Composition of Milk Fat Globules with Increased Linoleic Acid[1]

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ABSTRACT AND SUMMARY

Comparisons were made of the composition and distribution of the lipids in fat globules from conventional milks and polyunsaturated milks produced by cows fed protected lipid supplement. Washed creams were prepared from the milks of three individual cows fed a conventional ration, and three fed a protected sunflower-soybean supplement rich in linoleic acid. The washed creams were fractionated by treatment with sodium deoxycholate and centrifugation. Each washed cream and four fractions (designated as outer globule membrane, inner membrane, pellet, and globule core) were analyzed for protein, lipid, phospholipid, cholesterol, tocopherols, carotenoids, and fatty acid composition. The outer and inner membrane fractions were further fractionated into neutral and polar (phospholipid) lipid classes by thin layer chromatography. For both types of washed cream the approximate weight distribution of total solids was: outer membrane, 1%; inner membrane, 2%; pellet, 0.1%; and core, 96%. The percentages of protein, phospholipid, cholesterol, and carotenoids were all lower in the polyunsaturated than in the conventional creams. In the polyunsaturated creams, the percentages of both saturated and unsaturated C_{18} acids were higher, and of acids of C_{16} and shorter chain length lower, than in the conventional creams. The phospholipids in the outer and inner membranes from the polyunsaturated milks had larger proportions of linoleic acid than did the phospholipids from the conventional milks. However, this increase in unsaturation was less than that of the core neutral lipids. Pancreatic lipase hydrolysis of the core fractions showed that the increased linoleic acid was introduced preferentially at the 2-position of the triglycerides. In general, the observed changes in physical properties and in susceptibility of polyunsaturated milk to the development of oxidized flavor are consistent with the differences in the relative proportions of the various classes of lipids in the conventional and polyunsaturated milks.

[1]Data from thesis of D.H. Bianco submitted in partial fulfillment of the requirements for the M.S. Degree in Food Science, University of California, Davis. Presented in part at the AOCS Meeting, New Orleans, April, 1976.

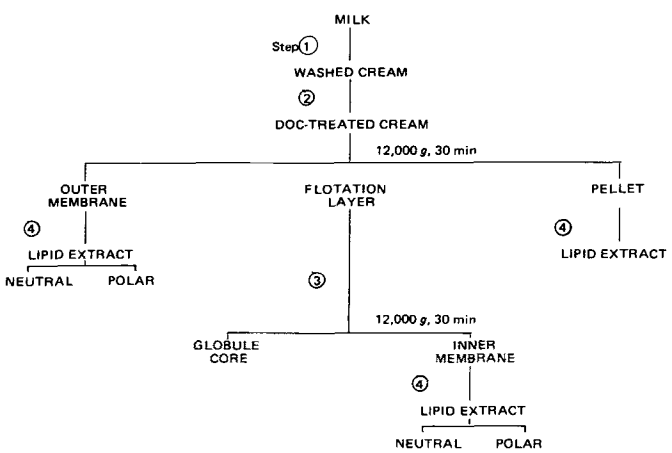

FIG. 1. Schematic outline of fractionation of fat globules from conventional and polyunsaturated milks. The procedure is based on the action of sodium deoxycholate (DOC) on uncooled washed cream. See text for description of procedure.

INTRODUCTION

The milk fat globule and the interfacial layer at its surface, known as the milk fat globule membrane (MFGM), continue to be of scientific and technological interest. The core of each globule consists mainly of triglycerides whereas the MFGM includes a complex mixture of polar and neutral lipids, proteins, glycoproteins, enzymes, and trace elements. The origin, composition, and structure of the bovine milk fat globule and its membrane have been reviewed recently by Mulder and Walstra (1) and Brunner (2). These reviews point out many areas where knowledge is still lacking.

The fatty acid composition of the globule glycerides is influenced by the composition of the cow's diet (3). The feeding of vegetable oils protected by formaldehyde-treated protein results in the production of milk much higher in polyunsaturated fatty acids than regular milk. This process and the production of foods from ruminants with elevated content of linoleic acid have been reviewed by McDonald and Scott (4).

Little has been reported on effects of diet on the composition and properties of the MFGM. Huang and Kuksis (5) found small quantitative differences in phospholipids and neutral lipids of MFGM prepared from summer and winter milk. Anderson (6) fed coconut oil in protected and unprotected forms. The protected supplement resulted in less MFGM and less neutral fat in the MFGM but proportions of individual phospholipids were unaffected. However, Sleigh and Burley (7) found increased levels of unsaturated fatty acids in MFGM phospholipids when cows were fed supplements of protected polyunsaturated vegetable oils.

The objective of the present research was to study the changes in the lipids of the milk fat globule and its membrane when a protected lipid supplement was fed to cows. This work was part of a broader project concerned with the production, processing, and human acceptability of polyunsaturated meat and milk products, conducted by several departments of the University of California, Davis (8-10).

EXPERIMENTAL PROCEDURES

Samples

Milk samples were the complete morning milking from each of six high-producing Holstein cows in the University herd. Three were on a conventional hay and grain ration, and three were being fed a complete milled ration containing hay, a grain concentrate mixture, and a protected lipid supplement provided by Alta Lipids (USA) Ltd., Boise, ID. The supplement, equal to 35% of the total ration, contained a 7:3 mixture of sunflower seeds and soybeans treated with formaldehyde generally in accordance with the Australian protected lipid process (11).

Fractionation of Fat Globules

Figure 1 outlines the principal steps in the procedure used to prepare washed cream and four fractions designated as outer fat globule membrane, inner membrane, pellet, and globule core. Step 1 included the separation of cream from each individual uncooled milk without delay at 37 C, and the washing of the cream at the same temperature in a DeLaval cream separator, Model 518. Each cream was washed twice with a volume of buffer equal to the volume of skim milk discarded at the initial separation. The buffer was an aqueous solution of 0.25 *M* sucrose and 0.15 *M* sodium chloride buffer at pH 7 with 0.01 *M* sodium phosphate.

In step 2, the washed cream was treated with sodium deoxycholate solution (DOC) as described by Hayashi and Smith (12) and by Erickson et al. (13). Briefly, 30 g aliquots of washed cream were weighed into 50 ml centrifuge tubes and sufficient DOC solution was added to give 1% DOC in the aqueous phase of the washed cream. The mixture was held 1 hr at 37 C with gentle shaking. Under these conditions, DOC releases some of the membrane material from the outside of the fat globules. The mixture was centrifuged (Sorvall RC-2B, type SS-34 rotor) at 12,000 *g* for 30 min at 37 C, and was then cooled to 0 C to solidify the flotation layer. Aqueous layers, containing the outer membrane fraction, and the pellets were collected separately and refrigerated until they could be analyzed.

In step 3, the flotation layers were pooled, melted at 60 C under nitrogen, and recentrifuged at 12,000 *g* for 30 min at 40 C. After the tubes were chilled to 0 C, the white layer or inner membrane fraction was scraped from the bottom of the plug of solidified fat or globule core fraction.

In step 4, the total lipids in the fractions designated as outer membrane, pellet, and inner membrane, were extracted with chloroform-methanol (2:1, v/v) and nonlipid material was removed by partitioning between chloroform and methanol-water phases. Aqueous phases were saved for protein determinations. Inner and outer membrane lipids were separated into neutral and polar fractions by preparative thin layer chromatography. Narrow bands of the lipids were applied to prewashed Silica Gel G plates. Development was with diethyl ether-acetic acid (100:1, v/v). The neutral and polar fractions were scraped off each plate and recovered from the gel with chloroform-methanol (2:1, v/v).

Analytical Methods

The fractions analyzed included: washed cream, outer membrane, pellet, inner membrane, and core. Protein content was estimated by the Biuret method of Torten and Whitaker (14). Lipid phosphorus was by the method of Smith et al. (15). Total tocopherols were extracted and determined by the colorimetric procedure of Low and Dunkley (16), and total carotenoids by absorptivity at 437 nm (17). Cholesterol and major phospholipid classes (phosphatidyl cholines, phosphatidyl ethanolamines, sphingomyelins) were determined essentially by the quantitative thin layer chromatographic (TLC) method of Blank et al. (18). A standard curve for each lipid class was run together with the sample on the same TLC plate to eliminate any variation in background density between plates.

The composition of the fatty acids in the various fractions was determined by gas chromatography of either methyl or butyl esters. Fractions expected to contain appreciable phospholipids but essentially no butyric acid (pellet, outer and inner membranes) were converted to methyl esters by the method of Metcalfe et al. (19). The mixture of esters was taken up in petroleum ether, washed with water, and then freed of cholesterol or other contaminants by TLC on silica gel G plates (20). The ester band was extracted from the gel with petroleum ether and dried with sodium sulfate. The gas chromatograph (Hewlett-Packard model 5700 A) was equipped with dual columns of stainless steel, 380 cm long X 0.32 cm ID packed with 10% Supelco SP-222-PS on 100/120 mesh Supelcoport. The instrument also had flame ionization detectors (model 1871-A), an automatic sampler (model 7671-A), and a laboratory data system (model 3352-A). Samples were run isothermally at 190 C, with nitrogen carrier flow rate 20 ml/min. Results were quantitated by the data system after calibration with known standards (Supelco, Inc., Bellefonte, PA).

Fractions expected to have short chain fatty acids (washed cream, globule core, membrane neutral lipids) were butyl esterified by a procedure based on that of Parodi (21). The esters in petroleum ether were washed with dilute aqueous sodium chloride and dried with sodium sulfate, before being separated by gas chromatography. The column was 170 cm X 0.32 cm ID stainless steel packed with 15% EGSS-X on 100/120 mesh Gas-Chrom P (Applied Science Laboratories, State College, PA). Column temperature was held 2 min at 70 C, then was programmed at the rate of 4 C/min to 180 C and held for 32 min.

The method used for the pancreatic lipase hydrolysis of triglycerides was similar to that of Smith and Hardjo (22). Following hydrolysis, the original and residual triglyceride and residual monoglyceride fractions were butylated and analyzed by gas chromatography by the procedures described above.

RESULTS AND DISCUSSION

Distribution of Components Among Washed Cream Fractions

In interpreting the results of the fractionation scheme shown in Figure 1, it is assumed that washed cream represents the original milk fat globules, the core fraction represents the inside of the globules, and the combined outer and inner membrane and pellet fractions represent the original MFGM material. The distribution of total solids, protein, lipid, phospholipid and cholesterol among the fractions obtained from conventional and polyunsaturated washed creams is shown in Table I. Results are expressed as percentage of each component originally present in each washed cream. For both types of cream, the approximate distribution of total solids by weight was: outer membrane, 1%; inner membrane, 2%; pellet, 0.1%; and core, 96%. Most of the protein and phospholipid was found in the two membrane fractions and in the pellet, whereas nearly all the total lipid and cholesterol was located in the core fraction. The outer or DOC-released membrane from each washed cream accounted for 51% of the protein and 65% of the phospholipid initially present in the creams. This agrees with the composition of the corresponding MFGM fraction isolated by Hayashi and Smith (12). The inner or DOC-insoluble part of the MFGM constituted about 30% of the phospholipid and 12-22% of the protein in the original washed creams. The phospholipid value corresponds to that of Hayashi and Smith (about 35%) but the amount of protein is low (average 17% vs. 32%). We concluded that the distribution of major lipid components was similar among the fractions prepared from conventional and polyunsaturated creams.

Distribution of Constituents in Each Globule Fraction

Protein, total lipid and phospholipid percentages for each fraction from the two creams are compared in Table II. Polyunsaturated cream had significantly less protein and less phospholipid than did conventional. This suggests that the MFGM of polyunsaturated milk is more susceptible to loss during the washing procedure or that there was less

TABLE I

Distribution of Total Solids, Protein, Lipids, Phospholipids, and Cholesterol among Fractions Obtained from Conventional and Polyunsaturated Washed Creams

Fraction[b]	Percent of total component[a]				
	Total solids	Protein	Lipid	Phospholipid	Cholesterol
Washed cream[c]					
conventional	100	100	100	100	100
polyunsaturated	100	100	100	100	100
Outer membrane					
conventional	1.1	50	0.6	68	1.3
polyunsaturated	0.9	52	0.3	63	1.2
Inner membrane					
conventional	1.9	12	1.9	26	2.2
polyunsaturated	1.8	22	1.5	35	2.4
Pellet					
conventional	0.1	4.5	<0.1	<0.1	–
polyunsaturated	0.1	6.0	<0.1	<0.1	–
Core					
conventional	96	–	98	0	101
polyunsaturated	93	–	92	0	116
Total recovered					
conventional	100	66	100	94	104
polyunsaturated	95	80	94	98	120

[a]Values are means of three samples.

[b]Refer to Figure 1 and text for description.

[c]Washed cream assumed to contain 100% of each component.

TABLE II

Gross Composition of Fractions Obtained from Conventional and Polyunsaturated Washed Creams[a]

Fraction	Protein, % of total solids	Lipid, % of total solids	Phospholipid, % of total lipid
Washed cream			
conventional	1.4	99	0.5
polyunsaturated	1.0[c]	99	0.3[d]
Outer membrane			
conventional	54.3	45.7	55.4
polyunsaturated	62.3	37.7	57.9
Inner membrane			
conventional	8.3	91.7	5.0
polyunsaturated	13.7	86.3	5.8
Pellet			
conventional	84.5	16.0	43.5
polyunsaturated	89.7	10.3	36.0
Core			
conventional	–[b]	100[b]	0
polyunsaturated	–[b]	100[b]	0

[a]Values are means of three samples.

[b]Assumed to be 100% neutral lipid.

[c]$P < 0.05$.

[d]$P < 0.001$.

MFGM present originally. The outer membrane for each cream was about 55-60% protein with remaining lipid being about 16-20% neutral lipids and 22-25% phospholipid. The inner membranes averaged only 11% protein with 81-87% neutral lipids and 5% phospholipid. These values are lower than those found by Hayashi and Smith (12) for a comparable fraction. However, in the present procedure, the inner membrane fraction was contaminated with neutral lipids when it was scraped from the lower surface of the globule core fraction. Total solids of the pellets from both creams were mostly protein (85-90%) and lipid (10-15%) that was rich in phospholipid. Because the core fractions are all lipid, they were not extracted.

Distribution of total cholesterol, tocopherols, and carotenoids is shown in Table III. Cholesterol concentration per gram of total lipid was greatest in the outer membrane, whereas tocopherols and carotenoids were concentrated in the core fraction of both types of cream.

Distribution of the three major phospholipid classes in the outer and inner membrane fractions is given in Table IV. The percentage of phosphatidyl cholines in the outer membrane was less in the polyunsaturated than in the conventional globules, but distribution of the phosphatidyl ethanolamines and sphingomyelins was similar.

The data for the original washed creams (Tables II, III) show that the amounts of protein, total phospholipid, cholesterol, and carotenoids were all lower in the polyunsaturated than in the conventional globules. Table IV indicates that the decrease in phospholipid could be attributed to a decrease in phosphatidyl cholines in the polyunsaturated outer membrane. Bitman et al. (23) reported no increase in milk fat cholesterol from cows fed protected lipids despite large increases in serum cholesterol. The relatively higher concentration of cholesterol in the outer membrane of both conventional and polyunsaturated globules suggests that it is important to the structure and

properties of the MFGM.

The tocopherol concentrations in washed cream and core fraction of about 25 μg/g lipid (Table III) were similar to those of Erickson et al. (13) who found about 20 μg/g lipid. We found very low values (7.3 and 4.2 μg/g lipid) for the inner membrane fraction, and the amount in the outer membrane was too small to measure, whereas Erickson et al. found that tocopherol was concentrated in the lipid of the milk fat globule membrane. This suggests that the labile tocopherols associated with the two membrane fractions in our study were either largely removed by washing or were oxidized prior to assay. Polyunsaturated milk is extremely susceptible to autoxidation.

There were lower concentrations of carotenoids in both the polyunsaturated washed cream and core fraction compared to the conventional fractions. Australian investigators made the same observation (personal communication). This effect is probably due to the lower content of carotenoids in the ration of cows receiving the protected lipid supplement.

TABLE III

Distribution of Cholesterol, Tocopherols, and Carotenoids in Fractions Obtained from Conventional and Polyunsaturated Milk Fat Globules[a]

Fraction	Cholesterol, mg/g lipid	Tocopherols, μg/g lipid	Carotenoids, μg/g lipid
Washed cream			
conventional	4.0	23.3	1.8
polyunsaturated	2.8[b]	25.2	1.1[c]
Outer membrane			
conventional	9.0	<0.1	<0.1
polyunsaturated	9.3	<0.1	<0.1
Inner membrane			
conventional	4.2	7.3	<0.1
polyunsaturated	4.4	4.2	<0.1
Core			
conventional	4.0	26.0	1.7
polyunsaturated	3.5[b]	28.9	1.4

[a]Values are means of three samples.
[b]$P < 0.01$
[c]$P < 0.05$.

Fatty Acid Composition of Globule Fractions

Fatty acid composition of total lipids from conventional and polyunsaturated creams and corresponding globule fractions is summarized in Table V. Because about 98% of milk lipid is in the globule core, the composition of this fraction should be very similar to that of washed cream, and such was the case for both types of globules. In comparing conventional with polyunsaturated fractions, highly significant increases in linoleic (3 to 23%), oleic (20 to 29%), and stearic (9 to 17%) acids were found in washed cream and core fractions. Compensating decreases occurred in the amount of each fatty acid of less than 18 carbons in chain length. Cook et al. (24) reported a larger increase in linoleic, no change in stearic, and decrease in oleic acid following feeding of a protected lipid supplement. Such discrepancies can be explained in part by differences in degree of lipid protection—i.e. if our supplement were not as effectively protected from ruminal activity as that of Cook et al., hydrogenation of some linoleate would yield

TABLE IV

Distribution of Major Phospholipid Classes in Membrane Fractions from Conventional and Polyunsaturated Milk Fat Globules[a]

Fraction	Percent of total phospholipid		
	Phosphatidylcholines	Phosphatidylethanolamines	Sphingomyelins
Outer membrane			
conventional	25.0	32.0	27.0
polyunsaturated	18.7[b]	27.3	25.3
Inner membrane			
conventional	24.7	30.7	19.7
polyunsaturated	21.3	31.3	21.0

[a]Values are means of three samples.
[b]$P < 0.01$.

TABLE V

Major Fatty Acid Composition of Total Lipids from Fractions Obtained from Conventional and Polyunsaturated Milk Fat Globules[a]

	Wt % fatty acids							
	Washed cream		Core		Outer membrane		Inner membrane	
Fatty acid	conv.	poly.	conv.	poly.	conv.	poly.	conv.	poly.
4:0	5.2	3.6	3.8	4.0	–	–	–	–
6:0	3.2	1.5	2.6	1.6	–	–	–	–
8:0	2.0	1.0	1.5	0.7	–	–	0.6	–
10:0	3.8	1.0	3.6	1.1	0.5	0.1	2.7	0.7
12:0	4.2	1.0	4.2	1.1	1.3	0.2	4.1	1.0
14:0	11.5	3.5	11.5	3.5	6.8	2.0	13.2	4.3
14:1	2.1	0.4	2.1	0.4	1.3	0.3	3.5	0.5
16:0	26.4	13.6	27.0	13.7	24.9	15.4	33.3	18.6
16:1	3.0	1.3	2.9	1.3	2.6	1.1	3.1	1.6
18:0	9.0	17.1	9.5	17.0	14.4	25.0	11.3	23.8
18:1	20.1	29.3	20.8	29.5	30.2	28.8	20.7	29.6
18:2	3.0	22.6	3.3	22.3	8.7	22.0	2.5	17.6
18:3	1.1	1.9	1.2	1.7	1.1	1.3	0.7	1.0
Longer chain + minor	5.4	2.2	6.0	2.1	8.2	3.8	4.3	1.3

[a]Values are means of three samples of each fraction.

TABLE VI

Fatty Acid Composition of Neutral and Polar Lipids of Membrane Fractions Obtained from Conventional and Polyunsaturated Milk Fat Globules[a]

	Wt % of fatty acids[b]							
	Outer membrane				Inner membrane			
	Neutral		Polar		Neutral		Polar	
Fatty acid	conv.	poly.	conv.	poly.	conv.	poly.	conv.	poly.
4:0	–	–	–	–	3.6	3.5	–	–
6:0	1.0	0.6	–	–	2.4	1.5	–	–
8:0	1.1	0.4	–	–	1.5	0.6	–	–
10:0	3.6	1.2	0.1	–	3.5	1.0	–	–
11:0	0.3	–	–	–	0.4	0.1	–	–
12:0	5.2	1.5	0.4	0.1	4.2	1.0	0.2	–
12:1	0.5	0.2	–	–	0.1	–	–	–
14:0	13.3	4.6	3.7	0.9	11.7	3.5	2.7	1.2
14:1	0.9	0.2	0.3	–	2.0	0.4	0.1	0.2
15:0	3.1	0.9	1.0	0.3	1.4	0.5	0.6	–
16:0 br	0.5	0.3	0.3	0.1	0.6	0.2	0.2	–
16:0	32.7	18.6	18.0	11.2	27.7	14.6	16.9	13.0
16:1	3.0	1.7	2.4	0.9	2.9	1.3	2.2	1.0
17:0	1.1	0.5	0.9	0.4	1.2	0.6	0.8	0.3
18:0 br	0.3	–	0.4	0.1	0.5	0.1	0.3	–
18:0	10.3	20.4	13.7	21.8	9.9	20.0	16.1	27.4
18:1	18.0	28.7	36.0	28.8	20.2	27.8	40.1	29.1
18:2	2.3	17.8	12.7	28.1	3.1	20.8	12.1	22.6
18:3	0.7	1.1	2.0	2.5	1.2	1.7	1.4	1.8
19:0	0.6	0.7	0.7	0.6	0.8	–	–	0.6
20:0	–	–	0.2	0.1	0.3	0.4	–	–
20:1	0.3	0.2	0.5	0.5	0.1	0.1	0.7	–
20:2	–	–	0.4	0.2	–	–	0.3	–
22:0	–	–	2.6	1.0	–	–	1.3	1.8
22:1	–	–	2.3	1.0	–	–	2.6	–
23:0	–	–	0.7	1.4	–	–	–	–
24:0	–	–	–	–	–	–	0.7	0.2

[a]Refer to Figure 1 and text for description.

[b]Values are means of three samples of each fraction.

increased oleate and stearate.

The fatty acid data for the membrane fractions from conventional globules agree generally with the results of Hladik and Forman summarized by Brunner (2). Compared to the original globules, butyric to capric acids were absent or present in lower percentages in the membrane fractions of both conventional and polyunsaturated creams. Comparing polyunsaturated core and membrane fractions to the corresponding conventional fractions, the percentages of C_{18} acids were increased with compensating decreases in C_{16} and acids of shorter chain length. A greater increase (+19%) in linoleic acid occurred in the core fraction of the polyunsaturated globules than in the lipids of the outer (+13%) and inner (+15%) membrane fractions. Fatty acid compositions of the pellet fraction for both types of globules were also determined, but are not reported. As might be predicted, each pattern corresponded closely to that of the respective outer membrane fraction.

To better locate changes in the lipids of milk fat globule membranes from the two types of cream, the fatty acid compositions of neutral and polar lipid subfractions from the outer and inner membrane fractions were determined (Table VI). The neutral and polar subfractions were predominantly triglycerides and phospholipids, respectively. The data for neutral lipids of conventional outer and inner membranes corresponded reasonably with each other, with data for the core fraction (Table V), and with the results of Hladik and Forman as cited in Brunner (2) for triglycerides of milk fat globule membrane. These results do not support the existence of a high melting triglyceride fraction as a component of the MFGM (25).

The polar lipids of conventional outer and inner membranes had fatty acid compositions consistent with those to be expected from the mixtures of phosphoglycerides and sphingolipids present in MFGM (26). The corresponding polyunsaturated polar lipids had higher percentages of long chain polyunsaturated fatty acids with compensating decreases in palmitic and shorter chain acids. The presence of such highly unsaturated phospholipids in the outer membrane, readily available to attack by dissolved oxygen, is consistent with the increased oxidative instability of polyunsaturated milk.

Triglyceride Structure of Globule Core

Most of the milk fat triglycerides are located within the globule core. The increase in total C_{18} acids from about 37% to about 74% requires changes in the intramolecular distribution of fatty acids in the triglycerides. The results obtained when the core triglycerides from conventional and polyunsaturated globules were analyzed by the pancreatic lipase technique (22) are given in Table VII. For each type of globule, the composition of the triglycerides remaining after lipolysis agreed reasonably with that of the original triglycerides. These results indicate that there was no significant preferential hydrolysis among the various types of triglycerides present (27). The proportion of each fatty acid at the 2-position in the triglycerides of each fat, as calculated by the method of Mattson and Volpenhein (28), is also indicated in Table VII. Theoretically, a value of 33.3% would indicate no preferential esterification of a particular acid to either the 2-position or 1,3-positions; but this makes no allowance for experimental error. We have considered that values within the arbitrary range of 28-38% suggest nonpreferential or random distribution (29). Less than 28% of a particular fatty acid esterified at the 2-position indicates that the fatty acid is located preferentially at the 1,3-position; and, conversely, more than 38% at the 2-position indicates preferential attachment at the 2-position.

The distribution pattern of the major fatty acids in the conventional globules agrees generally with the results of Freeman et al. (27), except that caprylic and capric acids were found to be preferentially esterified at the 2-position.

TABLE VII

Pancreatic Lipase Hydrolysis of Triglycerides from the Core of Conventional and Polyunsaturated Milk Fat Globules

Source	Fatty acid, mole %											
	4:0	6:0	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Conventional												
Original triglycerides	7.9	4.4	2.4	5.1	5.2	13.7	28.1	2.4	8.0	18.6	2.9	1.2
Residual triglycerides	4.4	4.1	2.0	4.5	4.3	13.0	29.5	3.3	9.5	20.5	3.6	1.4
Monoglycerides	–	1.2	3.0	8.5	9.1	24.9	32.8	2.5	4.7	12.0	1.3	–
Percent acid at 2-position[a]	–	9	41	56	58	61	39	34	20	21	16	–
Preferential esterification	–	1,3-	2-	2-	2-	2-	2-	none	1,3-	1,3-	1,3-	–
Polyunsaturated												
Original triglycerides	9.0	3.0	1.1	1.7	1.5	4.4	14.2	1.2	15.1	27.0	20.3	1.6
Residual triglycerides	5.3	2.5	0.9	1.2	1.2	4.0	14.4	2.0	16.0	28.7	21.8	2.2
Monoglycerides	–	0.9	1.6	3.2	3.0	8.8	18.6	1.4	10.7	25.4	25.1	1.2
Percent acid at 2-position[a]	–	9	51	61	67	67	44	41	24	31	41	25
Preferential esterification	–	1,3-	2-	2-	2-	2-	2-	2-	1,3-	none	2-	1,3-

[a]Calculated from M/3T x 100 = proportion (percent) of fatty acid type esterified at the 2-position, where M is mole percent of the acid in the monoglycerides and T is mole percent of the same acid in the original triglycerides.

The pattern for polyunsaturated globules shows that the additional linoleic acid was preferentially introduced at the 2-position. Additional oleic acid was also introduced at the 2-position to increase the proportion of this acid to 31%.

It is important to recognize that the pancreatic lipase technique gives the *average* concentration of a particular fatty acid at positions 1 and 3. Stereospecific analyses (30) have shown that butyric and caproic acids are esterified preferentially at position 3. The present research has demonstrated that the principal difference in the positioning of fatty acids in polyunsaturated milk fat, as compared to conventional milk fat, is the preferential introduction of additional linoleic and oleic acids at the 2-position of constituent triglycerides. This is in agreement with Morrison and Hawke (personal communication) who found the order of preference for positional introduction of linoleic acid as 2>1>3 in the triglycerides of polyunsaturated milk fat.

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