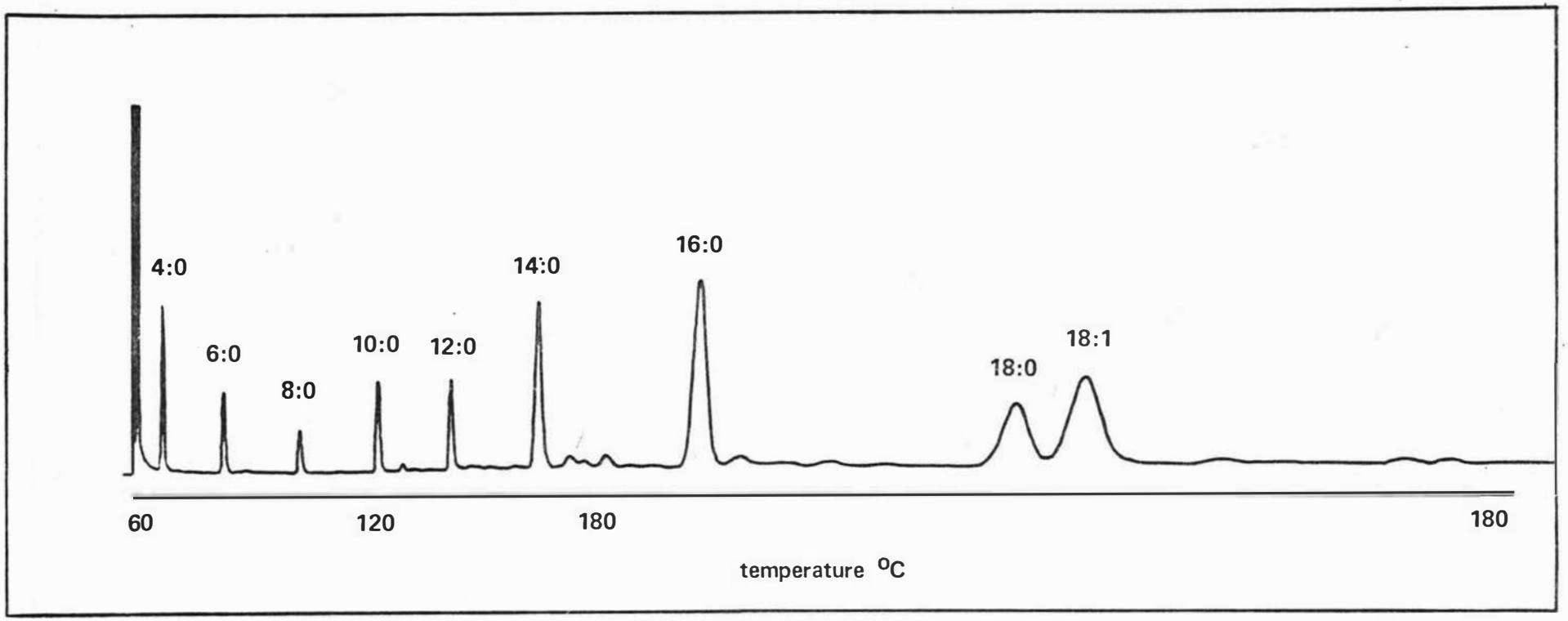


Figure 4. Gas-liquid chromatogram of the fatty acid methyl esters of the September sample of milk fat.

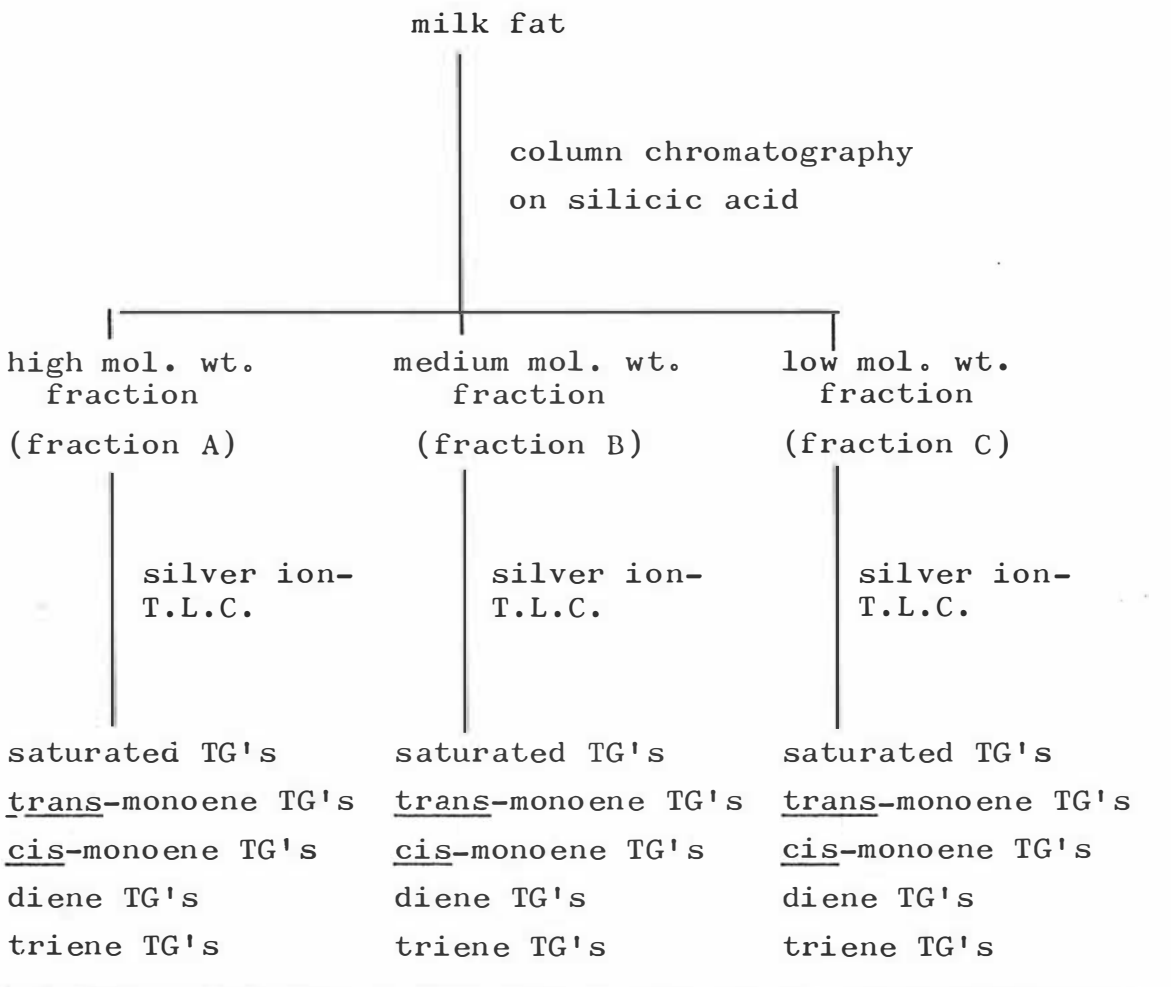
and the other from the month of January (summer). In addition a third milk fat sample from the month of March was selected because it had a FA composition similar to that of the selected January sample.

The FA compositions of the three milk fat samples selected are given in Table 7. The results presented are the means of duplicate determinations. In addition the proportions of the quantitatively more important FA's in the samples are compared in graphical form in Figure 3 and a gas-liquid chromatogram of the FA methyl esters of the September sample is presented in Figure 4. It can be seen that the FA compositions of the January and March samples resembled each other closely with values for corresponding FA's (as moles %) varying only slightly. On the other hand, the FA composition of the September sample differed considerably from those of the January and March samples in that it contained higher proportions of 4:0, 18:0 and 18:1 and a lower proportion of 16:0.

3.1.2. Fractionation of the three selected samples of milk fat.

The selected samples of milk fat were separated into TG fractions of high, medium and low mol. wt. (fractions A, B and C) by chromatography on silicic acid columns and these TG fractions were further resolved into TG classes of differing levels of unsaturation by silver ion-T.L.C. (Figure 5).

Figure 5. Triacylglycerol fractions and classes obtained from milk fat.



(a) Proportions of the triacylglycerol fractions of high, medium and low molecular weight

The proportions of the corresponding TG fractions prepared from the three samples of milk fat by silicic acid column chromatography (Table 8) were similar in that the TG fractions of high mol. wt. (fraction A's) comprised between 39.1 and 41.4% of the samples, the TG fractions of medium mol. wt. (fraction B's) comprised between 16.0

and 18.1%, and the TG fractions of low mol. wt. (fraction C's) comprised between 42.4 and 43.2%.

Table 8. Proportions of the triacylglycerol fractions of high, medium and low molecular weight (fractions A, B and C) obtained from each of the three samples of milk fat by silicic acid column chromatography.

Triacylglycerol fraction	Proportions (Mole %)[a]		
	September	January	March
Fraction A	40.1	39.1	41.4
Fraction B	16.6	18.1	16.0
Fraction C	43.2	42.8	42.4

[a]Triacylglycerol fractions were weighed and converted to mole % using their respective fatty acid compositions.

(b) Proportions of the triacylglycerol classes of differing levels of unsaturation

The proportions of TG classes of differing degrees of unsaturation, which were prepared from the TG fractions of high, medium and low mol. wt. by silver ion-T.L.C. are presented in Table 9. The data show that the saturated TG's, cis-monoene TG's and diene TG's were the more abundant component TG classes in fraction A while trans-monoene TG's and triene TG's were relatively minor constituent TG classes. In fraction B the saturated TG's,

Table 9. Proportions of the triacylglycerol classes of differing levels of unsaturation prepared from the triacylglycerol fractions of high, medium and low molecular weight (fractions A, B and C) by silver ion-T.L.C.

Triacylglycerol class	Proportions (mole %)[a]					
	September		January		March	
	% F[a]	% M[b]	% F[a]	% M[b]	% F[a]	% M[b]
Fraction A						
Saturated TG's	28.5	11.4	33.9	13.2	31.7	13.1
Trans-monoene TG's	11.2	4.5	10.0	3.9	8.2	3.4
Cis-monoene TG's	28.4	11.4	31.3	12.2	32.8	13.6
Diene TG's	21.5	8.6	17.5	6.8	17.5	7.2
Triene TG's	10.5	4.2	7.6	3.0	9.9	4.1
Fraction B						
Saturated TG's	45.4	7.6	50.4	9.1	49.1	7.8
Trans-monoene TG's	10.1	1.7	7.2	1.3	6.2	1.0
Cis-monoene TG's	27.2	4.5	23.2	4.2	26.7	4.3
Diene TG's	10.0	1.7	11.3	2.0	10.1	1.6
Triene TG's	6.8	1.1	8.2	1.5	8.0	1.3
Fraction C						
Saturated TG's	46.5	20.1	54.3	23.2	53.4	22.6
Trans-monoene TG's	9.4	4.0	7.6	3.2	5.8	2.4
Cis-monoene TG's	28.2	12.1	25.3	10.8	26.5	11.2
Diene TG's	9.9	4.3	8.4	3.6	8.9	3.8
Triene TG's	6.3	2.7	4.6	2.0	5.6	2.4

[a] percentage in triacylglycerol fraction.

[b] percentage in milk fat.

which comprise between 45.4 and 50.4% of the TG's present in these fractions, were the principal TG class. Cis-monoene TG's were present in appreciable amounts but trans-monoene TG's, diene TG's and triene TG's were of minor quantitative importance. In fraction C the saturated TG's were again the principal constituent TG class, comprising between 46.5 and 54.3% of the TG's. Cis-monoene TG's were of considerable quantitative importance but trans-monoene TG's, diene TG's and triene TG's were minor component TG classes.

The proportions of corresponding TG classes in the January and March samples of milk fat samples were comparable. In contrast each TG fraction of the September milk fat sample contained a lesser contribution by saturated TG's and a greater contribution by unsaturated TG's than the corresponding TG fractions of the January and March milk fat samples. In each sample of milk fat the contribution by saturated TG's increased in going from fraction A to fraction C and the converse applied for unsaturated TG's. As a result of this trend saturated TG's of low mol. wt. accounted for more than 50% of the total saturated TG's present in each sample of milk fat.

(c) Purity of the triacylglycerol classes of differing levels of unsaturation

In order to obtain an estimate of the purity of the TG classes separated by silver ion-T.L.C. the average number of double bonds per TG molecule was calculated from their FA compositions (Table 10). No unsaturated FA's

Table 10. Degree of unsaturation of the triacylglycerol classes prepared from the triacylglycerol fractions of high, medium and low molecular weight (fractions A, B and C) by silver ion-T.L.C.

Triacylglycerol class	Average number of double bonds per triacylglycerol molecule.[a]		
	September	January	March
Fraction A			
Saturated TG's	0.0	0.0	0.0
Trans-monoene TG's	0.95	0.93	0.91
Cis-monoene TG's	1.05	1.02	1.03
Diene TG's	1.91	1.90	2.09
Triene TG's	2.87	2.82	3.05
Fraction B			
Saturated TG's	0.0	0.0	0.0
Trans-monoene TG's	0.90	0.85	0.85
Cis-monoene TG's	1.07	0.98	1.03
Diene TG's	1.75	1.72	1.80
Triene TG's	2.76	2.62	2.72
Fraction C			
Saturated TG's	0.0	0.0	0.0
Trans-monoene TG's	0.94	0.94	0.88
Cis-monoene TG's	1.03	0.97	1.04
Diene TG's	1.87	1.77	1.80
Triene TG's	2.60	2.68	2.95

[a] calculated from the respective fatty acid compositions.

were detected in any of the saturated TG's. In all cases values for trans-monoene TG's were slightly less than unity suggesting that these TG classes were contaminated with small amounts of saturated TG's. Values for cis-monoene TG's were equal to unity, within experimental error, indicating no contamination by diene TG's. T.L.C. analysis of methyl esters prepared from the constituent TG's of bands containing monoene TG's showed that trans-monoene TG's contained no detectable amounts of oleic acid and that no elaidic acid could be detected in the cis-monoene TG's, thus ruling out partial overlapping of the bands containing monoene TG's. With the exception of fraction A of the March sample of milk fat, values for diene TG's were less than two suggesting partial contamination of these TG classes by cis-monoene TG's. Again, with the exception of fraction A of the March sample of milk fat, values for triene TG's were less than three which suggests partial contamination of triene TG's by diene TG's.

Section 3.2. Composition of the triacylglycerol fractions of high, medium and low molecular weight prepared from each of the three samples of milk fat.

3.2.1. Triacylglycerol compositions of the triacylglycerol fractions prepared from the March sample of milk fat.

The TG compositions of the March sample of milk fat and the TG fractions of high, medium and low mol. wt. (fractions A, B and C), which were prepared from the March sample by silicic acid column chromatography, are given in Table 11. In addition gas-liquid chromatograms of the constituent TG's of these samples are presented in Figure 6 and the TG compositions of the TG fractions are shown in graphical form in Figure 7.

Reference to Table 11 shows that the following molecular types of TG's all contributed appreciable amounts to the March sample of milk fat; C_{34} (5.2%), C_{36} (13.9%), C_{38} (17.2%), C_{40} (12.0%), C_{42} (6.3%), C_{44} (4.9%), C_{46} (5.3%), C_{48} (7.2%), C_{50} (9.6%), and C_{52} (7.7%). In fraction A the more abundant molecular types of TG's; C_{44} (9.0%), C_{46} (11.7%), C_{48} (16.9%), C_{50} (23.0%) and C_{52} (18.7%), comprised 79.3% of the TG's which make up this TG fraction. The major molecular types of TG's in fraction B; C_{38} (25.0%), C_{40} (33.3%) and C_{42} (20.9%) comprised 79.2% of the TG's present. In fraction C the two principal molecular types of TG's were C_{36} (31.3%) and C_{38} (30.9%), while C_{34} (12.2%) and C_{40} (13.9%) were of considerable quantitative importance.

Table 11. Triacylglycerol compositions of the March sample of milk fat and its triacylglycerol fractions of high, medium and low molecular weight (fractions A, B and C).

	Composition of molecular types of triacylglycerols (mole %)							
TG[a]	Fraction A		Fraction B		Fraction C		Milk fat	
	% F[b]	% M[c]	% F[b]	% M[c]	% F[b]	% M[c]	Rec.	Orig.
28	-	-	-	-	0.4	0.2	0.2	0.2
30	-	-	-	-	1.1	0.5	0.5	0.4
32	-	-	-	-	3.3	1.4	1.4	1.3
34	-	-	-	-	12.2	5.1	5.1	4.4
35	-	-	-	-	1.0	0.4	0.4	0.5
36	-	-	3.4	0.5	31.3	13.2	13.7	12.2
37	-	-	-	-	2.5	1.1	1.1	0.9
38	-	-	25.0	4.0	30.9	13.1	17.1	15.3
39	-	-	1.9	0.3	1.2	0.5	0.8	0.8
40	1.8	0.7	33.3	5.4	13.9	5.9	12.0	12.4
41	0.3	0.1	1.3	0.2	0.6	0.3	0.6	0.7
42	5.2	2.2	20.9	3.3	1.6	0.7	6.2	6.9
43	0.7	0.3	1.0	0.2	-	-	0.5	0.4
44	9.0	3.7	7.6	1.2	-	-	4.9	5.6
45	1.1	0.5	0.5	0.1	-	-	0.6	0.4
46	11.7	4.8	3.1	0.5	-	-	5.3	5.6
47	1.6	0.7	-	-	-	-	0.7	0.6
48	16.9	7.0	1.3	0.2	-	-	7.2	7.9
49	1.6	0.7	-	-	-	-	0.7	0.8
50	23.0	9.5	0.6	0.1	-	-	9.6	10.4
51	1.6	0.7	-	-	-	-	0.7	0.9
52	18.7	7.7	-	-	-	-	7.7	8.1
53	0.9	0.4	-	-	-	-	0.4	0.5
54	5.7	2.4	-	-	-	-	2.4	2.9
	99.8	41.4	99.9	16.0	100.0	42.4	99.8	100.1

[a] carbon number of triacylglycerols

[b] percentage in triacylglycerol fraction

[c] percentage in milk fat

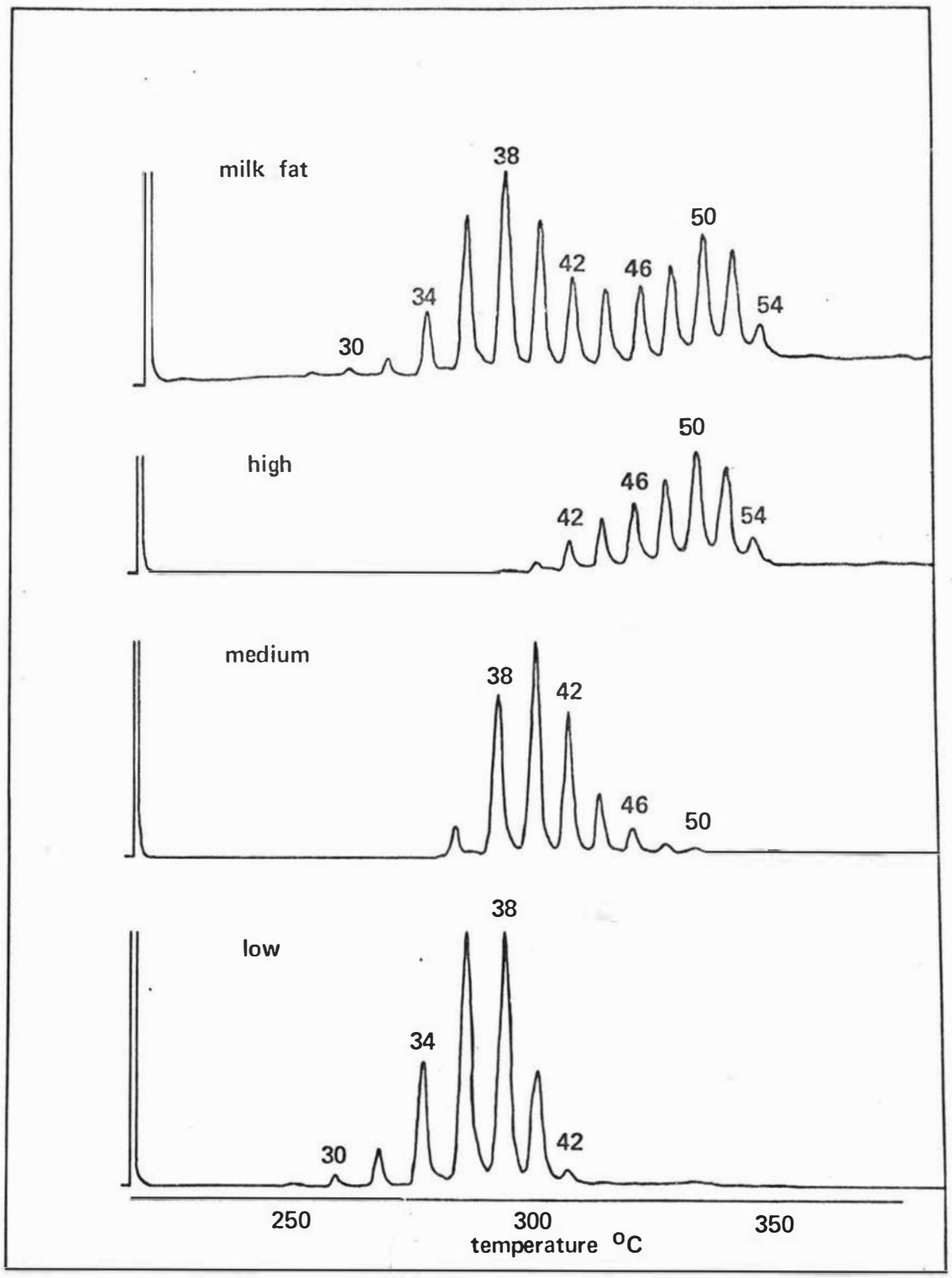


Figure 6. Gas-liquid chromatograms of the triacylglycerols of the March sample of milk fat and its triacylglycerol fractions of high, medium and low molecular weight.

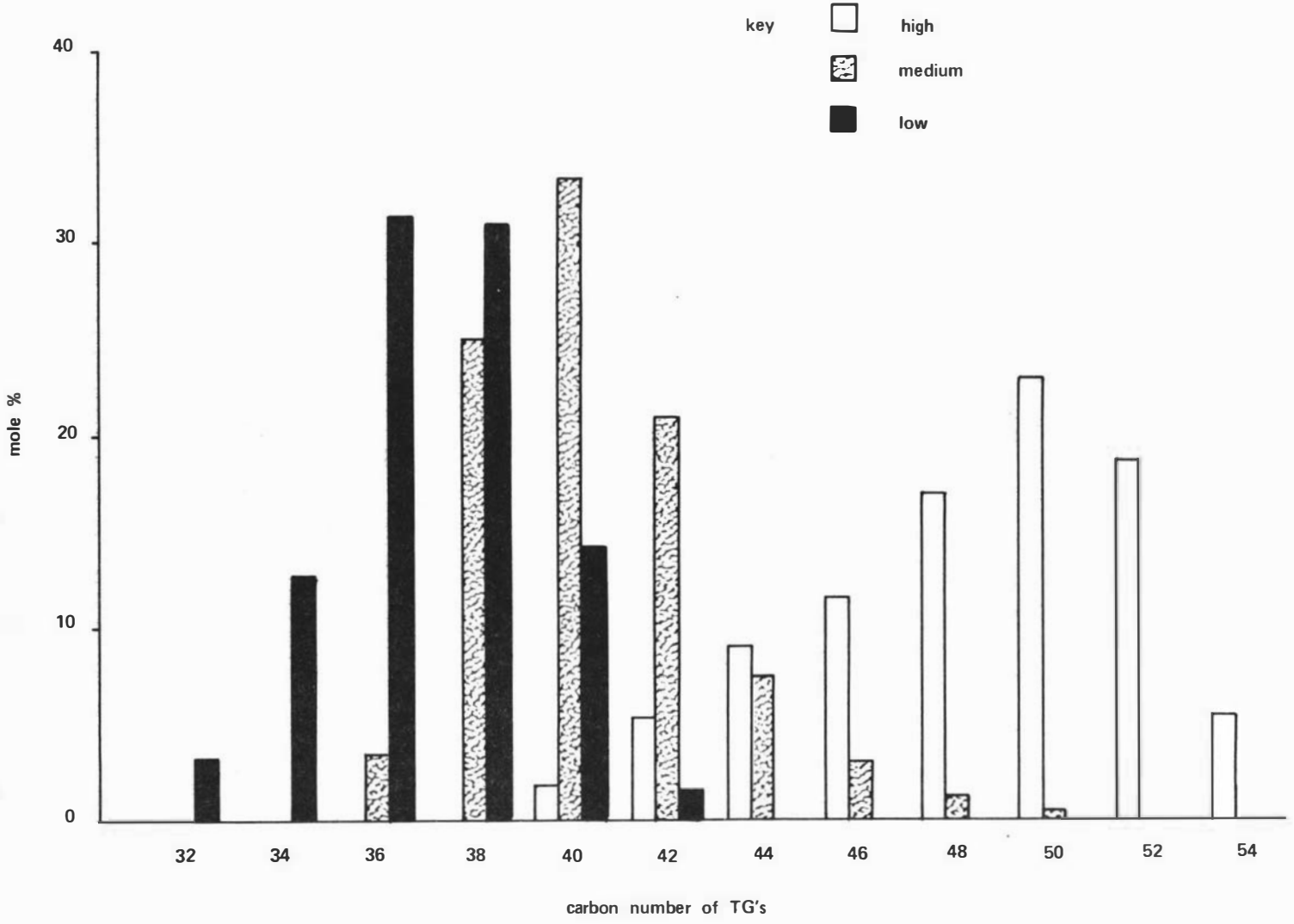


Figure 7. Triacylglycerol compositions of the triacylglycerol fractions of high, medium and low molecular weight obtained from the March sample of milk fat.

It is evident from the data presented in Table 11 and Figure 7 that the March sample of milk fat was effectively separated into three TG fractions on the basis of mol. wt. The molecular types of TG's C_{46} to C_{54} were concentrated almost exclusively in fraction A, the molecular types C_{28} to C_{36} were concentrated almost exclusively in fraction C while fraction B contained the greatest proportions of the molecular types C_{40} and C_{42}. The average mol. wt. (calculated from TG compositions) of the total March sample and fractions A, B and C prepared from it were respectively; 734, 814, 702 and 652. These values provided additional evidence of the effectiveness of the fractionation procedure.

3.2.2. Fatty acid compositions of the triacylglycerol fractions of high, medium and low molecular weight.

The FA compositions of the TG fractions of high, medium and low mol. wt. (fractions A, B and C), which were prepared from each of the three samples of milk fat by silicic acid column chromatography, are given in Tables 12, 13 and 14. The results shown are the means of duplicate determinations. A graphical representation of the more abundant FA's in the three TG fractions of the March sample of milk fat is presented in Figure 8.

An inspection of Tables 12, 13 and 14 shows that for each sample of milk fat the FA composition reconstructed from the proportions of FA's in the respective TG fractions closely resembled the FA composition determined

Table 12. Fatty acid compositions of the triacylglycerol fractions of high, medium, and low molecular weight (fractions A, B and C) obtained from the sample of September milk fat by silicic acid column chromatography.

FA	Fatty acid composition (mole %)							
	Fraction A		Fraction B		Fraction C		Milk fat	
	% F[a]	% M[b]	% F[a]	% M[b]	% F[a]	% M[b]	Rec.	Orig.
4:0	-	-	6.1	1.0	24.9	10.7	11.7	12.0
6:0	0.7	0.3	11.2	1.9	6.1	2.6	4.8	4.5
8:0	1.7	0.7	4.9	0.8	2.1	0.9	2.4	2.3
10:0	4.1	1.6	6.2	1.0	3.5	1.5	4.1	4.2
10:1	-	-	0.6	0.1	0.4	0.2	0.3	0.3
12:0	4.1	1.6	3.9	0.6	3.7	1.6	3.8	4.0
14:0	11.5	4.6	10.2	1.7	10.5	4.5	10.8	10.9
14:1	0.9	0.4	0.8	0.1	0.6	0.3	0.8	0.8
15:0	1.6	0.6	1.2	0.2	1.1	0.5	1.3	1.4
16:0	23.2	9.3	21.7	3.7	20.2	8.7	21.7	22.1
16:1	1.7	0.7	1.1	0.2	1.2	0.5	1.4	1.4
17:0	0.9	0.4	0.7	0.1	0.3	0.1	0.6	0.6
18:0	17.5	7.0	13.3	2.2	8.2	3.5	12.7	13.1
18:1	28.8	11.5	16.6	2.8	15.9	6.9	21.2	21.5
18:2	2.0	0.8	0.8	0.1	1.1	0.5	1.4	0.7
18:3	1.0	0.4	0.6	0.1	0.5	0.2	0.7	0.3
20:2	0.4	0.2	0.3	0.0	-	-	0.2	0.2
	100.1	40.1	100.2	16.6	100.3	43.2	99.9	100.3

[a]percentage in fraction

[b]percentage in milk fat

Table 13. Fatty acid compositions of the triacylglycerol fractions of high, medium, and low molecular weight (fractions A, B and C) obtained from the sample of January milk fat by silicic acid column chromatography.

FA	Fatty acid composition (mole %)							
	Fraction A		Fraction B		Fraction C		Milk fat	
	% F[a]	% M[b]	% F[a]	% M[b]	% F[a]	% M[b]	Rec.	Orig.
4:0	-	-	0.5	0.1	23.1	9.9	10.0	9.7
6:0	-	-	9.6	1.7	7.0	3.0	4.7	4.5
8:0	0.6	0.2	6.4	1.2	1.8	0.8	2.2	2.2
10:0	3.7	1.4	8.7	1.6	3.0	1.3	4.3	4.2
10:1	-	-	0.9	0.2	0.5	0.2	0.4	0.4
12:0	4.3	1.7	5.2	0.9	3.4	1.5	4.1	4.1
14:0	13.0	5.1	11.7	2.1	10.4	4.4	11.6	11.5
14:1	1.3	0.5	1.4	0.3	0.8	0.4	1.2	1.2
15:0	1.9	0.7	1.2	0.2	1.3	0.6	1.5	1.7
16:0	30.8	12.1	27.8	5.0	24.4	10.4	27.5	27.6
16:1	2.4	0.9	1.8	0.3	1.9	0.8	2.0	2.1
17:0	1.6	0.6	1.0	0.2	0.9	0.4	1.2	0.8
18:0	14.5	5.7	8.8	1.6	6.9	2.9	10.2	10.1
18:1	23.5	9.2	13.4	2.4	12.7	5.4	17.0	17.8
18:2	1.6	0.6	1.1	0.2	1.2	0.5	1.3	1.4
18:3	0.9	0.4	0.7	0.1	0.8	0.3	0.8	0.8
	100.1	39.1	100.2	18.1	100.0	42.8	100.0	100.1

[a] percentage in fraction

[b] percentage in milk fat

Table 14. Fatty acid compositions of the triacylglycerol fractions of high, medium, and low molecular weight (fractions A, B and C) obtained from the sample of March milk fat by silicic acid column chromatography.

FA	Fatty acid composition (mole %)							
	Fraction A		Fraction B		Fraction C		Milk fat	
	% F[a]	% M[b]	% F[a]	% M[b]	% F[a]	% M[b]	Rec.	Orig.
4:0	-	-	2.0	0.3	23.5	10.0	10.3	10.0
6:0	0.2	0.1	11.7	1.9	6.7	2.8	4.8	4.6
8:0	0.9	0.4	5.0	0.8	2.0	0.8	2.0	2.3
10:0	3.5	1.5	7.7	1.2	2.9	1.2	3.9	4.2
10:1	-	-	0.8	0.1	0.4	0.2	0.3	0.3
12:0	3.8	1.6	4.6	0.7	3.6	1.5	3.8	3.9
14:0	12.4	5.1	11.6	1.9	10.3	4.4	11.4	10.9
14:1	1.4	0.6	1.4	0.2	1.0	0.4	1.2	1.1
15:0	1.9	0.8	1.0	0.2	1.4	0.6	1.6	1.6
16:0	29.2	12.1	26.2	4.2	24.0	10.2	26.5	26.4
16:1	2.0	0.8	1.0	0.2	1.7	0.7	1.7	1.9
17:0	1.1	0.4	0.3	-	0.6	0.3	0.7	0.9
18:0	15.6	6.4	9.8	1.6	7.4	3.2	11.2	11.5
18:1	24.9	10.3	15.1	2.4	13.5	5.7	18.4	18.4
18:2	2.1	0.9	1.2	0.2	0.6	0.2	1.3	1.1
18:3	0.9	0.4	0.8	0.1	0.5	0.2	0.7	0.8
	99.9	41.4	100.2	16.0	100.1	42.4	99.8	99.9

[a]percentage in fraction

[b]percentage in milk fat

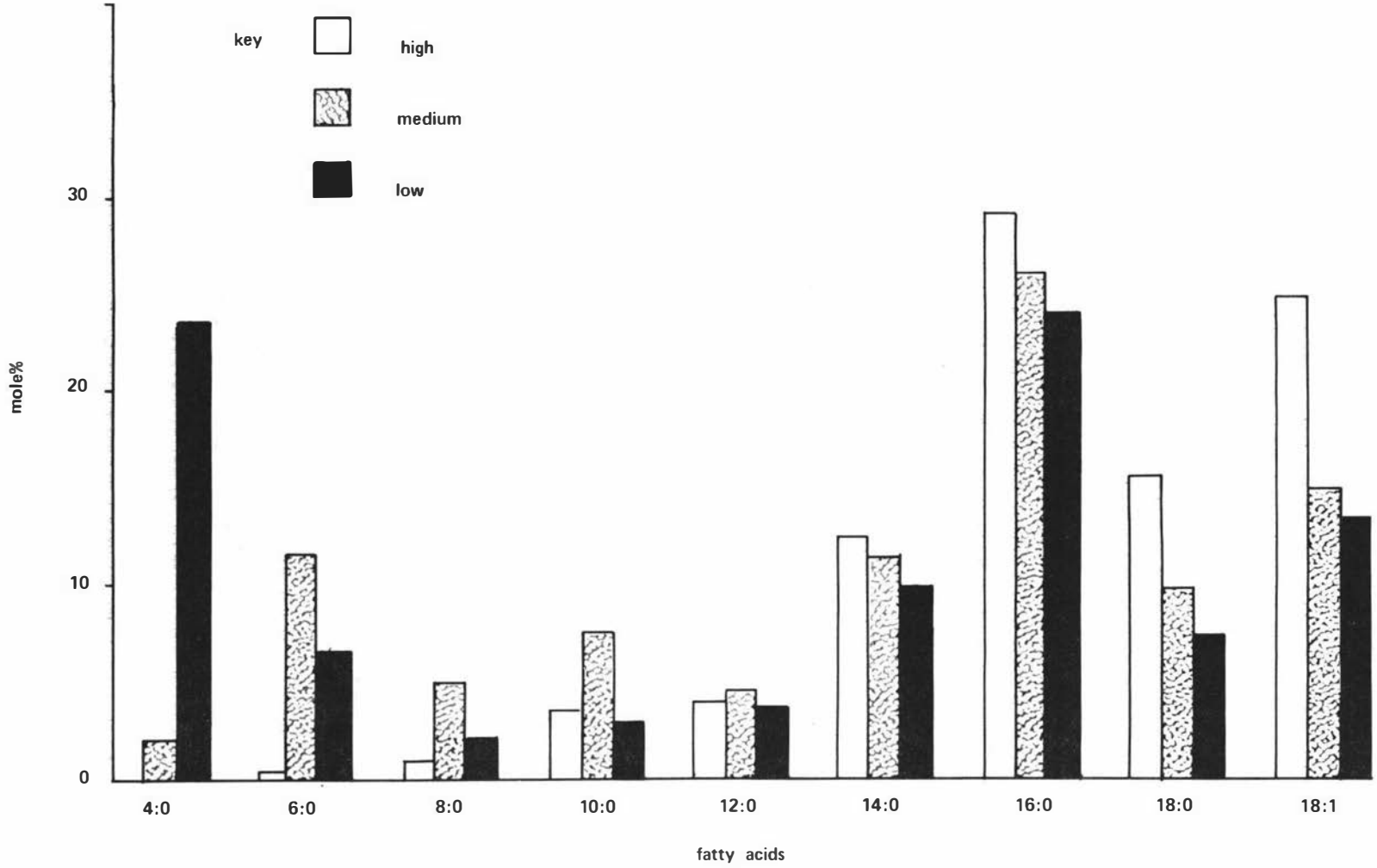


Figure 8. Fatty acid compositions of the triacylglycerol fractions of high, medium and low molecular weight obtained from the March sample of milk fat.

experimentally.

(a) The September sample of milk fat

Fraction A of the September sample contained no 4:0 and very little 6:0 while 14:0 (11.5%), 16:0 (23.2%), 18:0 (17.5%), and 18:1 (28.8%) together comprised 81.0% of the component FA's (Table 12). 18:0 and 18:1 together accounted for 34.6% of the FA's in the milk fat sample and of this 18.5% (i.e. 53% of total 18:0 + 18:1) was concentrated in fraction A. In fraction B 6:0 (11.2%), 14:0 (10.2%), 16:0 (21.7%), 18:0 (13.3%) and 18:1 (16.6%) together accounted for 73.0% of the FA's present. 4:0 (24.9%), 14:0 (10.5%), 16:0 (20.2%), and 18:1 (15.9%) comprised 71.5% of the FA constituents of fraction C. The short-chain FA's; 4:0, 6:0 and 8:0, accounted for 33.1% of the FA's present in this fraction. 90% of 4:0 present in the September sample was concentrated in fraction C.

(b) The January sample of milk fat

In fraction A of the January sample 14:0 (13.0%), 16:0 (30.8%), 18:0 (14.5%) and 18:1 (23.5%) together constituted 81.8% of the FA's present (Table 13). No detectable amounts of 4:0 or 6:0 and only a small amount of 8:0 were found in this TG fraction. More than 50% of the 18:0 and 18:1 present in the January sample was found in fraction A. 6:0 (9.6%), 14:0 (11.7%), 16:0 (27.8%) and 18:1 (13.4%) comprised 62.5% of the component FA's of fraction B. In fraction C 4:0 (23.1%), 14:0 (10.4%), 16:0 (24.4%) and 18:1 (12.7%) accounted for 70.6% of the constituent FA's. Together the short-chain

FA's; 4:0, 6:0, and 8:0, contributed 31.9% of FA's present in this TG fraction. The 4:0 present in the January sample was concentrated almost exclusively in fraction C.

(c) The March sample of milk fat

In the March sample fraction A contained no 4:0 and only small amounts of 6:0 and 8:0 while 14:0 (12.4%), 16:0 (29.2%), 18:0 (15.6%) and 18:1 (24.9%) comprised 82.1% of the component FA's (Table 14). Together 18:0 and 18:1 accounted for 29.9% of the FA's in the sample of milk fat and of this 16.7% was concentrated in fraction A. In fraction B 6:0 (11.7%), 14:0 (11.6%), 16:0 (26.2%), 18:0 (9.8%) and 18:1 (15.1%) accounted for 74.4% of FA's present. In fraction C 71.3% of the FA's consisted of 4:0 (23.5%), 14:0 (10.3%), 16:0 (24.0%) and 18:1 (13.5%). Together the short-chain FA's; 4:0, 6:0 and 8:0 accounted for 32.2% of the FA's present in this TG fraction. The 4:0 present in the sample of milk fat was again concentrated almost exclusively in fraction C.

(d) Comparison of the September, January and March samples of milk fat

From the above description it is evident that corresponding TG fractions contained the same major FA's. In the TG fractions of high mol. wt. (fraction A's) the major constituent FA's were 14:0, 16:0, 18:0 and 18:1; in the TG fractions of medium mol. wt. (fraction B's) 6:0, 14:0, 16:0, 18:0 and 18:1 made large contributions to the total FA's; and the major FA components in the TG

fractions of low mol. wt. (fraction C's) were 4:0, 14:0, 16:0 and 18:1.

Tables 12, 13 and 14 show that the distribution of FA's in the three TG fractions of differing mol. wt. was similar for each of the three samples of milk fat. 4:0 occurred almost exclusively in fraction C's with the exception of the September sample where it comprised 6% of the total FA's in fraction B. Values of 6:0, 8:0 and 10:0 (mole %) were greatest in fraction B. In the January and March samples the proportions of 14:0 decreased from fraction A to fraction C but in the September sample the proportions of 14:0 in fractions B and C were 10.2% and 10.5% respectively. In each of the three samples proportions for 16:0 decreased from fraction A to fraction C. 18:0 showed a similar but greater decrease from fraction A to fraction C while the proportions of 18:1 in each fraction A was considerably greater than those in the respective fractions B and C.

As noted previously (Section 3.1.1.) the January and March samples of milk fat had similar FA compositions which differed from the FA composition of the September sample in that the September sample contained greater proportions of 4:0, 18:0 and 18:1 and a lesser proportion of 16:0 (Table 7). These similarities and differences in FA compositions were, in general, reflected in the FA compositions of the TG fractions of differing mol. wt. prepared from each of the three samples of milk fat (Tables 12, 13 and 14). Fraction A of the September sample contained higher proportions of 18:0 and 18:1 and

a lower proportion of 16:0 than the A fractions of the January and March samples, fraction B of the September sample had higher proportions of 4:0 and 18:0 and a lower proportion of 16:0 than the January and March B fractions, and fraction C of the September sample contained higher proportions of 4:0 and 18:1 and a lower proportion of 16:0 than the C fractions of the January and March samples. In addition to the above differences in the proportions of FA's between corresponding TG fractions most of the other more abundant FA's in the B fractions varied slightly between the respective fraction B's.

These findings suggest that, in the main, the entire mol. wt. range of TG's present in milk fat is influenced by changes in the FA composition of milk fat. Furthermore, milk fat samples of similar FA composition contain TG fractions of comparable mol. wt. which have similar FA compositions.

Table 15 shows the average mol. wt. of the component FA's of each sample of milk fat and each TG fraction. Values of 256-257, 224-226, and 200-202 were obtained for the A fractions, the B fractions and the C fractions respectively. Thus it is evident that the A fractions contained TG's which possessed on average six carbons more than TG's of the B fractions and 12 carbons more than TG's of the C fractions. Despite the difference between the FA compositions of the September sample and its TG fractions, on the one hand, and the FA compositions of the January and March samples and their TG fractions, on the other, it is clear that the three samples of milk

fat and the corresponding TG fractions consisted of FA's which had closely similar average mol. wts.

Table 15. The average molecular weight of the fatty acid constituents of each sample of milk fat and each triacylglycerol fraction.

	Average molecular weight[a]		
	September	January	March
Milk fat	227	229	229
fraction A	256	257	257
fraction B	224	226	224
fraction C	202	200	202

[a]calculated from the respective fatty acid compositions.

3.2.3. Stereospecific analysis of the triacylglycerol fractions of high, medium and low molecular weight.

The TG fractions of high, medium and low mol. wt. (fractions A, B and C), which were prepared from each of the three samples of milk fat by silicic acid column chromatography, were subjected to stereospecific analysis. The results obtained are shown in Tables 16 to 24. Included in the Tables are the FA compositions of (i) the original TG's, (ii) the 1,2(2,3)-DG's, 1,3-DG's and 2-MG's isolated from the reaction products following deacylation

of the TG samples, (iii) the 2,3-PL's and 1-PL's isolated from the reaction products after the digestion of 1,2(2,3)-PL's with phospholipase A_2, (iv) the 1,2(2,3)-DG's and 1,3-DG's determined by calculation, and (v) position 3 determined by two different calculations.

(a) Accuracy of the stereospecific analyses

For the results of the stereospecific analysis to be quantitatively correct the 1,2(2,3)-DG's formed as intermediates during the first step of the analysis must be representative of the FA's present in the original TG's (Section 2.4.1.).

Data presented in Tables 16, 17 and 18 show that for each TG fraction of high mol. wt. (fraction A) the FA composition of the 1,2(2,3)-DG's, prepared by deacylation of TG's with a Grignard reagent, was similar to the FA composition determined for the 1,2(2,3)-DG's by calculation. This would suggest that 1,2(2,3)-DG's determined experimentally were representative of the FA's present in the original TG's. Furthermore, it was found that for each fraction A the FA compositions of the 1,3-DG's obtained experimentally deviated slightly from the FA composition determined for the 1,3-DG's by calculation. However the differences between each set of FA compositions did not indicate that the 1,3-DG's obtained experimentally were partially contaminated with 1,2(2,3)-DG's.

Data obtained for TG fractions of medium and low mol. wt. (fractions B and C) which is presented in Tables 19 to 24 showed that for each TG fraction the FA composition of

the 1,2(2,3)-DG's, prepared by digestion of TG's with pancreatic lipase, deviated to some extent from the FA composition determined by calculation. In each case the proportions of 4:0 and 6:0 obtained by calculation were 10-20% lower. This would suggest that the 1,2(2,3)-DG's obtained experimentally were not strictly representative of the constituent FA's of the original TG's.

A further check of the accuracy of each stereospecific analysis may be obtained by comparing the two FA compositions determined for position 3. Both FA compositions were determined indirectly by calculation and the nature of the calculations magnified the experimental errors. Consequently some variation in the two sets of data would be expected and in each stereospecific analysis it was found that these two FA compositions varied appreciably. However, the uncertainty introduced into this aspect of the stereospecific analysis did not prevent valid qualitative comparison between the distribution of FA's in positions 1, 2 and 3 of TG's.

(b) <u>The arrangement of fatty acids in positions 1, 2 and 3 of the triacylglycerols of high molecular weight</u>

The similarities between the FA compositions of the A fractions of the January and March samples (Section 3.2.2.) were reflected in the FA compositions of positions 1, 2 and 3 of their constituent TG's with corresponding positions in each fraction having similar FA compositions (Tables 17 and 18). Furthermore the differences in the

Table 16. Stereospecific analysis of the triacylglycerol fraction of high molecular weight (fraction A) obtained from the sample of September milk fat by silicic acid column chromatography.

	Fatty acid composition (mole %)									
							1[a]	2[a]	3[a]	
FA	TG's Orig.	1,3-DG's Exp.	1,3-DG's Calc.	1,2(2,3)-DG's Exp.	1,2(2,3)-DG's Calc.	2,3-PL's	1-PL's	2-MG's	3A[b]	3B[c]
6:0	0.7	0.2	1.0	1.0	0.5	1.0	-	-	2.0	2.1
8:0	1.7	1.0	2.3	1.2	1.4	1.7	-	0.4	3.0	4.7
10:0	4.1	4.2	4.6	3.6	3.9	5.4	0.8	3.1	7.7	8.4
12:0	4.1	3.5	3.7	4.2	4.3	4.6	1.7	4.9	4.3	5.7
14:0	11.5	8.2	7.7	13.4	13.4	13.9	7.1	19.0	8.8	8.4
14:1	0.9	0.8	1.1	0.9	0.8	1.0	0.6	0.6	1.4	1.5
15:0	1.6	1.4	1.7	1.5	1.6	1.2	1.1	1.5	0.9	2.2
16:0	23.2	18.7	16.7	26.3	26.4	23.8	27.6	36.2	11.4	5.8
16:1	1.7	1.4	1.8	1.8	1.7	1.6	0.9	1.6	1.6	2.6
17:0	0.9	1.7	1.1	0.7	0.8	0.6	0.5	0.4	0.8	1.8
18:0	17.5	22.5	21.4	15.8	15.5	15.4	25.2	9.7	21.1	17.6
18:1	28.8	34.3	32.4	28.2	27.0	28.1	33.6	21.8	34.4	31.0
18:2	2.0	1.4	2.6	1.3	1.7	1.6	0.9	0.7	2.5	4.4
18:3	1.0	0.6	1.5	-	0.7	-	-	-	-	3.0
20:2	0.4	-	0.6	-	0.3	-	-	-	-	1.2
	100.1	99.9	100.2	99.9	100.0	99.9	100.0	99.9	99.9	100.4

[a] position relative to sn - glycerol 3- phosphate

[b] 2x(2,3-PL's) - (2-MG's)

[c] 3x(TG's) - (1-PL's) - (2-MG's)

Table 17. Stereospecific analysis of the triacylglycerol fraction of high molecular weight (fraction A) obtained from the sample of January milk fat by silicic acid column chromatography.

	Fatty acid composition (mole %)									
							1[a]	2[a]	3[a]	
FA	TG's Orig.	1,3-DG's Exp.	1,3-DG's Calc.	1,2(2,3)-DG's Exp.	1,2(2,3)-DG's Calc.	2,3-PL's	1-PL's	2-MG's	3A[b]	3B[c]
8:0	0.6	0.4	0.9	0.8	0.5	0.7	-	-	1.4	1.8
10:0	3.7	3.7	4.4	3.8	3.4	4.7	1.2	2.3	7.1	7.6
12:0	4.3	3.3	3.7	4.7	4.6	5.5	1.8	5.6	5.4	5.5
14:0	13.0	10.1	8.3	16.5	15.4	16.9	7.0	22.5	11.3	9.5
14:1	1.3	1.2	1.5	1.3	1.2	1.6	0.6	1.0	2.2	2.3
15:0	1.9	1.6	2.0	2.0	1.9	1.8	1.1	1.7	1.9	2.9
16:0	30.8	26.4	23.8	35.9	34.3	28.9	38.6	44.9	12.9	8.9
16:1	2.4	1.6	2.8	1.7	2.2	1.7	1.2	1.6	1.8	4.4
17:0	1.6	1.4	2.1	0.8	1.4	1.2	1.3	0.7	1.7	2.8
18:0	14.5	18.9	18.6	12.1	12.5	12.2	23.0	6.4	18.0	14.1
18:1	23.5	29.7	29.6	19.7	20.5	23.8	23.5	11.3	36.3	35.7
18:2	1.6	1.8	1.8	0.8	1.5	1.0	0.6	1.3	0.7	2.9
18:3	0.9	-	1.1	-	0.8	-	-	0.6	-0.6	2.1
	100.1	100.1	100.6	100.1	100.2	100.0	99.9	99.9	100.1	100.5

[a] position relative to sn - glycerol 3- phosphate

[b] 2x(2,3-PL's) - (2-MG's)

[c] 3x(TG's) - (1-PL's) - (2-MG's)

Table 18. Stereospecific analysis of the triacylglycerol fraction of high molecular weight (fraction A) obtained from the sample of March milk fat by silicic acid column chromatography.

	Fatty acid composition (mole %)									
							1[a]	2[a]	3[a]	
FA	TG's Orig.	1,3-DG's Exp.	1,3-DG's Calc.	1,2(2,3)-DG's Exp.	1,2(2,3)-DG's Calc.	2,3-PL's	1-PL's	2-MG's	3A[b]	3B[c]
6:0	0.2	-	0.3	-	0.2	-	-	-	-	0.6
8:0	0.9	0.6	1.1	0.9	0.8	1.2	-	0.5	1.9	2.2
10:0	3.5	3.4	3.6	3.5	3.5	4.1	1.5	3.3	4.9	5.7
12:0	3.8	3.6	2.9	4.5	4.3	4.3	2.2	5.6	3.0	3.6
14:0	12.4	8.9	8.1	15.5	14.6	15.6	9.1	21.1	10.1	7.0
14:1	1.4	1.3	1.6	1.1	1.3	1.3	0.8	1.1	1.5	2.3
15:0	1.9	1.4	1.9	1.6	1.9	2.0	1.4	2.0	2.0	2.3
16:0	29.2	25.2	23.6	33.8	32.0	27.3	38.1	40.4	14.2	9.1
16:1	2.0	1.5	1.9	1.3	2.1	1.6	1.1	2.3	0.9	2.6
17:0	1.1	1.7	1.5	1.1	0.9	1.3	1.0	0.4	2.2	1.9
18:0	15.6	19.4	20.0	13.7	13.4	15.2	20.3	6.8	23.6	19.7
18:1	24.9	31.1	30.5	21.2	22.1	24.8	24.1	13.8	35.8	36.8
18:2	2.1	1.5	2.3	1.3	2.0	1.2	0.6	1.7	0.7	4.0
18:3	0.9	0.4	0.8	0.6	1.0	-	-	1.2	-1.2	1.5
	99.9	100.0	100.1	100.1	100.1	99.9	100.2	100.2	99.6	99.3

[a] position relative to sn - glycerol 3- phosphate

[b] 2x(2,3-PL's) - (2-MG's)

[c] 3x(TG's) - (1-PL's) - (2-MG's)

proportions of 16:0, 18:0 and 18:1 between fraction A of the September sample, on the one hand, and the A fractions of the January and March samples, on the other, (Section 3.2.2.) were reflected in the differences in the proportions of FA's which occupied positions 1 and 2 of their constituent TG's (Tables 16, 17 and 18). For example, in fraction A of each of the September, January and March samples of milk fat the proportions of 18:1 in the constituent TG's were 28.8%, 23.5% and 24.9% respectively; the proportions of 18:1 esterified at position 1 were 33.6%, 23.5% and 24.1% respectively, the proportions at position 2 were 21.8%, 11.3% and 13.8% respectively, and the proportions at position 3 were (31.0-34.4%), (35.7-36.3%) and (35.8-36.8%) respectively.

As expected these differences involving 16:0, 18:0 and 18:1, were superimposed on broad similarities in the pattern of distribution of FA's in positions 1, 2 and 3 of each fraction A (Tables 16, 17 and 18). 10:0 was preferentially esterified at position 3 while similar proportions of 12:0 were esterified at positions 2 and 3. Only small amounts of these two FA's occupied position 1. 14:0 was preferentially esterified at carbon 2 of the glycerol molecules while smaller amounts were esterified at carbons 1 and 3. Proportions of 14:0 at carbons 1, 2 and 3 were (7.0-9.1%), (19.0-22.5%) and (8.4-11.3%) respectively. 16:0 was preferentially incorporated at position 1 (27.6-38.6%) and at position 2 (36.2-44.9%) with only a relatively minor amount incorporated at position 3 (5.8-14.2%). 18:0 exhibited a pattern of

distribution which was the reverse of that shown by 14:0. It was preferentially esterified at positions 1 and 3 with only a small amount esterified at position 2. Proportions of 18:0 at positions 1, 2 and 3 were (20.3-25.2%), (6.4-9.7%) and (14.1-21.1%) respectively. The distribution pattern of 18:1 was similar to that for 18:0 with proportions of (23.5-33.6%), (11.3-21.8%) and (31.0-36.8%) esterified at positions 1, 2 and 3 respectively.

As a result of this non-random distribution of FA's in the TG fractions of high mol. wt., position 1 was largely occupied by 16:0, 18:0 and 18:1; position 2 by 14:0 and 16:0; and position 3 by 18:0 and 18:1. In addition considerable amounts of 18:1 were esterified at position 2 in fraction A of the September sample. The long-chain FA's (14:0, 16:0, 18:0 and 18:1) which comprise the major FA's in each fraction A were present in significant proportions in all three positions.

(c) <u>The arrangement of fatty acids in positions 1, 2 and 3 of the triacylglycerols of medium molecular weight</u>

Fraction B of the September sample contained greater proportions of 4:0 and 18:0 and a lesser proportion of 16:0 than the B fractions of the January and March samples. In addition the proportions of most of the other more abundant component FA's varied slightly between the respective B fractions (Section 3.2.2.). The differences in the proportions of 4:0, 16:0 and 18:0 between fraction B of the September sample, on the one hand, and the B fractions of the January and March samples, on the other, were reflected

Table 19. Stereospecific analysis of the triacylglycerol fraction of medium molecular weight (fraction B) obtained from the sample of September milk fat by silicic acid column chromatography.

	Fatty acid composition (mole %)							
					1[a]	2[a]	3[a]	
FA	TG's Orig.	1,2 (2,3) - DG's Exp.	1,2 (2,3) - DG's Calc.	2,3-PL's	1-PL's	2-MG's	3A[b]	3B[c]
4:0	6.1	5.5	4.6	10.1	-	-	20.2	18.3
6:0	11.2	10.1	8.4	16.9	2.6	-	33.8	31.0
8:0	4.9	4.7	4.5	6.4	1.5	3.5	9.3	9.7
10:0	6.2	6.8	7.2	7.8	2.3	10.4	5.2	5.9
10:1	0.6	0.5	0.6	0.6	0.5	0.8	0.4	0.5
12:0	3.9	4.3	5.0	4.5	4.8	8.4	0.6	-1.5
14:0	10.2	12.5	13.1	10.8	8.8	21.8	-0.2	0.0
14:1	0.8	0.6	0.8	0.6	0.5	0.9	0.3	1.0
15:0	1.2	1.3	1.4	1.6	1.5	2.0	1.2	0.1
16:0	21.7	24.1	23.3	18.7	29.3	28.2	9.2	7.6
16:1	1.1	1.0	1.2	1.3	0.8	1.7	0.9	0.8
17:0	0.7	0.3	0.6	0.4	0.8	0.4	0.4	0.9
18:0	13.3	11.2	11.5	7.4	20.5	6.0	8.8	13.4
18:1	16.6	16.3	16.2	12.8	26.2	15.1	10.5	8.5
18:2	0.8	0.8	0.8	-	-	0.8	-0.8	1.6
18:3	0.6	-	0.4	-	-	-	-	1.8
20:2	0.3	-	0.2	-	-	-	-	0.9
	100.2	100.0	99.8	99.9	100.1	100.0	99.8	100.5

[a] position relative to sn - glycerol 3- phosphate

[b] 2x(2,3-PL's) - (2-MG's)

[c] 3x(TG's) - (1-PL's) - (2-MG's)

Table 20. Stereospecific analysis of the triacylglycerol fraction of medium molecular weight (fraction B) obtained from the sample of January milk fat by silicic acid column chromatography.

	Fatty acid composition (mole %)							
					1[a]	2[a]	3[a]	
FA	TG's Orig.	1,2 (2,3) - DG's Exp.	1,2 (2,3) - DG's Calc.	2,3-PL's	1-PL's	2-MG's	3A[b]	3B[c]
4:0	0.5	0.5	0.4	1.2	-	-	2.4	1.5
6:0	9.6	9.2	7.5	13.4	4.1	1.1	25.7	23.6
8:0	6.4	5.6	5.4	7.4	2.4	2.6	12.2	14.2
10:0	8.7	8.0	8.2	9.8	4.0	6.7	12.9	15.4
10:1	0.9	0.5	0.8	0.5	0.6	0.5	0.5	1.6
12:0	5.2	5.9	5.8	4.8	6.9	7.7	1.9	1.0
14:0	11.7	14.5	14.1	13.4	7.9	21.1	5.7	6.1
14:1	1.4	1.2	1.4	0.9	1.0	1.6	0.2	1.6
15:0	1.2	1.5	1.4	1.5	2.4	2.0	1.0	-0.8
16:0	27.8	31.4	30.2	26.3	35.8	37.6	15.0	10.0
16:1	1.8	1.2	1.7	1.1	1.7	1.3	0.9	2.4
17:0	1.0	0.4	0.9	0.6	0.8	0.5	0.7	1.7
18:0	8.8	7.6	7.8	6.9	13.8	4.8	9.0	7.8
18:1	13.4	11.9	12.8	11.8	18.1	10.9	12.7	11.2
18:2	1.1	0.6	1.0	0.4	0.6	0.8	0	1.9
18:3	0.7	-	0.7	-	-	0.7	-0.7	1.4
	100.2	100.0	100.1	100.0	100.1	99.9	100.1	100.6

[a] position relative to sn - glycerol 3- phosphate

[b] 2x(2,3-PL's) - (2-MG's)

[c] 3x(TG's) - (1-PL's) - (2-MG's)

Table 21. Stereospecific analysis of the triacylglycerol fraction of medium molecular weight (fraction B) obtained from the sample of March milk fat by silicic acid column chromatography.

	Fatty acid composition (mole %)							
					1[a]	2[a]	3[a]	
FA	TG's Orig.	1,2 (2,3) - DG's Exp.	1,2 (2,3) - DG's Calc.	2,3-PL's	1-PL's	2-MG's	3A[b]	3B[c]
4:0	2.0	1.8	1.5	2.9	-	-	5.8	6.0
6:0	11.7	11.0	8.8	15.0	4.5	-	30.0	30.6
8:0	5.0	4.4	4.1	5.1	3.0	1.5	8.7	10.5
10:0	7.7	7.4	7.4	7.3	5.1	6.5	8.1	11.5
10:1	0.8	0.5	0.7	0.4	0.6	0.4	0.4	1.4
12:0	4.6	4.9	5.2	4.5	3.8	6.9	2.1	3.1
14:0	11.6	14.1	14.4	13.5	9.6	22.7	4.3	2.5
14:1	1.4	1.2	1.5	1.2	0.9	2.0	0.4	1.3
15:0	1.0	1.6	1.3	1.3	1.5	2.4	0.2	-0.9
16:0	26.2	28.6	28.8	24.8	35.1	36.6	13.0	6.9
16:1	1.0	1.0	1.2	1.1	1.0	1.8	0.4	0.2
17:0	0.3	0.3	0.3	0.6	0.5	0.3	0.9	0.1
18:0	9.8	8.2	8.7	8.8	15.2	5.5	12.1	8.7
18:1	15.1	13.7	14.3	12.9	19.1	12.0	13.8	14.2
18:2	1.2	0.8	1.0	0.6	-	0.6	0.6	3.0
18:3	0.8	0.5	0.8	-	-	0.8	-0.8	1.6
	100.2	100.0	100.0	100.0	99.9	100.0	100.0	100.7

[a] position relative to sn - glycerol 3- phosphate

[b] 2x(2,3-PL's) - (2-MG's)

[c] 3x(TG's) - (1-PL's) - (2-MG's)

in the differences in the proportions of these FA's in positions 1, 2 and 3 of their constituent TG's (Tables 19, 20 and 21). However, the effect was not constant throughout all three positions in that the differences in the proportions of 4:0 affected only position 3, the differences in 16:0 affected positions 1 and 2 and the differences in 18:0 affected position 1. The small differences in the proportions of other FA's between the respective B fractions were also reflected in the differences in the proportions of FA's esterified at carbons 1, 2 and 3 of their constituent TG's.

Despite these differences between the FA compositions of corresponding positions of the respective B fractions the overall pattern of distribution of FA's in positions 1, 2 and 3 was similar for each fraction B (Tables 19, 20 and 21). The short-chain FA's, 4:0 and 6:0, were esterified almost exclusively at position 3 while 8:0 was preferentially incorporated at this position. In the January and March B fractions 10:0 was preferentially esterified at position 3 but in fraction B of the September sample 10:0 was preferentially esterified at position 2. Proportions of 12:0 were greatest at carbon 2 and least at carbon 3. Approximately twice as much 14:0 was esterified at position 2 as at position 1 with small to negligible amounts esterified at position 3. The proportions of 14:0 at positions 1, 2 and 3 were (7.9-9.6%), (21.1-22.7%) and (-0.2 - 6.1%) respectively. 16:0 was concentrated at carbon 1 (29.3-35.8%) and at carbon 2 (28.2-37.6%) with comparatively minor amounts

esterified at carbon 3 (6.9-15.0%). The greatest proportions of 18:0 were esterified at position 1 (13.8-20.5%) considerable proportions were esterified at position 3 (7.8-13.4%) but only small proportions were esterified at position 2 (4.8-6.0%). 18:1 was preferentially incorporated at carbon 1 of the glycerol molecules (18.1-26.2%) while smaller and comparable amounts were incorporated at carbon 2 (10.9-15.1%) and at carbon 3 (8.5-14.2%).

From the above description it is clear that in each TG fraction of medium mol. wt. the more abundant constituent FA's were distributed in a non-random manner within the constituent TG molecules. 16:0, 18:0 and 18:1 comprised about 70% of the acyl groups at position 1; 14:0 and 16:0 accounted for 50-60% of the FA's esterified at position 2, and 4:0, 6:0, 8:0 and 10:0 accounted for 50-70% of the acyl groups which occupied position 3.

(d) <u>The arrangement of fatty acids in positions 1, 2 and 3 of the triacylglycerols of low molecular weight</u>

The similarities between the FA compositions of the C fractions of the January and March samples (Section 3.2.2.) were reflected in the proportions of FA's in positions 1, 2 and 3 of their constituent TG's and almost without exception corresponding positions in these fractions had similar FA compositions (Tables 23 and 24). The slightly greater proportions of 4:0, and 18:1 and the lesser proportions of 16:0 in fraction C of the September

Table 22. Stereospecific analysis of the triacylglycerol fraction of low molecular weight (fraction C) obtained from the sample of September milk fat by silicic acid column chromatography.

FA	Fatty acid composition (mole %)							
	TG's	1,2 (2,3) - DG's		2,3-PL's	1[a]	2[a]	3[a]	
	Orig.	Exp.	Calc.		1-PL's	2-MG's	3A[b]	3B[c]
4:0	24.9	21.2	18.7	33.6	-	-	67.2	74.7
6:0	6.1	5.5	4.6	7.6	1.9	-	15.2	16.4
8:0	2.1	2.6	2.2	2.2	1.2	2.7	1.7	2.4
10:0	3.5	5.3	5.2	4.4	1.5	9.7	-0.9	-0.7
10:1	0.4	0.3	0.5	0.3	-	0.6	0	0.6
12:0	3.7	4.7	5.0	4.8	1.4	8.8	0.8	0.9
14:0	10.5	14.3	14.0	12.1	8.7	24.6	-0.4	-1.8
14:1	0.6	0.6	0.9	0.6	0.3	1.7	-0.5	-0.2
15:0	1.1	1.2	1.4	1.6	2.6	2.2	1.0	-1.5
16:0	20.2	20.4	22.2	16.7	31.2	28.4	5.0	1.0
16:1	1.2	1.0	1.4	1.1	0.9	1.8	0.4	0.9
17:0	0.3	0.4	0.3	0.4	0.4	0.4	0.4	0.1
18:0	8.2	6.8	7.1	3.6	21.7	3.9	3.3	-1.0
18:1	15.9	14.3	15.5	10.6	28.1	14.1	7.1	5.5
18:2	1.1	0.7	1.0	0.5	-	0.8	0.2	2.5
18:3	0.5	0.6	0.5	-	-	0.4	-0.4	1.1
	100.3	99.9	100.5	100.1	99.9	100.1	100.1	100.9

[a] position relative to sn - glycerol 3- phosphate

[b] 2x(2,3-PL's) - (2-MG's)

[c] 3x(TG's) - (1-PL's) - (2-MG's)

Table 23. Stereospecific analysis of the triacylglycerol fraction of low molecular weight (fraction C) obtained from the sample of January milk fat by silicic acid column chromatography.

	Fatty acid composition (mole %)							
					1[a]	2[a]	3[a]	
FA	TG's Orig.	1,2 (2,3) - DG's Exp.	1,2 (2,3) - DG's Calc.	2,3-PL's	1-PL's	2-MG's	3A[b]	3B[c]
4:0	23.1	19.4	17.3	32.5	1.7	-	65.0	67.6
6:0	7.0	6.4	5.7	8.1	2.4	2.0	14.2	16.6
8:0	1.8	1.7	1.9	1.6	1.0	2.3	0.9	2.1
10:0	3.0	3.8	4.2	2.9	1.5	7.9	-2.1	-0.4
10:1	0.5	0.4	0.5	0.4	-	0.7	0.1	0.8
12:0	3.4	4.3	4.6	4.2	3.0	8.4	0.0	-1.2
14:0	10.4	14.4	13.5	11.6	9.7	22.9	0.3	-1.4
14:1	0.8	1.1	1.1	0.9	0.7	2.0	-0.2	-0.3
15:0	1.3	1.4	1.6	1.5	1.1	2.4	0.6	0.4
16:0	24.4	27.4	26.7	21.1	38.8	33.5	8.7	0.9
16:1	1.9	1.4	1.9	1.6	1.8	2.0	1.2	1.9
17:0	0.9	0.5	0.8	0.4	1.0	0.4	0.4	1.3
18:0	6.9	5.4	6.0	3.0	16.4	3.4	2.6	0.9
18:1	12.7	11.3	12.3	9.6	20.6	11.0	8.2	6.5
18:2	1.2	0.6	1.1	0.5	0.4	0.7	0.3	2.5
18:3	0.8	0.4	0.7	-	-	0.4	-0.4	2.0
	100.1	99.9	99.9	99.9	100.1	100.0	99.8	100.2

[a] position relative to sn - glycerol 3- phosphate

[b] 2x(2,3-PL's) - (2-MG's)

[c] 3x(TG's) - (1-PL's) - (2-MG's)

Table 24. Stereospecific analysis of the triacylglycerol fraction of low molecular weight (fraction C) obtained from the sample of March milk fat by silicic acid column chromatography.

	Fatty acid composition (mole %)							
					1[a]	2[a]	3[a]	
FA	TG's Orig.	1,2 (2,3) - DG's Exp.	1,2 (2,3) - DG's Calc.	2,3-PL's	1-PL's	2-MG's	3A[b]	3B[c]
4:0	23.5	20.4	17.6	32.2	3.6	-	64.4	66.9
6:0	6.7	5.8	5.0	8.2	2.6	-	16.4	17.5
8:0	2.0	1.7	1.9	1.7	0.8	1.7	1.7	3.5
10:0	2.9	3.6	4.0	3.5	1.9	7.5	-0.5	-0.7
10:1	0.4	0.3	0.5	0.3	0.2	0.8	-0.2	0.2
12:0	3.6	4.1	4.8	4.0	2.2	8.4	-0.4	0.2
14:0	10.3	13.6	13.4	10.8	10.6	22.7	-1.1	-2.4
14:1	1.0	1.1	1.3	0.8	0.5	2.3	-0.7	0.2
15:0	1.4	1.8	1.7	1.6	1.6	2.8	0.4	-0.2
16:0	24.0	26.5	26.2	20.3	37.2	32.8	7.8	2.0
16:1	1.7	1.2	1.9	1.5	1.3	2.4	0.6	1.4
17:0	0.6	0.2	0.5	0.3	0.6	0.3	0.3	0.9
18:0	7.4	5.8	6.5	3.8	15.9	3.7	3.9	2.6
18:1	13.5	13.2	13.5	10.4	20.8	13.4	7.4	6.3
18:2	0.6	0.5	0.7	0.5	0.3	0.9	0.1	0.6
18:3	0.5	0.3	0.5	-	-	0.4	-0.4	1.1
	100.1	100.0	100.0	99.9	100.1	100.1	99.7	100.1

[a] position relative to sn - glycerol 3- phosphate

[b] 2x(2,3-PL's) - (2-MG's)

[c] 3x(TG's) - (1-PL's) - (2-MG's)

sample, compared to the C fractions of the January and March samples (Section 3.2.2.) were reflected in the differences in the proportions of FA's which occupied positions 1, 2 and 3 of their constituent TG's (Tables 22, 23 and 24). However the effect was not constant throughout all three positions in that the differences in the proportions of 4:0 affected only position 3, the differences in 16:0 affected positions 1 and 2 and the differences in 18:1 affected only position 1.

Despite these differences the overall pattern of distribution of FA's in positions 1, 2 and 3 was similar for each fraction C (Tables 22, 23 and 24). The short-chained FA's, 4:0 and 6:0, were esterified almost entirely at position 3. In each fraction C 4:0 comprised about 65% and 6:0 about 15% of the acyl groups at position 3. In contrast the medium-chain FA's, 10:0 and 12:0, were esterified almost exclusively at position 2 with each FA comprising 7.5-10.0% of the component FA's of this position. The long-chain FA's, 14:0, 16:0, 18:0 and 18:1, were preferentially esterified at carbons 1 and 2 of the glycerol molecules. Approximately twice as much 14:0 was esterified at position 2 as at position 1 with negligible amounts esterified at position 3. Proportions of 14:0 at positions 1, 2 and 3 were (8.7-10.6%), (22.7-24.6%) and (-2.4 - 0.3%) respectively. 16:0 was preferentially esterified at position 1 (31.2-38.8%) and at position 2 (28.4-33.5%) while only a relatively minor proportion was esterified at position 3 (0.9-8.7%). 18:0 was preferentially esterified at carbon 1 (15.9-21.7%) with only small amounts

at carbon 2 (3.4-3.9%) and at carbon 3 (-1.0 - 3.9%). 18:1 was also concentrated at position 1 although appreciable amounts were esterified at positions 2 and 3. Proportions of 18:1 at positions 1, 2 and 3 were, (20.6-28.1%), (11.0-14.1%) and (5.5-8.2%) respectively.

It is clear from the above description that in each TG fraction of low mol. wt. the component FA's were arranged in a highly selective manner within the constituent TG molecules. 4:0 and 6:0 comprised 80-90% of the FA's esterified at position 3, 14:0 and 16:0 accounted for about 55% of the acyl groups at position 2, and 16:0, 18:0 and 18:1 constituted 75-80% of FA's at position 1.

(e) The arrangement of fatty acids in positions 1, 2 and 3 of the constituent triacylglycerols of each sample of milk fat.

The FA compositions at positions 1, 2 and 3 of the TG's of the three total milk fats were reconstructed from the proportions of FA's at positions 1, 2 and 3 of their respective TG fractions of high, medium and low mol. wt. In each case the FA composition of position 3 was reconstructed from data determined by the following calculation:

$$\text{position 3} = 2 \times (\text{2,3-PL's}) - (\text{2-MG's}).$$

The detailed results are shown in Tables 25, 26 and 27 and the arrangement of the more abundant FA's in positions 1, 2 and 3 of the September sample of milk fat

Table 25. Stereospecific analysis of the triacylglycerols of the sample of September milk fat. Data reconstructed from analyses carried out on the high, medium, and low molecular weight fractions.

	Fatty acid composition (mole %)				
				Milk fat	
FA	1[a]	2[a]	3[a]	Rec.	Orig.
4:0	-	-	32.4	10.8	12.0
6:0	1.3	-	13.1	4.8	4.5
8:0	0.8	1.9	3.5	2.1	2.3
10:0	1.4	7.2	3.2	3.9	4.2
10:1	0.1	0.4	0.1	0.2	0.3
12:0	2.1	7.2	2.2	3.8	4.0
14:0	8.1	21.9	3.3	11.1	10.9
14:1	0.5	1.1	0.4	0.7	0.8
15:0	1.8	1.9	1.0	1.6	1.4
16:0	29.4	31.5	8.3	23.1	22.1
16:1	0.9	1.7	1.0	1.2	1.4
17:0	0.5	0.4	0.6	0.5	0.6
18:0	22.9	6.6	11.3	13.6	13.1
18:1	30.0	17.3	18.3	21.9	21.5
18:2	0.4	0.8	1.0	0.7	0.7
18:3	-	0.2	0.5	0.2	0.3
20:2	-	-	-	-	0.2
	100.2	100.1	100.2	100.2	100.3

[a]position relative to sn - glycerol 3- phosphate

Table 26. Stereospecific analysis of the triacylglycerols of the sample of January milk fat. Data reconstructed from analyses carried out on the high, medium, and low molecular weight fractions.

	Fatty acid composition (mole %)				
				Milk fat	
FA	1[a]	2[a]	3[a]	Rec.	Orig.
4:0	0.7	-	28.2	9.6	9.7
6:0	1.8	1.1	10.7	4.5	4.5
8:0	0.9	1.5	3.1	1.8	2.2
10:0	1.8	5.5	4.2	3.8	4.2
10:1	0.1	0.4	0.1	0.2	0.4
12:0	3.2	7.2	2.5	4.3	4.1
14:0	8.3	22.4	5.6	12.1	11.5
14:1	0.7	1.5	0.8	1.0	1.2
15:0	1.3	2.1	1.2	1.5	1.7
16:0	38.2	38.7	11.5	29.5	27.6
16:1	1.6	1.7	1.4	1.5	2.1
17:0	1.1	0.5	1.0	0.9	0.8
18:0	18.5	4.8	9.8	11.0	10.1
18:1	21.3	11.1	20.0	17.4	17.8
18:2	0.5	1.0	0.4	0.6	1.4
18:3	-	0.5	-0.5	0.0	0.8
	100.0	100.0	100.0	99.7	100.1

[a]position relative to sn - glycerol 3- phosphate

Table 27. Stereospecific analysis of the triacylglycerols of the sample of March milk fat. Data reconstructed from analyses carried out on the high, medium, and low molecular weight fractions.

	Fatty acid composition (mole %)				
				Milk fat	
FA	1[a]	2[a]	3[a]	Rec.	Orig.
4:0	1.5	-	28.4	10.0	10.0
6:0	1.8	-	11.8	4.6	4.6
8:0	0.8	1.2	2.9	1.6	2.3
10:0	2.3	5.6	3.1	3.7	4.2
10:1	0.2	0.4	0.0	0.2	0.3
12:0	2.5	7.0	1.4	3.6	3.9
14:0	9.8	22.0	4.4	12.1	10.9
14:1	0.7	1.8	0.4	0.9	1.1
15:0	1.5	2.4	1.0	1.6	1.6
16:0	37.2	36.6	11.3	28.4	26.4
16:1	1.2	2.3	0.7	1.4	1.9
17:0	0.8	0.4	1.2	0.8	0.9
18:0	17.6	5.3	13.4	12.1	11.5
18:1	21.9	13.3	20.2	18.5	18.4
18:2	0.4	1.2	0.4	0.7	1.1
18:3	-	0.8	-0.8	0.0	0.8
	100.2	100.3	99.8	100.2	99.9

[a]position relative to sn - glycerol 3-phosphate

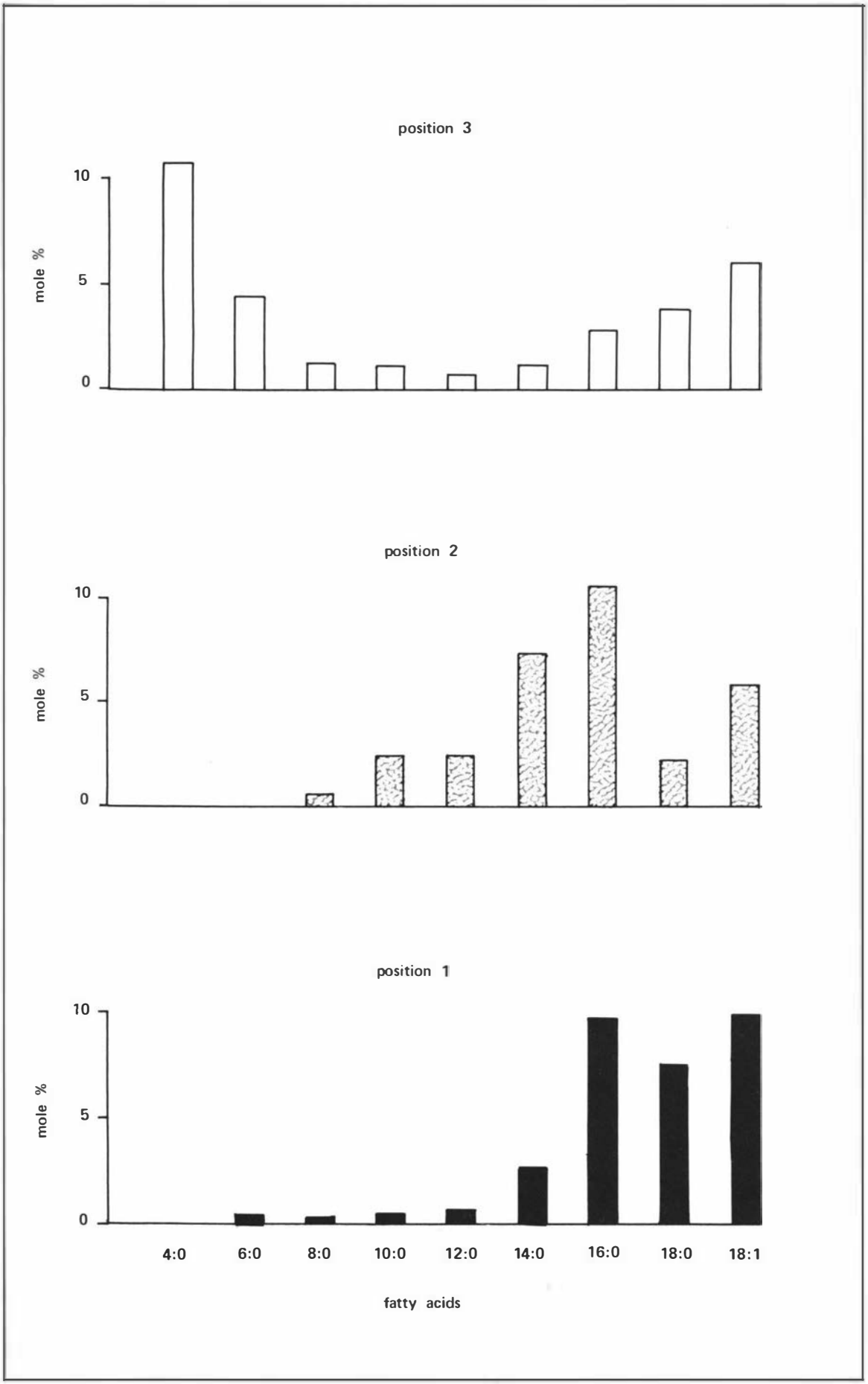


Figure 9. The arrangement of fatty acids within the triacylglycerols of the September sample of milk fat.

is presented in Figure 9.

The data obtained show that of the short-chain FA's, 4:0 and 6:0 were esterified almost exclusively at position 3 while 8:0 was preferentially esterified at position 3. On the other hand, the medium-chain FA's 10:0 and 12:0 were preferentially esterified at position 2 with, in general, more than 50% of each FA at this position. In contrast to the short- and medium-chain FA's, the major long-chain FA's, 14:0, 16:0, 18:0 and 18:1, were not as a class preferentially esterified at a specific position. More than twice as much 14:0 was esterified at carbon 2 as at carbon 1 with only relatively small amounts at carbon 3. About 10% of 16:0 in the milk fat samples was esterified at position 3 while the remainder was esterified in equal proportions at positions 1 and 2. Proportions of 18:0 were greatest at carbon 1 and least at carbon 2. In the March and January samples about 20-25% of 18:1 in the milk fat samples was esterified at position 2 while the remainder was esterified in equal proportions at positions 1 and 3. However, in the September sample of milk fat 18:1 was preferentially esterified at position 1 with lesser and comparable proportions esterified at positions 2 and 3.

As a result of this arrangement of FA's within the constituent TG's of the three samples of milk fat 16:0, 18:0, and 18:1 comprised about 80% of the FA's esterified at position 1, 14:0 and 16:0 accounted for 50-60% of the acyl groups at position 2, and 4:0 and 18:1 accounted for about 50% of the FA's esterified at position 3.

It should be emphasised that the pattern of distribution of FA's described above was the overall arrangement of FA's in positions 1, 2 and 3 of the TG's of the samples of milk fat. As discussed above certain FA's (e.g. 18:1) exhibited different arrangements in different TG fractions.

Section 3.3. Composition of the triacylglycerol classes of differing degrees of unsaturation prepared from the triacylglycerol fractions of high, medium and low molecular weight.

3.3.1. Fatty acid compositions of the triacylglycerol classes of high molecular weight.

The FA compositions of TG classes of differing levels of unsaturation, which were prepared from each of the three TG fractions of high mol. wt. (fraction A) by silver ion-T.L.C., are shown in Tables 28, 29 and 30. The results presented in Table 28 are the means of duplicate determinations.

(a) The September sample of milk fat

In the saturated TG's of fraction A of the September sample 14:0 (17.3%), 16:0 (35.1%) and 18:0 (26.9%) comprised 79.3% of the FA's present (Table 28). *Trans*- and *cis*-monoene TG's had similar FA compositions and the respective proportions of their major FA's were 14:0 (8.5% and 10.2%), 16:0 (25.5% and 25.8%), 18:0 (20.9% and 18.0%) and 18:1 (29.9% - elaidic acid and 32.8% - oleic acid). In the diene TG's 18:1 (56.6%) was the principal FA constituent while 14:0 (6.8%), 16:0 (15.8%) and 18:0 (9.3%) were present in appreciable amounts but 18:2 (1.7%) was quantitatively unimportant. 18:1 (49.9%) was the principal FA in the triene TG's while 16:0 (12.1%), 18:0 (8.8%), 18:2 (10.4%) and 18:3 (7.0%) were all present in

Table 28. Fatty acid compositions of the triacylglycerol classes of differing degrees of unsaturation obtained from the high molecular weight fraction of the sample of September milk fat by silver ion - T.L.C.

FA	Fatty acid composition (mole %)											
	Saturated TG's		Trans-monoene TG's		Cis-monoene TG's		Diene TG's		Triene TG's		Fraction	
	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	Rec.	Orig.
6:0	1.7	0.5	1.1	0.2	0.6	0.2	0.2	-	-	-	0.9	0.7
8:0	2.6	0.8	1.6	0.2	1.3	0.4	0.7	0.1	0.2	-	1.5	1.7
10:0	7.9	2.2	5.0	0.6	3.8	1.1	2.3	0.5	0.8	0.1	4.5	4.1
10:1	-	-	-	-	-	-	0.3	0.1	0.3	-	0.1	-
12:0	6.7	1.9	4.3	0.5	3.7	1.1	2.2	0.5	1.5	0.2	4.2	4.1
14:0	17.3	4.9	8.5	0.9	10.2	2.9	6.8	1.5	4.7	0.5	10.7	11.5
14:1	-	-	-	-	1.0	0.3	1.0	0.2	1.1	0.1	0.6	0.9
15:0	1.9	0.5	1.4	0.2	1.4	0.4	0.6	0.1	0.6	0.1	1.3	1.6
16:0	35.1	10.0	25.5	2.8	25.8	7.3	15.8	3.4	12.1	1.3	24.8	23.2
16:1	-	-	0.7	0.1	1.5	0.4	2.5	0.5	2.6	0.3	1.3	1.7
17:0	-	-	-	-	-	-	-	-	-	-	-	0.9
18:0	26.9	7.7	20.9	2.3	18.0	5.1	9.3	2.0	8.8	0.9	18.0	17.5
18:1	-	-	29.9	3.3	32.8	9.2	56.6	12.2	49.9	5.2	29.9	28.8
UNK[c]	-	-	1.0	0.1	-	-	-	-	-	-	0.1	-
18:2	-	-	-	-	-	-	1.7	0.4	10.4	1.1	1.5	2.0
18:3	-	-	-	-	-	-	-	-	7.0	0.7	0.7	1.0
20:2	-	-	-	-	-	-	-	-	-	-	-	0.4
	100.1	28.5	99.9	11.2	100.1	28.4	100.0	21.5	100.0	10.5	100.1	100.1

[a] percentage in triacylglycerol class

[b] percentage in fraction

[c] identity not determined

Table 29. Fatty acid compositions of the triacylglycerol classes of differing degrees of unsaturation obtained from the high molecular weight fraction of the sample of January milk fat by silver ion - T.L.C.

FA	Fatty acid composition (mole %)											
	Saturated TG's		Trans-monoene TG's		Cis-monoene TG's		Diene TG's		Triene TG's		Fraction	
	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	Rec.	Orig.
8:0	1.3	0.4	0.6	0.1	0.6	0.2	-	-	-	-	0.7	0.6
10:0	6.1	2.1	3.3	0.3	2.6	0.8	1.3	0.2	0.4	0.0	3.4	3.7
12:0	6.7	2.3	4.1	0.4	3.8	1.2	2.2	0.4	1.0	0.1	4.4	4.3
14:0	20.3	6.9	12.6	1.3	12.0	3.8	7.6	1.3	5.4	0.4	13.7	13.0
14:1	-	-	1.0	0.1	1.7	0.5	2.2	0.4	1.7	0.1	1.1	1.3
15:0	2.6	0.9	1.2	0.1	1.1	0.4	0.9	0.2	0.8	0.1	1.7	1.9
16:0	42.5	14.4	32.0	3.2	32.1	10.0	18.7	3.3	17.0	1.3	32.2	30.8
16:1	-	-	1.4	0.1	3.2	1.0	3.3	0.6	3.0	0.2	1.9	2.4
17:0	-	-	-	-	-	-	-	-	-	-	-	1.6
18:0	20.5	6.9	15.1	1.5	13.5	4.2	8.1	1.4	8.9	0.7	14.7	14.5
18:1	-	-	26.7	2.7	29.4	9.2	52.9	9.2	41.1	3.1	24.2	23.5
UNK[c]	-	-	2.0	0.2	-	-	-	-	-	-	0.2	-
18:2	-	-	-	-	-	-	2.5	0.4	14.4	1.1	1.5	1.6
18:3	-	-	-	-	-	-	-	-	6.6	0.5	0.5	0.9
20:2	-	-	-	-	-	-	0.4	0.1	-	-	0.1	-
	100.0	33.9	100.0	10.0	100.0	31.3	100.1	17.5	100.3	7.6	100.3	100.1

[a] percentage in triacylglycerol class

[b] percentage in fraction

[c] identity not determined

Table 30. Fatty acid compositions of the triacylglycerol classes of differing degrees of unsaturation obtained from the high molecular weight fraction of the sample of March milk fat by silver ion - T.L.C.

FA	Fatty acid composition (mole %)											
	Saturated TG's		Trans-monoene TG's		Cis-monoene TG's		Diene TG's		Triene TG's		Fraction	
	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	Rec.	Orig.
6:0	0.9	0.3	-	-	-	-	-	-	-	-	0.3	0.2
8:0	2.2	0.7	0.9	0.1	1.0	0.3	-	-	-	-	1.1	0.9
10:0	6.7	2.1	2.9	0.2	3.4	1.1	1.4	0.2	0.6	0.1	3.7	3.5
12:0	6.8	2.2	3.9	0.3	3.8	1.2	2.0	0.4	1.4	0.1	4.2	3.8
14:0	17.9	5.7	14.4	1.2	12.7	4.2	7.0	1.2	5.2	0.5	12.8	12.4
14:1	-	-	1.1	0.1	2.3	0.7	2.4	0.4	1.6	0.2	1.4	1.4
15:0	2.6	0.8	1.3	0.1	1.6	0.5	1.0	0.2	0.7	0.1	1.7	1.9
16:0	41.7	13.2	29.0	2.4	29.3	9.6	18.2	3.2	16.6	1.6	30.0	29.2
16:1	-	-	1.1	0.1	2.0	0.7	3.2	0.6	2.8	0.3	1.7	2.0
17:0	-	-	-	-	-	-	-	-	-	-	-	1.1
18:0	21.2	6.7	17.3	1.4	13.9	4.6	8.5	1.5	8.9	0.9	15.1	15.6
18:1	-	-	26.0	2.1	30.1	9.9	48.3	8.4	38.8	3.8	24.2	24.9
UNK[c]	-	-	2.3	0.2	-	-	-	-	-	-	0.2	-
18:2	-	-	-	-	-	-	7.8	1.4	11.8	1.2	2.6	2.1
18:3	-	-	-	-	-	-	-	-	11.6	1.1	1.1	0.9
	100.0	31.7	100.2	8.2	100.1	32.8	99.8	17.5	100.0	9.9	100.1	99.9

[a] percentage in triacylglycerol class

[b] percentage in fraction

[c] identity not determined

significant amounts.

(b) The January sample of milk fat

Table 29 shows that in fraction A of the January sample the following FA's comprised 83.3% of the FA's present in the saturated TG's: 14:0 (20.3%), 16:0 (42.5%) and 18:0 (20.5%). Trans- and cis-monoene TG's had similar FA compositions and the respective proportions of their more abundant FA components were 14:0 (12.6% and 12.0%), 16:0 (32.0% and 32.1%), 18:0 (15.1% and 13.5%) and 18:1 (26.7% - elaidic acid and 29.4% - oleic acid). In the diene TG's 18:1 (52.9%) was the principal FA constituent, 14:0 (7.6%), 16:0 (18.7%), and 18:0 (8.1%) were present in appreciable amounts but 18:2 (2.5%) was only a minor component. In the triene TG's 18:1 (41.1%) was the principal FA while 16:0 (17.0%) and 18:2 (14.4%) were of considerable quantitative importance.

(c) The March sample of milk fat

The saturated TG's of fraction A of the March sample contained 14:0 (17.9%), 16:0 (41.7%) and 18:0 (21.2%) which accounted for 80.8% of the constituent FA's (Table 30). 14:0 (14.4% and 12.7%), 16:0 (29.0% and 29.3%), 18:0 (17.3% and 13.9%) and 18:1 (26.0% - elaidic acid and 30.1% - oleic acid) were the major FA's in the trans- and cis-monoene TG's respectively. 18:1 (48.3%) was the principal FA in the diene TG's and 14:0 (7.0%), 16:0 (18.2%), 18:0 (8.5%) and 18:2 (7.8%) were present in considerable amounts. In the triene TG's 18:1 (38.8%) was the principal FA and 16:0 (16.6%), 18:2 (11.8%) and

18:3 (11.6%) were of considerable quantitative importance.

(d) Comparison of the September, January and March samples of milk fat

It is evident from the above description of Tables 28, 29 and 30 that the same major FA's were found in corresponding TG classes of the TG fractions of high mol. wt. In the saturated TG's 14:0, 16:0 and 18:0 made large contributions to the total FA's, in the trans- and cis-monoene TG's the major constituent FA's were 14:0, 16:0, 18:0 and 18:1, while in the diene TG's and triene TG's 18:1 was the principal FA.

Table 31. Ratios of the saturated fatty acids present in the triacylglycerol classes prepared from the high molecular weight triacylglycerol fraction of the September milk fat sample.

Triacylglycerol class	Fatty acids expressed as a proportion of the total saturated fatty acids		
	14:0	16:0	18:0
Saturated TG's	0.17	0.35	0.27
Trans-monoene TG's	0.12	0.37	0.30
Cis-monoene TG's	0.16	0.40	0.28
Diene TG's	0.18	0.42	0.25
Triene TG's	0.16	0.42	0.31

When 14:0, 16:0 and 18:0 are expressed as a proportion of the total saturated FA's present in the respective TG classes it was found that for each of these FA's the values were relatively constant throughout the TG classes. The relevant values are given for fraction A of the September sample in Table 31 and similar trends were observed for the A fractions of the January and March samples.

3.3.2. Fatty acid compositions of the triacylglycerol classes of medium molecular weight.

The FA compositions of TG classes of differing levels of unsaturation, which were prepared from each of the three TG fractions of medium mol. wt. (fraction B) by silver ion-T.L.C., are given in Tables 32, 33 and 34.

(a) The September sample of milk fat

In the saturated TG's of fraction B of the September sample 6:0 (12.6%), 14:0 (12.7%), 16:0 (30.0%) and 18:0 (18.8%) comprised 74.1% of the FA's present (Table 32). Trans- and cis-monoene TG's had similar FA compositions and the respective proportions of their major component FA's were 6:0 (12.5% and 11.3%), 16:0 (15.9% and 15.4%), 18:0 (10.7% and 9.3%), and 18:1 (27.9% - elaidic acid and 32.6% - oleic acid). In the diene TG's 18:1 (41.8%) was the principal FA while none of the remaining 12 FA's exceeded 11.7%. The more abundant constituent FA's of

Table 32. Fatty acid compositions of the triacylglycerol classes of differing degrees of unsaturation obtained from the medium molecular weight fraction of the sample of September milk fat by silver ion - T.L.C.

FA	Fatty acid composition (mole %)											
	Saturated TG's		Trans-monoene TG's		Cis-monoene TG's		Diene TG's		Triene TG's		Fraction	
	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	Rec.	Orig.
4:0	8.5	3.8	5.4	0.5	4.6	1.2	1.2	0.1	-	-	5.6	6.1
6:0	12.6	5.7	12.5	1.3	11.3	3.1	11.7	1.2	1.6	0.1	11.4	11.2
8:0	4.8	2.2	5.3	0.5	4.8	1.3	5.1	0.5	2.5	0.2	4.7	4.9
10:0	6.7	3.1	6.9	0.7	6.5	1.8	6.3	0.6	6.4	0.4	6.6	6.2
10:1	-	-	-	-	0.4	0.1	3.3	0.3	1.9	0.1	0.5	0.6
12:0	4.9	2.2	4.4	0.5	3.5	1.0	3.4	0.3	4.6	0.3	4.3	3.9
14:0	12.7	5.8	8.7	0.9	8.1	2.2	5.8	0.6	9.1	0.6	10.1	10.2
14:1	-	-	-	-	1.1	0.3	2.1	0.2	2.3	0.2	0.7	0.8
15:0	1.0	0.5	0.3	0.0	0.7	0.2	-	-	0.5	-	0.7	1.2
16:0	30.0	13.6	15.9	1.6	15.4	4.2	8.9	0.9	14.4	1.0	21.3	21.7
16:1	-	-	0.7	0.1	1.6	0.4	3.0	0.3	2.6	0.2	1.0	1.1
17:0	-	-	-	-	-	-	-	-	-	-	-	0.7
18:0	18.8	8.5	10.7	1.1	9.3	2.5	3.3	0.3	6.2	0.4	12.8	13.3
18:1	-	-	27.9	2.8	32.6	8.9	41.8	4.3	25.7	1.8	17.8	16.6
UNK[c]	-	-	1.3	0.1	-	-	-	-	-	-	0.1	-
18:2	-	-	-	-	-	-	4.1	0.4	7.1	0.5	0.9	0.8
18:3	-	-	-	-	-	-	-	-	15.2	1.0	1.0	0.6
20:2	-	-	-	-	-	-	-	-	-	-	-	0.3
	100.0	45.4	100.0	10.1	99.9	27.2	100.0	10.0	100.1	6.8	99.5	100.2

[a] percentage in triacylglycerol class

[b] percentage in fraction

[c] identity not determined

Table 33. Fatty acid compositions of the triacylglycerol classes of differing degrees of unsaturation obtained from the medium molecular weight fraction of the sample of January milk fat by silver ion - T.L.C.

FA	Fatty acid composition (mole %)											
	Saturated TG's		Trans-monoene TG's		Cis-monoene TG's		Diene TG's		Triene TG's		Fraction	
	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	Orig.	Rec.
4:0	1.0	0.5	-	-	-	-	-	-	-	-	0.5	0.5
6:0	11.7	5.9	10.0	0.7	9.0	2.1	5.0	0.6	-	-	9.6	9.3
8:0	6.9	3.5	7.0	0.5	6.9	1.6	5.1	0.6	1.0	0.1	6.4	6.3
10:0	9.2	4.6	9.3	0.7	9.2	2.1	7.1	0.8	4.4	0.4	8.7	8.6
10:1	-	-	-	-	0.2	0.1	5.3	0.6	2.1	0.2	0.9	0.9
12:0	5.8	2.9	4.8	0.3	5.2	1.2	4.1	0.5	4.9	0.4	5.2	5.3
14:0	14.2	7.2	10.6	0.7	10.1	2.3	7.9	0.9	11.4	0.9	11.7	12.0
14:1	-	-	-	-	2.7	0.6	4.0	0.5	2.4	0.2	1.4	1.3
15:0	1.6	0.8	0.8	0.1	1.2	0.3	0.9	0.1	1.2	0.1	1.2	1.4
16:0	37.1	18.7	21.3	1.5	20.9	4.9	17.0	1.9	22.1	1.8	27.8	28.8
16:1	-	-	1.1	0.1	2.2	0.5	3.2	0.4	2.3	0.2	1.8	1.2
17:0	-	-	-	-	-	-	-	-	-	-	1.0	-
18:0	12.5	6.3	8.0	0.6	4.7	1.1	3.4	0.4	3.9	0.3	8.8	8.7
18:1	-	-	25.0	1.8	27.7	6.4	29.9	3.2	22.6	1.8	13.4	13.2
UNK[c]	-	-	2.3	0.2	-	-	-	-	-	-	-	0.2
18:2	-	-	-	-	-	-	7.4	0.8	7.1	0.6	1.1	1.4
18:3	-	-	-	-	-	-	-	-	14.6	1.2	0.7	1.2
	100.0	50.4	100.2	7.2	100.0	23.2	100.3	11.3	100.0	8.2	100.2	100.3

[a] percentage in triacylglycerol class

[b] percentage in fraction

[c] identity not determined

Table 34. Fatty acid compositions of the triacylglycerol classes of differing degrees of unsaturation obtained from the medium molecular weight fraction of the sample of March milk fat by silver ion - T.L.C.

FA	Fatty acid composition (mole %)											
	Saturated TG's		Trans-monoene TG's		Cis-monoene TG's		Diene TG's		Triene TG's		Fraction	
	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	Rec.	Orig.
4:0	2.8	1.4	-	-	-	-	-	-	-	-	1.4	2.0
6:0	14.2	7.0	12.2	0.8	11.5	3.1	6.1	0.6	-	-	11.5	11.7
8:0	5.9	2.9	5.9	0.4	5.8	1.5	4.0	0.4	1.3	0.1	5.3	5.0
10:0	7.8	3.8	7.2	0.4	7.1	1.9	5.7	0.6	5.1	0.4	7.1	7.7
10:1	-	-	-	-	0.4	0.1	4.5	0.5	2.1	0.2	0.8	0.8
12:0	4.8	2.4	4.2	0.3	4.5	1.2	3.9	0.4	4.7	0.4	4.7	4.6
14:0	13.8	6.8	10.4	0.6	9.3	2.5	7.9	0.8	11.1	0.9	11.6	11.6
14:1	-	-	-	-	2.7	0.7	3.8	0.4	2.7	0.2	1.3	1.4
15:0	1.7	0.8	0.8	0.1	1.5	0.4	1.1	0.1	1.6	0.1	1.5	1.0
16:0	35.5	17.4	22.3	1.4	19.6	5.3	14.4	1.4	20.2	1.6	27.1	26.2
16:1	-	-	1.7	0.1	2.0	0.5	2.7	0.3	2.4	0.2	1.1	1.0
17:0	-	-	-	-	-	-	-	-	-	-	-	0.3
18:0	13.5	6.6	8.6	0.5	6.5	1.7	3.1	0.3	3.7	0.3	9.4	9.8
18:1	-	-	25.0	1.5	29.2	7.8	36.7	3.7	23.6	1.9	14.9	15.1
UNK[c]	-	-	1.7	0.1	-	-	-	-	-	-	0.1	-
18:2	-	-	-	-	-	-	6.1	0.6	4.9	0.4	1.0	1.2
18:3	-	-	-	-	-	-	-	-	16.7	1.3	1.3	0.8
	100.0	49.1	100.0	6.2	100.1	26.7	100.0	10.1	100.1	8.0	100.1	100.2

[a] percentage in triacylglycerol class

[b] percentage in fraction

[c] identity not determined

the triene TG's were 16:0 (14.4%), 18:1 (25.7%) and 18:3 (15.2%).

(b) The January sample of milk fat

In the saturated TG's of fraction B of the January sample 6:0 (11.7%), 14:0 (14.2%), 16:0 (37.1%) and 18:0 (12.5%) accounted for 75.5% of the FA's present (Table 33). As in the September sample the FA compositions of trans- and cis-monoene TG's resembled each other closely and their respective major FA's were 6:0 (10.0% and 9.0%), 10:0 (9.3% and 9.2%), 14:0 (10.6% and 10.1%), 16:0 (21.3% and 20.9%) and 18:1 (25.0% - elaidic acid and 27.7% - oleic acid). In the diene TG's the more abundant constituent FA's were 16:0 (17.0%) and 18:1 (29.9%) while none of the remaining 11 FA's exceeded 7.9% of the total. In the triene TG's, 14:0 (11.4%), 16:0 (22.1%), 18:1 (22.6%) and 18:3 (14.6%) comprised 70.7% of the total FA's.

(c) The March sample of milk fat

Table 34 shows that the following FA's comprised 77.0% of FA's present in the saturated TG's obtained from fraction B of the March sample: 6:0 (14.2%), 14:0 (13.8%), 16:0 (35.5%) and 18:0 (13.5%). Trans- and cis-monoene TG's had similar FA compositions and the respective proportions of their major FA constituents were 6:0 (12.2% and 11.5%), 14:0 (10.4% and 9.3%), 16:0 (22.3% and 19.6%) and 18:1 (25.0% - elaidic acid and 29.2% - oleic acid). In the diene TG's the major FA's were 16:0 (14.4%) and 18:1 (36.7%) while the remaining 11 constituent FA's, varied between 1.1 and 7.9%. The

more abundant FA components in the triene TG's were 14:0 (11.1%), 16:0 (20.2%), 18:1 (23.6%) and 18:3 (16.7%) which together accounted for 71.6% of FA's present.

(d) Comparison of the September, January and March samples of milk fat

From the above description of Tables 32, 33 and 34, it can be seen that, with few exceptions, corresponding TG classes of the TG fractions of medium mol. wt. contained the same major FA's. In the saturated TG's the major FA components were 6:0, 14:0, 16:0 and 18:0 in the trans- and cis-monoene TG's 6:0, 14:0, 16:0, and 18:1 were of considerable quantitative importance, in the diene TG's the more abundant FA's were 16:0 and 18:1, and in the triene TG's the major constituent FA's were 14:0, 16:0, 18:1 and 18:3.

When comparing the contributions of saturated FA's to each TG class it is observed that, for each fraction B, values of 10:0 and 12:0 (moles %) remained approximately constant and that values of 16:0 and 18:0 (moles %) decreased as the proportion of saturated FA's in TG classes decreased.

3.3.3. Fatty acid compositions of the triacylglycerol classes of low molecular weight.

The FA compositions of TG classes of differing levels of unsaturation, which were prepared from each of the three TG fractions of low mol. wt. (fraction C) by silver

Table 35. Fatty acid compositions of the triacylglycerol classes of differing degrees of unsaturation obtained from the low molecular weight fraction of the sample of September milk fat by silver ion - T.L.C.

FA	Fatty acid composition (mole %)											
	Saturated TG's		Trans-monoene TG's		Cis-monoene TG's		Diene TG's		Triene TG's		Fraction	
	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	Rec.	Orig.
4:0	25.1	11.7	24.1	2.2	24.6	6.9	24.3	2.4	23.0	1.4	24.6	24.9
6:0	6.0	2.8	6.2	0.6	5.1	1.4	5.3	0.5	7.7	0.5	5.8	6.1
8:0	2.4	1.1	1.7	0.2	1.8	0.5	1.5	0.1	2.5	0.2	2.1	2.1
10:0	4.8	2.2	2.7	0.2	2.4	0.7	1.6	0.2	2.8	0.2	3.5	3.5
10:1	-	-	-	-	0.5	0.2	1.6	0.2	1.4	0.1	0.5	0.4
12:0	5.2	2.4	3.2	0.3	2.7	0.8	1.3	0.1	2.1	0.1	3.7	3.7
14:0	14.8	6.9	8.7	0.8	7.8	2.2	3.3	0.3	4.9	0.3	10.5	10.5
14:1	-	-	-	-	1.1	0.3	1.7	0.1	0.6	-	0.4	0.6
15:0	1.5	0.7	0.8	0.1	0.9	0.3	0.2	-	-	-	1.1	1.1
16:0	28.0	13.0	15.0	1.4	15.3	4.3	5.7	0.6	8.9	0.6	19.9	20.2
16:1	-	-	1.6	0.2	2.2	0.6	3.6	0.4	1.5	0.1	1.3	1.2
17:0	-	-	-	-	-	-	-	-	-	-	-	0.3
18:0	12.2	5.7	6.2	0.6	5.0	1.4	2.2	0.2	3.6	0.2	8.1	8.2
18:1	-	-	28.1	2.6	30.7	8.6	40.1	4.0	16.1	1.0	16.2	15.9
UNK[c]	-	-	1.7	0.2	-	-	-	-	-	-	0.2	-
18:2	-	-	-	-	-	-	7.7	0.8	8.1	0.5	1.3	1.1
18:3	-	-	-	-	-	-	-	-	17.0	1.1	1.1	0.5
	100.0	46.5	100.0	9.4	100.1	28.2	100.1	9.9	100.2	6.3	100.3	100.3

[a] percentage in triacylglycerol class

[b] percentage in fraction

[c] identity not determined

Table 36. Fatty acid compositions of the triacylglycerol classes of differing degrees of unsaturation obtained from the low molecular weight fraction of the sample of January milk fat by silver ion - T.L.C.

FA	Fatty acid composition (mole %)											
	Saturated TG's		Trans-monoene TG's		Cis-monoene TG's		Diene TG's		Triene TG's		Fraction	
	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	Rec.	Orig.
4:0	24.4	13.3	21.2	1.6	24.9	6.3	20.7	1.7	11.6	0.5	23.4	23.1
6:0	6.6	3.6	6.9	0.5	7.3	1.8	7.7	0.7	6.4	0.3	6.9	7.0
8:0	1.7	0.9	1.4	0.1	1.5	0.4	1.5	0.1	2.2	0.1	1.6	1.8
10:0	3.6	1.9	2.2	0.2	2.0	0.5	1.7	0.2	2.9	0.1	2.9	3.0
10:1	-	-	-	-	0.3	0.1	2.8	0.2	1.2	0.1	0.4	0.5
12:0	4.6	2.5	2.8	0.2	2.7	0.7	1.5	0.1	2.1	0.1	3.6	3.4
14:0	14.8	8.0	9.2	0.7	7.8	2.0	4.4	0.4	6.4	0.3	11.4	10.4
14:1	-	-	0.2	0.0	1.7	0.4	2.3	0.2	0.8	0.0	0.6	0.8
15:0	1.8	1.0	1.3	0.1	0.8	0.2	0.4	0.0	0.5	0.0	1.3	1.3
16:0	33.4	18.1	18.9	1.4	17.1	4.3	9.4	0.8	18.6	0.9	25.5	24.4
16:1	-	-	1.6	0.1	2.6	0.7	4.6	0.4	2.2	0.1	1.3	1.9
17:0	-	-	-	-	-	-	-	-	-	-	-	0.9
18:0	9.2	5.0	4.9	0.4	3.6	0.9	2.8	0.2	4.1	0.2	6.7	6.9
18:1	-	-	27.4	2.1	27.8	7.0	31.2	2.6	16.0	0.7	12.4	12.7
UNK[c]	-	-	2.0	0.2	-	-	-	-	-	-	0.2	-
18:2	-	-	-	-	-	-	9.0	0.8	6.3	0.3	1.1	1.2
18:3	-	-	-	-	-	-	-	-	18.8	0.9	0.9	0.8
	100.1	54.3	100.0	7.6	100.1	25.3	100.0	8.4	100.1	4.6	100.2	100.1

[a] percentage in triacylglycerol class

[b] percentage in fraction

[c] identity not determined

Table 37. Fatty acid compositions of the triacylglycerol classes of differing degrees of unsaturation obtained from the low molecular weight fraction of the sample of March milk fat by silver ion - T.L.C.

FA	Fatty acid composition (mole %)											
	Saturated TG's		Trans-monoene TG's		Cis-monoene TG's		Diene TG's		Triene TG's		Fraction	
	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	% C[a]	% F[b]	Rec.	Orig.
4:0	23.5	12.5	22.3	1.3	22.8	6.0	18.7	1.7	15.1	0.8	22.3	23.5
6:0	6.4	3.4	6.3	0.4	5.7	1.5	7.0	0.6	8.0	0.5	6.4	6.7
8:0	1.9	1.0	1.6	0.1	1.4	0.4	1.5	0.1	2.5	0.1	1.7	2.0
10:0	3.4	1.8	2.0	0.1	2.0	0.5	1.8	0.2	3.2	0.2	2.8	2.9
10:1	-	-	-	-	0.3	0.1	2.5	0.2	1.2	0.1	0.4	0.4
12:0	4.5	2.4	2.5	0.1	2.5	0.7	1.4	0.1	2.3	0.1	3.4	3.6
14:0	14.7	7.9	8.5	0.5	8.1	2.1	4.5	0.4	5.6	0.3	11.2	10.3
14:1	-	-	0.3	-	2.1	0.6	2.3	0.2	1.3	0.1	0.9	1.0
15:0	1.9	1.0	1.4	0.1	1.1	0.3	0.3	-	0.4	-	1.4	1.4
16:0	33.6	18.0	20.1	1.1	17.4	4.6	9.8	0.9	13.5	0.8	25.4	24.0
16:1	-	-	1.6	0.1	2.8	0.8	4.3	0.4	2.3	0.1	1.4	1.7
17:0	-	-	-	-	-	-	-	-	-	-	-	0.6
18:0	10.1	5.4	6.1	0.4	4.2	1.1	3.8	0.3	3.4	0.2	7.4	7.4
18:1	-	-	26.2	1.5	29.6	7.8	33.4	3.0	12.3	0.7	13.0	13.5
UNK[c]	-	-	1.2	0.1	-	-	-	-	-	-	0.1	-
18:2	-	-	-	-	-	-	8.7	0.8	5.9	0.3	1.1	0.6
18:3	-	-	-	-	-	-	-	-	23.1	1.3	1.3	0.5
	100.0	53.4	100.1	5.8	100.0	26.5	100.0	8.9	100.1	5.6	100.2	100.1

[a] percentage in triacylglycerol class

[b] percentage in fraction

[c] identity not determined

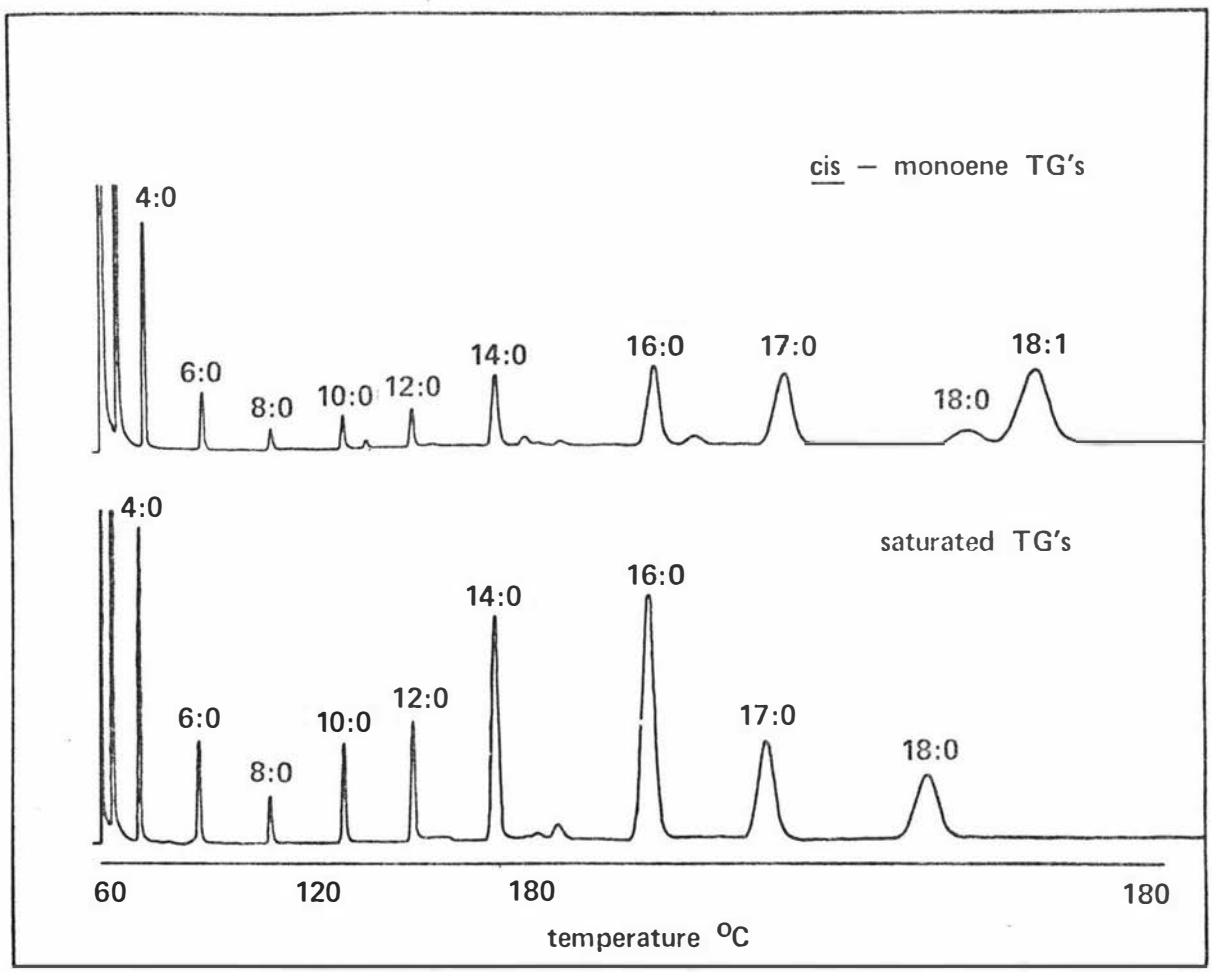


Figure 10. Gas-liquid chromatograms of the fatty acid methyl esters of the saturated and cis-monoene triacylglycerols obtained from the low molecular weight triacylglycerol fraction of the September milk fat sample.

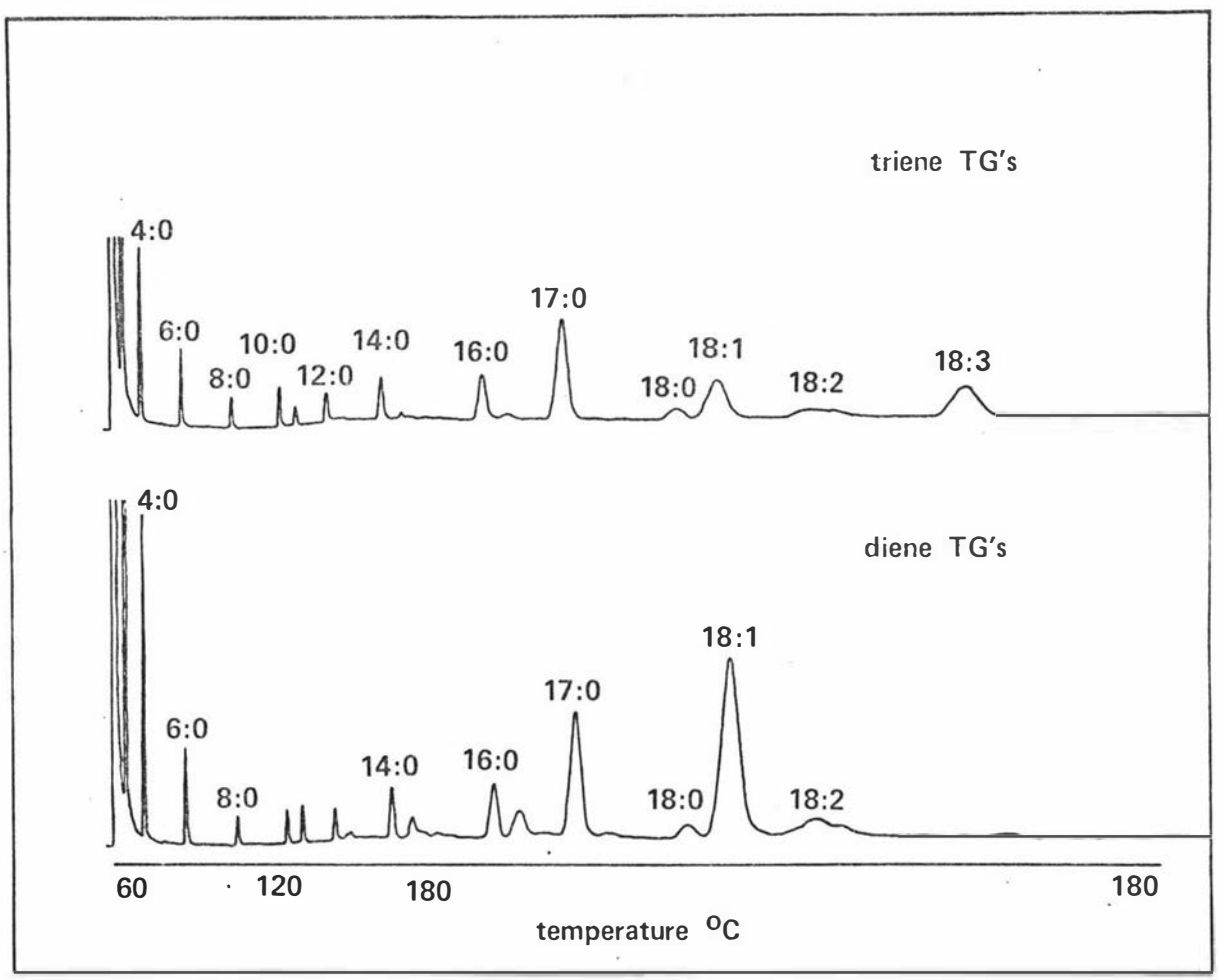


Figure 11. Gas-liquid chromatograms of the fatty acid methyl esters of the diene and triene triacylglycerols obtained from the low molecular weight triacylglycerol fraction of the September milk fat sample.

ion-T.L.C., are shown in Tables 35, 36 and 37. The results given in Table 35 are the means of duplicate determinations. Gas-liquid chromatograms of FA methyl esters of TG classes prepared from fraction C of the September sample are presented in Figures 10 and 11.

(a) The September sample of milk fat

In the saturated TG's of fraction C of the September sample 4:0 (25.1%), 14:0 (14.8%), 16:0 (28.0%) and 18:0 (12.2%) comprised 80.1% of the total FA's present (Table 35). 4:0 (24.1% and 24.6%), 16:0 (15.0% and 15.3%) and 18:1 (28.1% - elaidic acid and 30.7% - oleic acid) were the major FA's in the trans- and cis-monoene TG's respectively. In the diene TG's the major FA constituents were 4:0 (24.3%) and 18:1 (40.1%) while the more abundant FA's in the triene TG's were 4:0 (23.0%), 18:1 (16.1%) and 18:3 (17.0%).

(b) The January sample of milk fat

Table 36 shows that the following FA's comprised 72.6% of the FA's present in the saturated TG's obtained from fraction C of the January sample: 4:0 (24.4%), 14:0 (14.8%) and 16:0 (33.4%). Again 4:0 (21.2% and 24.9%), 16:0 (18.9% and 17.1%) and 18:1 (27.4% - elaidic acid and 27.8% - oleic acid) were the more abundant FA's in the trans- and cis-monoene TG's. The major constituent FA's in the diene TG's were 4:0 (20.7%) and 18:1 (31.2%) while in the triene TG's the quantitatively more important FA's were 4:0 (11.6%), 16:0 (18.6%), 18:1 (16.0%) and 18:3 (18.8%).

(c) The March sample of milk fat

In the saturated TG's of fraction C of the March sample 4:0 (23.5%), 14:0 (14.7%), 16:0 (33.6%) and 18:0 (10.1%) accounted for 81.9% of the FA's present (Table 37). The FA compositions of trans- and cis-monoene TG's were similar and the respective proportions of the major FA components were 4:0 (22.3% and 22.8%), 16:0 (20.1% and 17.4%) and 18:1 (26.2% - elaidic acid and 29.6% - oleic acid). In the diene TG's the major constituent FA's were 4:0 (18.7%) and 18:1 (33.4%) while in the triene TG's 4:0 (15.1%), 16:0 (13.5%), 18:1 (12.3%) and 18:3 (23.1%) were of considerable quantitative importance.

(d) Comparison of the September, January and March samples of milk fat

It is clear from the above description of the results that the same major FA constituents were present in corresponding TG classes of the TG fractions of low mol. wt. In the saturated TG's 4:0, 14:0, 16:0 and 18:0 were of considerable quantitative importance, in the trans- and cis-monoene TG's the more abundant FA constituents were 4:0, 16:0 and 18:1, in the diene TG's 4:0 and 18:1 made large contributions to the constituent FA's, and in the triene TG's the major FA's were 4:0, 16:0, 18:1 and 18:3.

Reference to Table 35 shows that in fraction C of the September sample of milk fat the proportions of each of 4:0, 6:0 and 8:0 were similar in each TG class while

the proportions of each of 14:0, 16:0 and 18:0 decreased markedly as the proportion of unsaturated FA's in TG classes increased. These FA's showed a similar pattern of distribution in the C fractions of the January and March samples of milk fat (Tables 36 and 37).

From a further examination of the data presented in Tables 28 to 37 and from the above detailed description of these results it is possible to put forward the following general conclusions relating to the component TG classes of the three samples of milk fat examined:

(i) TG's of the same average degree of unsaturation, obtained from corresponding TG fractions, had the same major FA constituents and generally comparable FA compositions.

(ii) In each TG fraction trans- and cis-monoene TG's had similar FA compositions.

(iii) In the TG fractions of medium and low mol. wt. more than 50% of each saturated FA was found in the saturated TG's.

(iv) For each TG fraction close agreement was obtained between the FA composition reconstructed from the proportions of FA's in the respective TG classes and the FA composition determined experimentally.

3.3.4. Triacylglycerol compositions of selected triacylglycerol classes of the March sample of milk fat.

The TG compositions of the more abundant TG classes prepared from the March sample of milk fat are given in Table 38. The molecular types C_{40} (7.4%), C_{42} (16.0%), C_{44} (18.8%), C_{46} (17.4%), C_{48} (15.4%) and C_{50} (10.4%) were of considerable quantitative importance in the saturated TG's of fraction A and comprised 85.4% of the TG's present. TG's with 44,46,48,50 and 52 carbon atoms in their constituent FA's comprised 9.8, 12.1, 22.2, 27.1 and 13.8% respectively of the cis-monoene TG's of fraction A (85.0% of the TG's present). In fraction B C_{38} (42.2%) and C_{40} (29.3%) comprised 71.5% of the saturated TG's while in the cis-monoene TG's of fraction B the molecular types C_{40} (42.4%) and C_{42} (27.3%) together accounted for 69.7% of the TG's. In the saturated TG's of fraction C the major TG types were C_{34} (22.6%), C_{36} (41.4%) and C_{38} (16.0%) while in the cis-monoene TG's C_{36} (20.2%), C_{38} (47.6%) and C_{40} (15.9%) comprised 83.7% of the constituent TG's.

It is evident from Table 38, that for each of the three TG fractions obtained from the March sample the saturated TG's had a lower average mol. wt. than the cis-monoene TG's.

Table 38. Triacylglycerol compositions of the saturated and cis-monoene TG's obtained from the triacylglycerol fractions of high, medium and low molecular weight (fractions A, B and C) of the March milk fat sample.

TG[a]	Composition of molecular types of triacylglycerols (mole %)					
	Fraction A		Fraction B		Fraction C	
	Sat.[b]	Mon.[c]	Sat.[b]	Mon.[c]	Sat.[b]	Mon.[c]
26	-	-	-	-	0.3	-
28	-	-	-	-	1.2	-
30	-	-	-	-	2.6	0.4
32	-	-	-	-	8.5	1.8
34	-	-	0.5	-	22.6	5.7
35	-	-	-	-	2.6	0.9
36	-	-	10.7	-	41.4	20.2
37	-	-	2.2	-	2.7	3.4
38	0.7	-	42.2	6.4	16.0	47.6
39	-	-	2.8	1.7	0.7	2.4
40	7.4	-	29.3	42.4	1.4	15.9
41	1.0	-	1.2	2.4	-	0.5
42	16.0	3.9	8.4	27.3	-	0.9
43	1.7	0.6	0.7	1.9	-	-
44	18.8	9.8	1.5	11.6	-	-
45	2.1	1.2	0.3	0.9	-	-
46	17.4	12.1	0.2	3.9	-	-
47	2.0	1.6	-	0.4	-	-
48	15.4	22.2	-	0.8	-	-
49	1.6	2.9	-	-	-	-
50	10.4	27.1	-	0.5	-	-
51	1.0	1.6	-	-	-	-
52	3.7	13.8	-	-	-	-
53	0.4	1.0	-	-	-	-
54	0.3	2.2	-	-	-	-
	99.9	100.0	100.0	100.2	100.0	99.7

[a] carbon number of triacylglycerols

[b] saturated triacylglycerols

[c] cis-monoene triacylglycerols

Section 3.4. Thermal analysis of the triacylglycerol fractions and classes prepared from the March sample of milk fat.

3.4.1. Heating thermograms obtained by differential scanning calorimetry.

The heating thermograms of the March sample of milk fat and the TG fractions of high, medium and low mol. wt. (fractions A, B and C), which were prepared from the March sample by silicic acid column chromatography are shown in Figure 12. The saturated TG's and unsaturated TG's prepared from fractions A and C of the March sample by silver ion-T.L.C. were also examined by differential scanning calorimetry (Figure 13). The heating thermograms presented in Figures 12 and 13 were recorded after rapid cooling of the fat sample (Section 2.2.3.).

Figure 12 shows that the March sample of milk fat melted over the range -33 to 37°C with the main melting peak at 17°C, a small broad peak centred at 7°C, and a shoulder plateau between 22 and 37°C. No exothermic transitions were present although a dip occurred at 11°C. Fraction A melted over the range -2 to 42°C and had a single melting peak at 38-40°C which was preceded by an exothermic transition centred at 12°C. Reference to the heating thermogram, obtained after tempering, showed that about 80% of this fraction melted between 25 and 42°C. Fraction B melted over the range -18 to

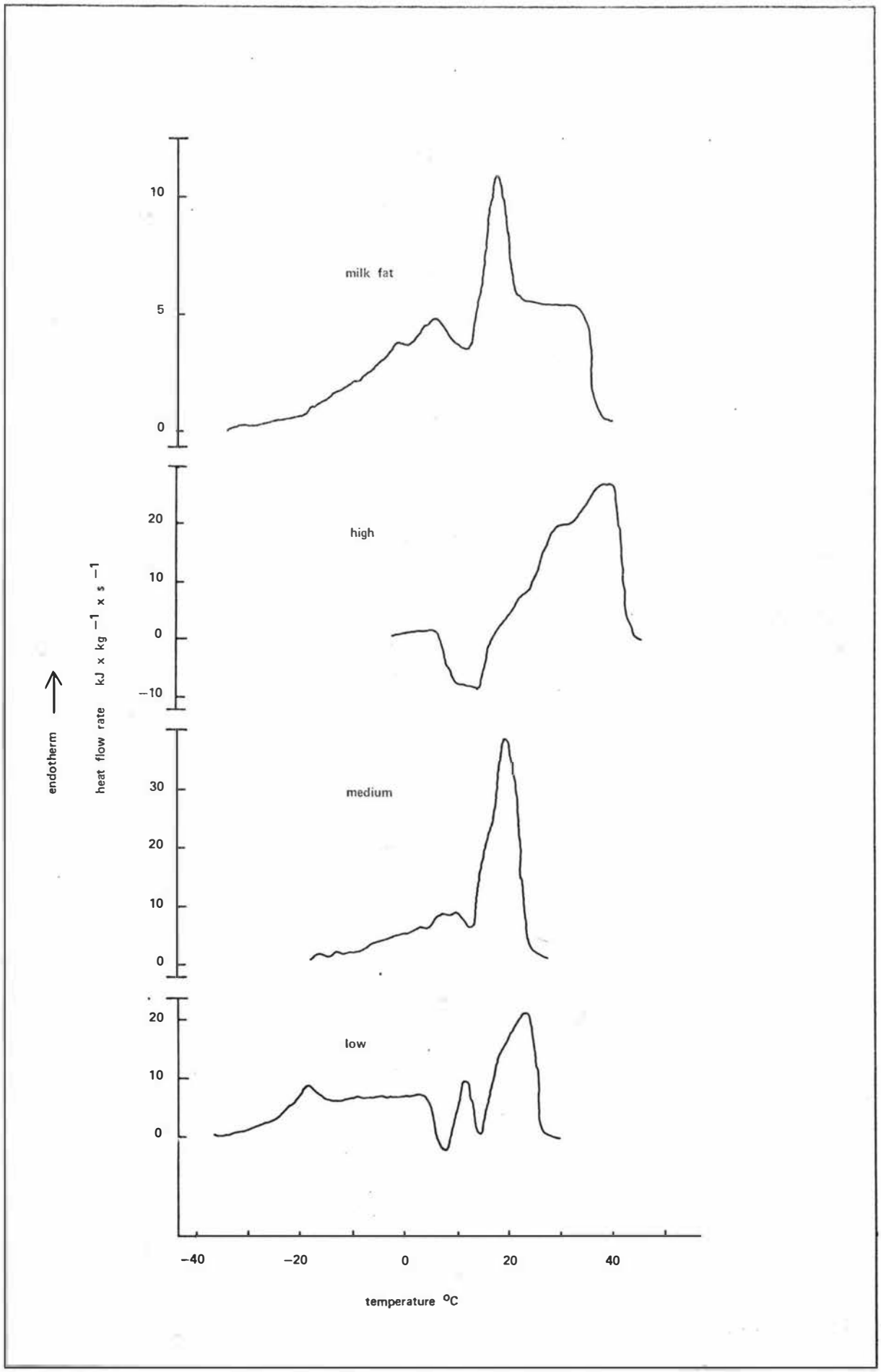


Figure 12. Heating thermograms of the March sample of milk fat and its triacylglycerol fractions of high, medium and low molecular weight.

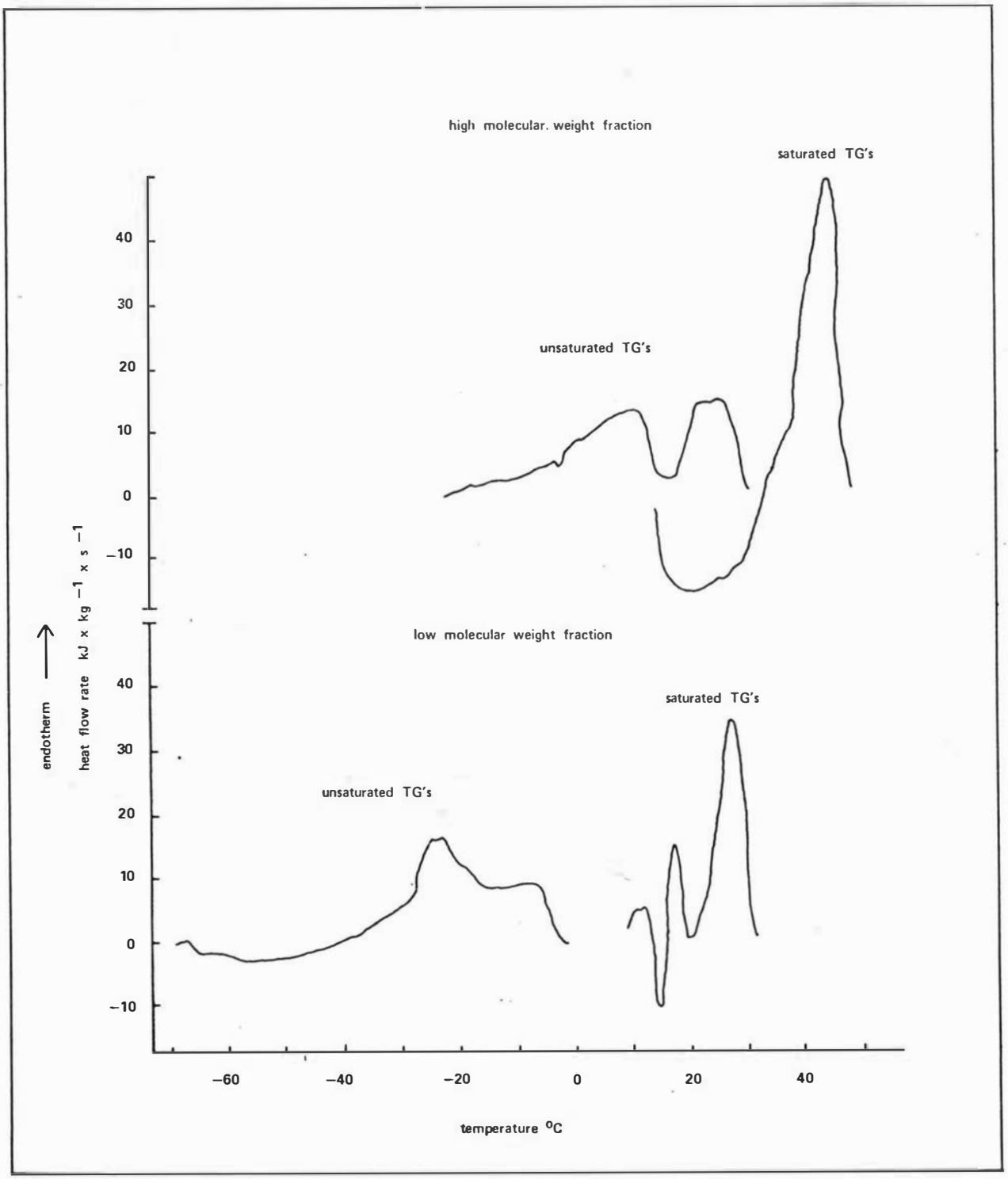


Figure 13. Heating thermograms of the saturated and unsaturated triacylglycerols obtained from the high and low molecular weight triacylglycerol fractions of the March milk fat sample.

23°C with a single large melting peak at 18°C. No exothermic transitions were present. Fraction C had the greatest melting range of the three fractions (-38 to 25°C). It had also the most complex heating thermogram with an exothermic transition at 8°C, a dip in the thermogram which almost reached zero at 15°C, and a main melting peak at 23°C. Approximately 30% of the most stable form of this fraction melted between -40 and 0°C.

The heating thermogram for the saturated TG's of fraction A (Figure 13) had a melting range from 14 to 45°C with an exothermic transition centred at 25°C and a single sharp melting peak at 43°C. It resembled the heating thermogram for fraction A although it had a sharper melting peak. The unsaturated TG's of fraction A melted over the range -23 to 29°C which was considerably below that of fraction A. The heating thermogram had a dip at about 17°C which almost reached zero. The heating thermograms for the saturated TG's and unsaturated TG's of fraction C had melting ranges from 8 to 30°C and from -68 to -3°C respectively. They did not overlap and each had a similar shape to the corresponding proportion of the heating thermogram for fraction C. However the unsaturated TG's exhibited a broad, shallow exothermic transition centred at -50°C which was not shown by fraction C.

The heating thermograms presented in Figures 12 and 13 show both polymorphism and solid solution formation. As noted in the introduction (Section 1.3.) polymorphism generally predominates in fats containing relatively few

TG species while solid solution formation predominates in fats which are complex mixtures of large numbers of TG's. An example of this trend is given in Figure 12. It can be seen that fractions A and C both exhibited obvious polymorphic behaviour (i.e. an exothermic transition) which was not evident in the total milk fat.

3.4.2. Effect of the tempering procedure.

Heating thermograms for fraction C of the March sample of milk fat obtained with the rapidly cooled and tempering procedures described in the Methods (Section 2.2.3.) are given in Figure 14. The exothermic transition at $8^{o}C$ and the dip at $15^{o}C$, which were present in the heating thermogram recorded after rapid cooling, were both removed by the tempering procedure with little alteration to the rest of the thermogram. This showed that both the exothermic transition and the dip at $15^{o}C$ were caused by polymorphic transitions.

The tempering procedure was applied to the TG samples whose heating thermograms are given in Figures 12 and 13. In the case of fraction A the tempering procedure removed the exothermic transition present in the heating thermogram recorded after rapid cooling (Figure 12) without significantly altering the shape of the rest of the thermogram thereby providing additional evidence that this exothermic transition was a polymorphic transition. Similarly all major dips present in the heating thermograms recorded after rapid cooling, for the saturated

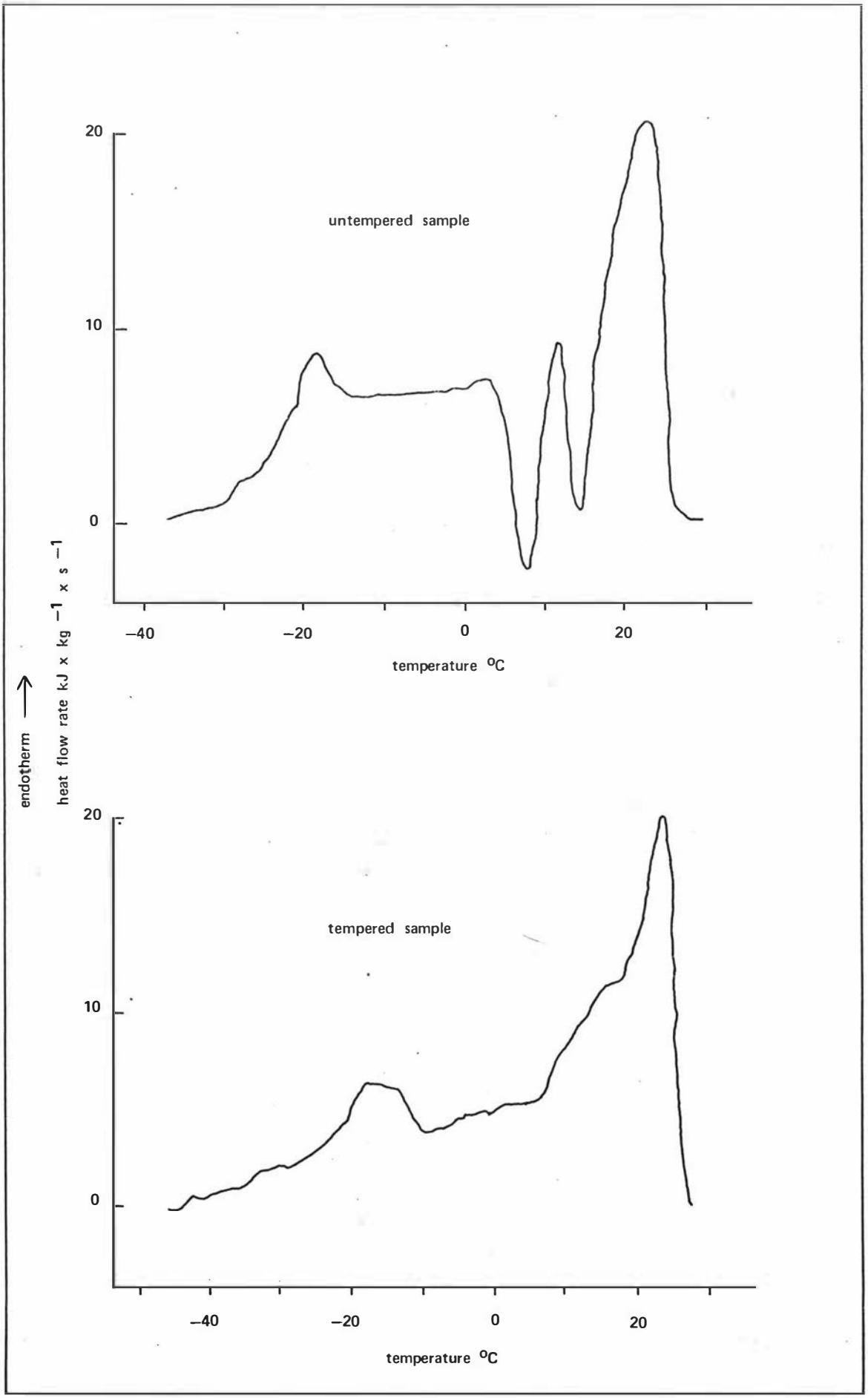


Figure 14. Heating thermograms of the low molecular weight triacylglycerol fraction of the March milk fat sample obtained with the rapidly cooled and tempering procedures.

TG's and unsaturated TG's of fractions A and C (Figure 13) were removed by the tempering procedure with, in each case, little change to the shape of the main melting peak. Hence these dips were the result of polymorphic transitions.

3.4.3. Relationship between composition and melting ranges of triacylglycerols.

Reference to Figure 13 shows that the greater the average mol. wt. and the lesser the average level of unsaturation of the TG sample the higher its melting range. It can be seen that the melting ranges of the different constituent TG classes of milk fat were spread over a wide temperature range (-68 to 45°C).

From the data given in Table 39 it is evident that although fraction A melted over the highest temperature range it contained the lowest proportion of saturated TG's. Conversely fraction C which melted over the lowest temperature range contained the highest proportion of saturated TG's. Thus in going from fraction A to fraction C of the March sample of milk fat the lowering effect on the melting range caused by the decrease in the average mol. wt. of constituent TG's outweighed the elevating effect on the melting range caused by the increase in the proportion of saturated TG's.

Table 39. The influence of composition on the melting ranges of the triacylglycerol fractions of high, medium and low molecular weight (fractions A, B and C) obtained from the March milk fat sample.

	Triacylglycerol class		
	Fraction A	Fraction B	Fraction C
Average mol. wt. of constituent TG's	814	702	652
Proportion (mole %) of saturated TG's in fraction	31.7	49.1	53.4
Melting range ^{o}C	-2 to 40	-18 to 23	-38 to 25

Chapter 4.

DISCUSSION

Section 4.1. Composition of milk fats.

It has been shown that marked and regular seasonal variations occur in the FA composition of New Zealand milk fat (Hansen and Shorland, 1952; Gray, 1973). It was considered that the effect of this seasonal variation of the FA's on the structure and composition of milk TG's could be best studied by (i) selecting samples of milk fat which showed large differences in their respective FA compositions, and (ii) selecting samples of milk fat with similar FA compositions which were produced at different stages of the dairying season. Reference to the studies carried out by Hansen and Shorland (1952) and by Gray (1973) shows that the September, January and March samples of milk fat used in the current investigation were representative of the two extremes which occur in FA composition of New Zealand milk fat during the dairying season. The September sample contained high proportions of 4:0, 18:0 and 18:1 and a low proportion of 16:0 and had a FA composition typical of soft spring milk fat while the January and March samples contained low proportions of 4:0, 18:0 and 18:1 and high proportions of 16:0 and had FA compositions typical of hard summer milk fat (Table 7).

When these three samples of milk fat were separated by adsorption chromatography on silica gel G into TG fractions of high, medium and low mol. wt. it was found that the proportions of corresponding TG fractions in the different samples were similar. Kuksis and Breckenridge (1968), using the same method of fractionation, prepared from a Canadian milk fat TG fractions whose proportions were comparable to those obtained above. For all samples the TG fractions of high mol. wt. comprised between 38.6 and 41.4% of the milk fat, the TG fractions of medium mol. wt. comprised between 16.0 and 18.1%, and the TG fractions of low mol. wt. comprised between 42.4 and 44.6%.

An examination of the FA compositions of these samples of milk fat and their TG fractions showed that, in general, the similarities and differences between FA compositions of the respective samples of milk fat were reflected in the FA compositions of their corresponding TG fractions. For example, the proportions of 16:0 in the Canadian (Kuksis and Breckenridge, 1968), September and March samples and their respective TG fractions were respectively: milk fat 23.4%, 22.1% and 26.4%, high mol. wt. fraction 24.4%, 23.2% and 29.2%, medium mol. wt. fraction 22.9%, 21.7% and 26.2%, low mol. wt. fraction 23.5%, 20.2% and 24.0%. Notable exceptions to the above trend were (i) the proportion of 4:0 in the low mol. wt. fraction of the Canadian sample which was lower than expected and (ii) the proportion of 18:1 in the high mol. wt. fraction of the same sample which

was higher than expected. This trend suggests that a change in FA composition of milk fat is reflected throughout the entire mol. wt. range of milk TG's.

When the TG fractions of differing mol. wt. were separated by silver ion chromatography to give TG classes of differing levels of unsaturation it was found that the low mol. wt. fractions contained a higher proportion of saturated TG's and consequently a lower proportion of unsaturated TG's than the medium mol. wt. fractions. In turn the medium mol. wt. fractions contained higher proportions of saturated TG's and lower proportions of unsaturated TG's than the high mol. wt. fractions (Table 40). For example, in the March sample of milk fat saturated TG's comprised 31.7%, 49.1% and 53.4% respectively of the high, medium and low mol. wt. fractions. In their investigations of North American milk fats Blank and Privett (1964) and Kuksis and Breckenridge (1968) noted a similar trend in the proportions of TG classes in the TG fractions (Table 40).

In so far as differences and similarities between the September, January and March samples of milk fat are concerned it was observed that (i) the proportions of corresponding TG classes in the January and March samples were similar and (ii) each TG fraction of the September sample contained a lower proportion of saturated TG's and a higher proportion of unsaturated TG's than the corresponding TG fractions of the January and March samples (Table 40). Comparable

fractionations carried out by other workers (Blank and Privett 1964, Kuksis and Breckenridge 1968, Shehata *et al*. 1972) showed that samples of milk fat from North American countries contained a much lower proportion of saturated TG's in TG fractions of high mol. wt. than New Zealand milk fats. However, similar proportions of the saturated, monoene, diene and triene TG's of low mol. wt. were found in the September sample and in the North American samples (Table 40).

Further examination showed that corresponding TG classes of these milk fats had generally comparable FA compositions. This finding was not unexpected since the TG classes were obtained by fractionating milk TG's on the basis of their constituent FA's.

Table 40. Proportions of triacylglycerol classes of differing degrees of unsaturation prepared from milk fats by a combination of adsorption chromatography and silver ion-T.L.C.

Triacylglycerol class	Proportions in triacylglycerol fractions of differing molecular weight: Present study September (mole %)	Present study January (mole %)	Present study March (mole %)	Ref.1[a] (wt. %)	Ref.2[b] (mole %)	Ref.3[c] (wt. %)
TG fraction of high mol. wt.						
Saturated TG's	28.5	33.9	31.7	19.6	16.5	17.0
Monoene TG's	39.6	41.3	41.0	58.3	36.7	40.6
Diene TG's	21.5	17.5	17.5	22.1	27.7	34.2
Triene TG's	10.5	7.6	9.9	-	12.9	4.0
Polyene TG's	-	-	-	-	6.2	4.2
TG fraction of medium mol. wt.						
Saturated TG's	45.4	50.4	49.1	-	38.7	-
Monoene TG's	37.3	30.4	32.9	-	38.3	-
Diene TG's	10.0	11.3	10.1	-	14.4	-
Triene TG's	6.8	8.2	8.0	-	8.7	-
TG fraction of low mol. wt.						
Saturated TG's	46.5	54.3	53.4	43.7	45.0	-
Monoene TG's	37.6	32.9	32.3	40.8	38.1	-
Diene TG's	9.9	8.4	8.9	9.8	11.7	-
Triene TG's	6.3	4.6	5.6	5.6	5.2	-

[a]Blank and Privett (1964)

[b]Breckenridge and Kuksis (1968a, 1969)

[c]Shehata et al. (1971, 1972)

Section 4.2. Stereospecific analyses of the triacyl-glycerols of milk fats.

A number of enzymatic methods have been proposed for the determination of the stereospecific placement of FA's in the constituent TG's of fats (Lands et al. 1966; Brockerhoff, 1965 and 1967). The initial step in each method is the preparation of DG's, either by hydrolysis with pancreatic lipase or by deacylation with a Grignard reagent, which must be representative of the original TG's for results of the stereospecific analysis to be strictly quantitative. Because a portion of milk TG's contain short-chain FA's the preparation of representative DG's from milk fat poses special problems for the following reasons:

(i) TG's containing 4:0 are hydrolysed more rapidly than trioleoyl-glycerol by pancreatic lipase (Sampugna et al. 1967).

(ii) Certain DG species formed by lipolysis of TG's containing 4:0 are preferentially hydrolysed by pancreatic lipase (Sampugna et al. 1967).

(iii) 1,2(2',3)-DG's and 1,3-DG's, obtained by deacylation of total milk fat with a Grignard reagent, cannot be completely separated by T.L.C. (Yurkowski and Brockerhoff, 1966).

In the present study TG fractions of high, medium and low mol. wt. prepared from milk fat were deacylated with a Grignard reagent in an effort to obtain

1,2(2,3)-DG's which were representative of the constituent FA's of the original TG's (Section 2.4.3.). This method produced representative 1,2(2,3)-DG's from TG fractions of high mol. wt. However when the TG fractions of medium and low mol. wt. were deacylated with a Grignard reagent the DG products formed (1,3-DG's and 1,2(2,3)-DG's) could not be completely separated. Consequently there remained no alternative but to prepare 1,2(2,3)-DG's by hydrolysis of TG samples with pancreatic lipase. Although there exists no data in the literature concerning the FA composition of 1,2(2,3)-DG's prepared in this manner from milk TG's of low mol. wt. the data obtained by Sampugna *et al.* (1967), which are mentioned above, suggest that the 1,2(2,3)-DG's formed would not be strictly representative of the FA's present in the original TG's. The experimental results obtained in the present study indicated that this was the case. It was found that for the TG fractions of medium and low mol. wt. the FA compositions of the 1,2(2,3)-DG's obtained experimentally contained some 10-20% more 4:0 and 6:0 than the FA compositions determined by calculation.

In the published studies relating to the stereospecific analysis of milk fat (Pitas *et al.* 1967) and milk TG's of low mol. wt. (Breckenridge and Kuksis, 1968b) the 1,2(2,3)-DG intermediates were prepared by hydrolysis of the TG sample with pancreatic lipase. Consequently these results and the data determined in the present study for milk TG's of medium and low mol.

wt. were not strictly quantitative. However, valid qualitative conclusions concerning the stereospecific arrangement of FA's can be obtained from these results.

An examination of the results of the stereospecific analyses of the high mol. wt. fractions of September, January and March samples of milk fat (Section 3.2.3.) and the molecular distillate of milk TG's of high mol. wt. (Breckenridge and Kuksis, 1969) showed that the overall pattern of distribution of FA's in positions 1, 2 and 3 was similar for each TG fraction. Similarly, when comparing the results of the stereospecific analyses of the low mol. wt. milk TG's determined in the present study and reported by Breckenridge and Kuksis (1968b) it was again observed that the overall pattern of distribution of FA's in positions 1, 2 and 3 was similar for each sample. Results of the stereospecific analyses of samples of milk fat obtained by Pitas *et al.* (1967) and reported in this work also showed that the stereospecific arrangement of FA's in these samples was similar.

These results showed that the FA's were distributed in a highly selective manner within the component TG's of milk fat and fractions of milk fat. In the constituent TG's of milk fat 4:0 and 6:0 were esterified almost entirely at position 3, 14:0 was concentrated at position 2, and 16:0 tended to be preferentially esterified at positions 1 and 2, but the patterns of distribution of 18:0 and 18:1 varied according to the

mol. wt. of the TG's. In milk TG's of low mol. wt. 18:0 and 18:1 were preferentially esterified at position 1 while in TG's of high mol. wt. they were concentrated at positions 1 and 3. The FA's clearly do not fit a 1,3-random-2-random distribution pattern. However, it has been suggested that the 1-random-2-random-3-random theory may hold for milk fat (Christie and Moore, 1970).

Section 4.3. Influence of the composition of milk triacylglycerols on the physical characteristics of milk fats.

In the preceding discussion it has been noted that:

(i) In the three samples of milk fat examined in the present study the stereospecific arrangement of FA's was similar.

(ii) Corresponding TG classes of these three samples of milk fat had generally comparable FA compositions.

(iii) Each TG fraction of the September sample contained a greater proportion of unsaturated TG's and consequently a lower proportion of saturated TG's than the corresponding TG fractions of the January and March samples.

Since the three samples of milk fat examined in the current work were selected from different stages during the dairying season it can be concluded that changes in the state of lactation and in feed conditions which occur with the progress of the dairying season in New Zealand affect the relative proportions of the constituent TG species of milk fat but do not significantly affect the nature of these TG species. This variation in the proportions of the constituent TG species of milk fat appears to be the overriding factor which influences the seasonal variation in the physical characteristics of New Zealand milk fat.

Similarly it can be concluded from the preceding discussion that in the Canadian milk fat examined by Kuksis and Breckenridge (1968) and in the milk fats investigated in the present work the nature of the constituent TG species is similar but the relative proportions of these TG species differ. The proportions of the saturated TG's of high mol. wt. were shown to be considerably lower in the Canadian milk fat.

Wood and Dolby (1965) noted that the hardness values of butter made in New Zealand were much higher than those reported by de Man and Wood (1958) for Canadian butter. When the hardness of butter made by conventional methods throughout the season was measured at 17°C the following hardness ranges were obtained: Canadian 1.5 - 3.2 Kg, New Zealand 5.6 - 7.5 Kg. Although some of the difference in hardness between New Zealand and Canadian butters is the result of different manufacturing techniques most of the difference may be attributed to the differences in the chemical composition of milk fat. The much higher hardness of New Zealand butter may therefore be largely attributed to the greater proportion of saturated TG's of high mol. wt. and the lesser proportion of unsaturated TG's of high mol. wt. present in New Zealand milk fat.

Section 4.4. Melting behaviour of the triacylglycerols of milk fat.

Studies of the thermal properties of synthetic TG's have shown that the melting points of TG's increase as the mol. wt. increases and as the level of unsaturation decreases (Bailey, 1950). As expected classes of TG's obtained from the March sample of milk fat (Figure 13) showed the same trend i.e. the greater the average mol. wt. and the lesser the degree of unsaturation of the constituent TG's of the TG class the higher its melting range. The unsaturated TG's of low mol. wt. which melted over the range -68 to -3°C were mainly responsible for the considerable proportion of milk fat which melted below 0°C. In contrast the saturated TG's of high mol. wt. had a relatively high melting range (14 to 45°C) and resembled that of the high melting glyceride fraction of milk fat (Norris et al. 1971).

By reference to Figure 12 it can be seen that the TG fraction of high mol. wt. had a narrower melting range than the TG fraction of low mol. wt. which implies that the constituent TG's of the high mol. wt. fraction fit together more easily than those which comprise the low mol. wt. fraction. This trend in melting ranges was also exhibited by the saturated and unsaturated TG's of the respective fractions (Figure 13). The heating thermograms of the saturated and unsaturated TG's of high mol. wt. showed considerable overlap indicating that solid solution effects are important. On the other

hand the heating thermograms of the saturated and unsaturated TG's of low mol. wt. did not overlap and each had a similar shape to the corresponding proportion of the TG fraction of low mol. wt. indicating that very little formation of solid solutions occurs. This means that the structural difference between the saturated and unsaturated TG's of low mol. wt. must be sufficiently large to prevent significant solid solution formation between these two TG classes. The unsaturated TG's of low mol. wt. are mainly TG's which consist of a long-chain saturated FA, a long-chain unsaturated FA and a short-chain saturated FA. In other words these TG's contain three FA's with widely different packing requirements which would not readily fit into the normal double or triple chain length TG lattices (Gunstone, 1964) and therefore would not be expected to exhibit significant solid solution formation with normal TG's. In contrast, the saturated and unsaturated TG's of the high mol. wt. fraction and the saturated TG's of the low mol. wt. fraction should all fit into either double or triple chain length TG structures and each would be expected to exhibit solid solution formation with one another.

As noted above the unsaturated TG's of the low mol. wt. fraction were mainly responsible for the considerable proportion of milk fat which melted below $0^{o}C$ and consequently for the wide melting range which is characteristic of milk fat. Thus the wide melting range of milk fat is due to both the large number of different TG

species and to the large structural difference between the various TG species.

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