CHEMISTRY OF MILK FAT: A REVIEW

E. L. JACK AND L. M. SMITH

Department of Dairy Industry, University of California, Davis

Current knowledge of milk fat is brought together here to provide a needed reference and to stimulate additional research in the field. Research on fats has tended to lag somewhat behind that on other biological materials—perhaps because the seeming simplicity of their composition and structure appears not to offer an opportunity for spectacular results, or because the nature of the materials makes research time-consuming and onerous. In any event, other biological compounds have attracted greater attention. But there has been a renewed interest in fats within the last few years, and knowledge of their chemistry has been greatly increased.

A single paper could not well contain all the data pertinent to milk fat, or butter fat, as it is frequently called—incorrectly, the authors believe. In the globular form as it occurs in milk, the lipid material is composed of triglycerides in which are dissolved free fatty acids, possibly mono- and diglycerides for which there are no published values, and a heterogeneous group of materials called unsaponifiable matter. Associated with the triglycerides at the fat globule surface, but probably not dissolved in them, are phospholipids. Aside from listing the amounts of these other materials, from sources believed to be reliable, this review will be confined to the chemistry of the triglycerides and constituent fatty acids.

Milk fat is not a uniform substance. This is evident from the discussion and the variations—often large—in the amounts of the individual constituents shown in the tables. Thus it is not possible to say that an exact quantity of any single component is representative of milk fat. It must rather be said that the amounts of individual constituents—and even of groups of constituents—are best expressed as average values or as ranges of values. To give a single value for any constituent is misleading, and any attempt to characterize milk fat on the basis of the quantity of a single constituent, or even of a group, is fallacious. It is, indeed, doubtful if available data permit one to assign reasonably narrow inclusive ranges of occurrence to most of the constituents.

I. NONGLYCERIDE COMPONENTS OF MILK FAT

A. PHOSPHOLIPIDS

Comparatively wide ranges of phospholipids associated with milk fat have been reported in the literature. Because the phospholipids form a part of the

Received for publication May 12, 1955.
fat globule membrane complex, the method of obtaining the fat will influence the amounts present. It is generally reported (165, 180), without reference to analytical data, that fat prepared by melting and filtering butter granules contains no phospholipid. Results obtained by the authors (162) and by El-Rafey et al. (34) indicate that fat prepared by this procedure contains no more than traces of phospholipids. When milk fat is prepared by extraction with solvent from products containing varying amounts of the membrane material and fat, a wide range of values is obtained. Reports in the literature (27, 58, 87, 143) indicate a range of 0.655 to 1.048% phospholipids in fat obtained from whole milk by the Mojonier extraction procedure. Horrall (87), Perlman (143), and Heinemann (58) have studied creams of different fat contents and report values of 0.256 to 0.493% phospholipids in the fat. Reference should be made to the original papers for specific values. Reports in the literature on a wide variety of other dairy products are not within the scope of this review.

The phospholipid material in milk has generally been referred to as lecithin, but there is an increasing amount of evidence that substantial quantities of cephalin are also present. The main difference between the two phospholipids is in the nitrogenous component; choline is present in lecithin, and ethanolamine in cephalin. Jack and Dahle (89) found the phospholipid material isolated from milk to have an isoelectric point at pH 2.0, which is lower than the isoelectric points generally reported for lecithin and conforms more nearly to the isoelectric point of cephalin. Crane and Horrall (27), analyzing milk phospholipids for choline content, found that lecithin accounted for less than half of the total phospholipids in most of the samples analyzed. Minor amounts of sphingomyelin and cerebrosides also have been reported (106) in milk phospholipids.

B. Free Fatty Acids

Although the free fatty acid content of milk fat depends to some extent on the age of the fat and any changes resulting from various treatments, the presence of free fatty acids can always be demonstrated in fresh milk fat. When the fat has been obtained by churning, as reported by Gould and Trout (43) and Herrington and Krukovsky (62), the amounts present in fresh fat range from 0.1 to 0.9 expressed as acid degree. The acid degree is the milliliters of 1 N alkali required to titrate 100 g. of fat to the phenolphthalein end-point.

Johnson and Gould (93) demonstrated that some free acids are lost in churning, and developed a solvent-extraction procedure (95) for obtaining the fat. Thomas et al. (173) applied this procedure to fresh milk and obtained acid degrees of 0.41 to 1.83, with an average of 0.82. Frankel and Tarassuk (40) described an extraction-titration procedure based on the solvent-extraction step of Johnson and Gould, and reported data, for freshly drawn milk that had never been cooled, ranging from 1.50 to 3.50 and averaging 2.01 expressed as free fat acidity (milliliters of 1 N alkali required to neutralize the ether extract from milk containing 100 g. of fat). Although solvent-extraction procedures reveal a larger proportion of the fatty acids in fresh milk than does the acid degree of the churned fat, these procedures probably still fall short of quantitative recovery of
all the fatty acids, especially butanoic, which is partly miscible with water. These procedures may also extract nonfat acidic materials.

C. UNSAPONIFIABLE MATTER

Representative values for the known constituents of the unsaponifiable matter are given in Table 1.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Range of occurrence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>2.74-3.34 mg/g fat</td>
<td>(134)</td>
</tr>
<tr>
<td></td>
<td>3.5 mg/g fat</td>
<td>(140)</td>
</tr>
<tr>
<td></td>
<td>3.0-3.7 mg/g fat</td>
<td>(136)</td>
</tr>
<tr>
<td>Lanosterol</td>
<td>Present</td>
<td>(123)</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>0.23-0.83 IU/g fat</td>
<td>(177)</td>
</tr>
<tr>
<td>Total tocopherols</td>
<td>42 γ/g fat</td>
<td>(57)</td>
</tr>
<tr>
<td></td>
<td>12-34 γ/g fat</td>
<td>(122)</td>
</tr>
<tr>
<td></td>
<td>23-50 γ/g fat</td>
<td>(115)</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>9.26 γ/g fat</td>
<td>(179)</td>
</tr>
<tr>
<td></td>
<td>9.45 γ/g fat</td>
<td>(121)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>7.37 γ/g fat</td>
<td>(179)</td>
</tr>
<tr>
<td></td>
<td>8.15 γ/g fat</td>
<td>(121)</td>
</tr>
<tr>
<td>Squalene</td>
<td>Present</td>
<td>(37)</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>Present</td>
<td>(152)</td>
</tr>
<tr>
<td>Unidentified waxes</td>
<td>Present</td>
<td>(123)</td>
</tr>
</tbody>
</table>

Whether cholesterol is present both as free cholesterol and as the ester is uncertain. Although Nataf et al. (131) found no evidence of cholesterol esters, Nieman et al. (136) reported values for them. In addition, the amounts of vitamin D depend upon feed and the amount of sunlight available to the cows, both of which vary from place to place and season to season. It should be noted that a substantial portion of the unsaponifiable matter has never been characterized.

II. CHEMISTRY OF THE TRIGLYCERIDES

It has been recognized since the work of Chevreul (22) that true fats are glycerol esters of fatty acids. The chemistry of the triglycerides depends on the component fatty acids, their configuration, and their arrangement in the glyceride molecule.

A. FATTY ACID COMPOSITION OF MILK FAT

Major Component Fatty Acids. Knowledge of the component fatty acids in milk fat has increased as analytical methods available to researchers in the field have improved.

1 The modification of the Geneva System of Nomenclature, as used by Chemical Abstracts, will be used where applicable throughout this review.
Table 2 shows the major component fatty acids and the range of values determined by several different investigators. Browne (21) was among the first investigators to make a detailed analysis of the fatty acid composition of milk fat. In his method the fatty acids were liberated from the glycerides and separated into water-soluble and water-insoluble groups. The composition was calculated from the neutralization number of each fraction. Ester distillation was subsequently introduced as an analytical tool, and the most complete data for this method among the early reports were those of Holland et al. (81). Hilditch and Longenecker (70) analyzed milk fat by the ester-fractionation method, adding the improvement of an electrically-heated, packed, distilling column. Hilditch and Paul (71) further improved the method by separating the fat into different fractions by precipitation from solvent before distillation.

Later reports in the literature are based upon the use of these improved techniques. Smith and Dastur’s studies (160) were concerned with the effects of inanition on the composition of milk fat; the data of Jack and Henderson (90) cover results obtained by more extensive preliminary fractionation; those of Hansen (49), and Hansen and Shorland (52) are representative of milk fat produced under New Zealand conditions.

One of the difficulties in the ester fractionation techniques has been to make an accurate estimation of the lower fatty acids. Chromatography is now an added

<table>
<thead>
<tr>
<th>Component acids—Mol %</th>
<th>Holland et al. (84)</th>
<th>Hilditch and Longenecker (70)</th>
<th>Hilditch and Paul (71)</th>
<th>Smith and Dastur (160)</th>
<th>Hansen (49)</th>
<th>Jack and Henderson (90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine value</td>
<td>27.999</td>
<td>37.5</td>
<td>46.9</td>
<td>36.6</td>
<td>39.6</td>
<td>32.42</td>
</tr>
<tr>
<td>Iodine value</td>
<td>27.999</td>
<td>37.5</td>
<td>46.9</td>
<td>36.6</td>
<td>39.6</td>
<td>32.42</td>
</tr>
</tbody>
</table>

(Calc. from wt. %)

**Saturated**

| C4 | 8.7 | 8.1 | 10.2 | 9.7 | 10.5 | 9.2 |
| C6 | 2.8 | 2.8 | 2.5  | 1.2 | 3.9  | 2.8 |
| C8 | 1.7 | 2.5 | 1.3  | 1.6 | 1.8  | 2.7 |
| C10| 2.6 | 3.7 | 1.5  | 2.5 | 3.3  | 3.5 |
| C12| 8.4 | 4.4 | 3.4  | 3.0 | 3.8  | 5.2 |
| C14| 24.2| 12.5| 8.6  | 12.5| 9.8  | 14.8|
| C16| 15.8| 23.2| 21.1 | 22.1| 23.3 | 27.2|
| C18| 10.0| 7.6 | 9.9  | 9.8 | 1/11.6| 8.5 |
| C20| --- | 1.0 | 0.7  | 0.8 | 1/11.6| 1.2 |

**Unsaturated**

| C18 | --- | 0.4 | 0.2  | 0.3 | 0.3  | 0.3 |
| C18 | --- | 0.9 | 0.2  | 0.3 | 0.3  | 0.2 |
| C18 | --- | 1.7 | 0.9  | 1.0 | 1.2  | 1.5 |
| C18 | --- | 3.7 | 2.8  | 3.0 | 1.9  | 5.2 |
| C18 | 27.2| 24.8| 31.4 | 30.5| 26.8 | 15.3|
| C18 | --- | 0.2 | 0.5  | 0.6 | 1.6  | 0.7 |
| Octadecadienoic | --- | 2.9 | 4.9  | 1.0 | ---  | 1.7 |
technique that improves the resolution of these components \((35, 137, 146, 147)\). Most of the developmental work in the field has been done by Holman and his associates \((85)\) and Kaufman et al. \((97, 98)\). James and Martin \((92)\) used a unique gas-liquid partition method for the separation and micro-estimation of volatile fatty acids. They obtained complete resolution, from formic through dodecanoic acid, by using a 4-ft. column.

Another limitation on ester fractionation by distillation has been the difficulty of separating into different fractions the methyl esters of the higher unsaturated acids that each contain the same number of carbon atoms. Brown and associates \((19)\) developed a low-temperature fractional crystallization technique, which has been widely employed to separate unsaturated fatty acids or their esters from mixtures. Schlenk \((154)\) reviewed the formation of urea inclusion compounds of fatty acids. Swern and Parker \((169)\) and others \((155)\) used the preferential formation of urea inclusion compounds to separate oleic acid or methyl oleate from polyethenoid materials. Holman et al. \((85)\) have also demonstrated the value of chromatography in separating the unsaturated as well as the saturated acids. Several investigators \((26, 60, 156, 163)\) employed combinations of the above techniques to concentrate the polyethenoid constituents of milk fats.

Although the actual isolation of most of the polyethenoid components of milk fat has not been accomplished, they have been estimated by ultraviolet spectrophotometric techniques. Table 3 shows the amounts of these constituents as determined in milk fat by different investigators. Traces of nonconjugated pentaenoic acids also have been detected \((158, 164)\).

The values for low molecular weight fatty acids and unsaturated fatty acids are not as precise as those for the others because of the limitations of current isolation procedures.

**Minor component fatty acids.** Data purporting to show the presence of vaccenic acid \((11-octadecenoic acid)\) in milk fat have been presented by several workers \((11, 12, 16, 41, 48)\) and special growth-promoting properties have been ascribed to this acid \((13)\). Later reports have shown that the special growth-promoting properties could not be substantiated \((30, 135)\), but the presence seems established of a transmonoethenoid acid with a double bond elsewhere than in the "9" position.

The thesis for the presence of vaccenic acid stems originally from a paper by Bertram \((11)\). He isolated from beef tallow \((jus)\) a solid unsaturated acid that he termed vaccenic acid \((vacc, for cow)\). He assigned the double bond to the "11" position because the molecular weights and melting points of the disruptive oxidation products \((ozonolysis)\) corresponded with those of heptanoic acid and nonanedicarboxylic acid. Bertram assigned a trans configuration to the acid because he was unable to convert it to a higher melting form with isomerizing agents. He was able to isolate from milk fat a small amount of material corresponding in melting point and iodine value with the acid obtained from beef tallow.

Gupta et al. \((48)\) verified Bertram's original observations except that, using potassium permanganate in acetone as the oxidizing agent, they were able to
### TABLE 3

**Polyethenoid fatty acids of milk fat**

<table>
<thead>
<tr>
<th></th>
<th>Conjugated</th>
<th></th>
<th></th>
<th>Nonconjugated</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diene</td>
<td>Triene</td>
<td>Tetraene</td>
<td>Diene</td>
<td>Triene</td>
<td>Tetraene</td>
<td></td>
</tr>
<tr>
<td>Mattson (119)</td>
<td>0.6-3.7</td>
<td></td>
<td></td>
<td>0.8-2.0</td>
<td>0.7-2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schaffer and Holm (153)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morris et al. (125)</td>
<td>0.64-1.45</td>
<td>0.010-0.030</td>
<td>0.0012-0.0044</td>
<td>2.62-2.71</td>
<td>0.77-1.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lembke et al. (110,111)</td>
<td>av. 1.00</td>
<td>av. 0.019</td>
<td>av. 0.0030</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McDowell (120)</td>
<td>0.65-2.1</td>
<td>0.035-0.07</td>
<td>0.0046-0.0072</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith and Jack (164)</td>
<td>0.74-1.08</td>
<td>0.02-0.04</td>
<td>0.002-0.004</td>
<td>1.16-1.59</td>
<td>0.73-0.97</td>
<td>0.28-0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>av. 0.89</td>
<td>av. 0.02</td>
<td>av. 0.003</td>
<td>av. 1.45</td>
<td>av. 0.83</td>
<td>av. 0.35</td>
<td></td>
</tr>
</tbody>
</table>
obtain nonanedicarboxylic acid (as a split product) only from milk fat, and then in very small quantities. On the other hand, they obtained not only heptanoic acid but also substantial proportions of octanoic acid. In fats other than milk fat, the amount of octanoic acid exceeded that of heptanoic. These workers believed that their data indicated not only trans-11-octadecenoic acid but also trans-10-octadecenoic. Gupta et al. suggested that there was good reason to believe that the cis forms of these acids should also occur. The techniques used for the trans forms would not reveal them.

If one follows this line of reasoning further, it seems probable that monoenethenoid acids might occur with the double bond in any one of several positions, and as either geometric isomer. Evidence on this point is not available, and perhaps the efforts to obtain such evidence would be disproportionate to the importance of the results at the present time.

The occurrence in milk fat of fatty acids with branched chains and uneven numbers of carbon atoms has been reported by New Zealand investigators. Hansen and Shorland (50, 51, 53) isolated two isomeric C17 acids—15-methyl-hexadecanoic and 14-methyl-hexadecanoic—and a C20 saturated acid fraction containing at least three, and possibly four, methyl groups. Hansen et al. (54, 55) isolated a C18 methyl branched-chain saturated fatty acid and two isomeric C15 acids, 12-methyl-tetradecanoic and 13-methyl-tetradecanoic. Shorland (157) isolated pure n-pentadecanoic acid from milk fat.

The possible presence of hydroxy fatty acids in milk fat has been suggested by Okey (141) to explain certain results obtained when cholesterol determinations were made on this fat. Henderson and Jack (60) also observed a solvation of some of their fractions that might be attributed to hydroxy fatty acids. Investigations in this direction might prove fruitful in revealing other undetermined constituents.

**Physical properties of the fatty acids.** For tables giving extensive lists of physical properties, the reader is referred to recent comprehensive monographs by Markley (118), Ralston (115), Bailey (5), and Deuel (29).

**Fatty acid configuration.** Figure 1 (1) shows a diagrammatic arrangement of atoms in a saturated fatty acid molecule but the principal interest in this subject centers around positional and geometrical isomerism in the unsaturated fatty acids.

Hilditch and Longenecker (70) showed by oxidation procedures that the double bond of the monoenethenoid series, which ranges from C10 (decenoic) to C18 (octadecenoic), was located between the ninth and tenth carbon atoms. The presence of a terminal double bond in decenoic acid has been confirmed recently by Smith et al. (161) using infrared spectroscopy. The basis for other locations of the double bond has been discussed above (vide vaccenic acid).

It is only recently that reasonably reliable methods have been available to investigate the possible conjugation of double bonds in the polyethenoid acids, all of which are 18 carbons or longer. The chemical method of analysis for the noneonjugated polyethenoid components, linoleic (9, 12-octadecadienoic), linolenic (9, 12, 15-octadecatrienoic), arachidonic (5, 8, 11, 14-eicosatetraenoic) acids,
based on the determination of iodine number and thiocyanogen number, is diffi-
cult; and when these compounds are present in small proportions, it is inaccu-
rate. Tests based on the formation and isolation of bromination products are
also insensitive and unreliable in such cases. A reliable chemical method is not
yet available to determine the minor conjugated constituents. However, recently
developed ultraviolet spectrophotometric methods have provided useful tools to
determine both conjugated and nonconjugated acids of milk fat (vide Table 3).

With the exception of vaccenic acid (trans-11-octadecenoic), the monoethenoid
fatty acids of milk fat have generally been assumed to be exclusively of cis
configuration. However, Cornwell et al. (26) and Smith et al. (161) reported
the presence in milk fat of trans isomers of octadecenoic acid by infrared spectro-
photometric analyses. The latter also presented evidence for the occurrence of
trans isomers of hexadecenoic, tetradecenoic, and dodecenoic acids. Cornwell et
al. suggested, on the basis of solubility, the presence of a unique trans acid with
the double bond probably in the "14" position or beyond.

Early workers (18, 63) postulated that milk fat–linoleic acid was actually a
mixture of cis-trans- and trans-cis-9, 12-octadecadienoic acids because no petro-
leum ether-insoluble tetrabromide could be isolated. White and Brown (178)
concentrated the octadecadienoic acids and found approximately two-thirds of
the material was ordinary linoleic, the remainder being presumably the cis-trans
and trans-cis isomers. On the other hand, Shorland (156) has reported that the
octadecadienoic acid of New Zealand milk fat is not linoleic, although the octa-
decatrienoic acid content consisted almost entirely of linolenic acid. Perhaps
the experimental difficulties involved and possible differences in the feed of the
cows may explain such conflicting reports in the literature.

Although ultraviolet and infrared spectrophotometric methods are valuable
tools for configuration study, they do not reveal the actual location of the double
bonds, except for terminal double bonds, or the order of arrangement of cis and
trans double bonds in the carbon chain.

B. INFLUENCE OF VARIOUS FACTORS ON FATTY ACID COMPOSITION

An evaluation of the effects of specific factors on the fatty acid composition
of milk fat is clouded by the fact that it has seldom been possible to control all
conditions surrounding the production of milk so that only one variable is opera-
tive at any one time. Usually when feed conditions have been varied there have
also been climatic or seasonal changes, differences in stage of lactation, and pos-
sibly also, although frequently obscured, variations in the plane of nutrition. In
spite of the uncertainties involved an attempt will be made to evaluate the effects
of these factors.

Feed. There is ample evidence that the character of feed influences the com-
position of the fat produced from it. Hilditch and associates (67, 68, 72, 71)
showed that concentrate feeds containing seed oils rich in octadecadienoic and
octadecatrienoic acids do not affect the composition of the milk fat produced from
them, but that when feeds containing dodecanoic and tetradecanoic acids are fed,
the proportions of these acids increase in the fat produced. Also, when feeds contain fish oils the characteristic polyethenoid $C_{20}$ and $C_{22}$ acids appear in the milk fat. There have been numerous other reports of the effects of different grains and concentrate feeds on the character of the fat produced, but these have been reported as changes in some chemical constant of the fat rather than being documented by fatty acid analysis.

Hansen and Shorland (52) considered that the influence of diet on the fatty acid composition of milk fat should remain substantially the same during the grazing season because the fatty acid content of grass is so small—2 to 4%—and its main unsaturated constituents are found in such small amounts in milk fat. Workers in Holland (1, 14, 15, 17) on the other hand, have made extensive studies of the effects of hays and grasses upon the resulting fat. Fluctuations in iodine value of the milk fat are closely allied with the octadecenoic acid content of the feed, which is considerably higher in grass and clover pastures than in hay. Brouwer and Freins (15) reported that nitrogen-fertilized pastures enabled the cows to produce fat with an iodine value 3.7 units higher than that from non-nitrogen fertilized pasture. Bartley et al. (7) substantiated the effect of pasture in increasing the unsaturation of milk fat; they attributed the increase mainly to octadecenoic acid. Mattsson (119) reported that summer-pasture fat contains more conjugated $C_{18}$ acids than that produced on winter fodder.

It seems well established, therefore, that the composition of feed will influence to some extent the fatty acid composition of the fat produced from it.

**Plane of nutrition.** Several investigators (23, 32, 150, 160) have shown that underfeeding affects the chemical composition of milk fat. The results have been reported generally in terms of chemical and physical constants, except for Smith and Dastur (160), who presented fatty acid composition analyses. The results agree that a lowered plane of nutrition results in a decrease in the volatile, or low molecular weight, fatty acids and an increase in the unsaturated fatty acids, principally octadecenoic.

**Environmental temperature.** Regan and Richardson (148) showed that temperatures of 85°F. and above affect the composition of milk by decreasing the solids-not-fat, and that at 95°F. the Reichert-Meissl value of the fat decreases and the iodine value increases sharply. The question arises as to whether it is the effect of temperature per se or whether the changes are the result of "hyper-thermic undernutrition." Eckles and Palmer (32) pointed out that all types of underfeeding cause a decline in the volatile acids and an increase in the degree of unsaturation of the fat. This viewpoint is supported by fatty acid analyses by Smith and Dastur. The data of Bartley et al. are in agreement with respect to the effects of temperature in experiments where the daily temperatures did not exceed 83°F. They could find no correlation between temperature and the iodine value of the fat, which lack of correlation would be expected from the temperatures experienced under their conditions.

**Stage of lactation.** Data purporting to relate to the effects of stage of lactation are so confused by the possible influence of other factors—such as changes in diet and plane of nutrition, i.e., whether or not the animal is using body fat—that
a clear-cut analysis is not possible. Some data (33, 84, 149) appear to show that there is a steady decrease in volatile acid content and a gradual increase in iodine value as lactation progresses. These are the results that would also be expected from a steady decline in the plane of nutrition, a condition that frequently accompanies the progress of lactation. Was this the operative factor in these results, or was it lactation per se? Bartley et al. and Hansen and Shorland (52) report that there is a rather rapid decrease in the unsaturated acids during the early stages of lactation, followed by a leveling off and gradual increase toward the end of the period. The latter authors conclude that it is not possible to differentiate between the effects of stage of lactation and plane of nutrition.

In considering the interrelationships among the factors discussed above, two conclusions seem to be justified: The composition of the feed from which milk is produced may affect the composition of the fat; and a lowered plane of nutrition that causes the animal to use body fat for milk fat production will result in a decrease in the volatile fatty acid content and an increase in the degree of unsaturation of the milk fat. At the present time the evidence does not justify an attempt to distinguish among the effects of lactation, environmental temperature, and plane of nutrition.

C. GLYCERIDE COMPOSITION OF MILK FAT

The composition of the individual glycerides will depend on the number of fatty acids available for glyceride constitution. Because there are undoubtedly at least twenty different fatty acids present in milk, the number of glycerides theoretically possible has been shown to be $20^5$, or 8,000; and even when one considers only the possibilities resulting from those acids present in substantial quantities—about 13—there will be $13^5$, or 2,197, different glycerides. Obviously it is impossible to determine all of the exact glyceride combinations that might be present. However, because of the value of such knowledge to the understanding of milk fat synthesis, the specificity of lipase activity, and the probable mechanisms of absorption and utilization in the human body, it has seemed worthwhile to numerous investigators to determine specific groupings and then to apply generalizations.

Constituent glycerides. It was many years after Chevreul (22) showed that fats were composed of glyceride esters of the fatty acids before it was realized that these were not simple triglycerides, even though logical reasoning might have suggested the fact and should have provoked investigation. The first real evidence that milk fat was not composed of simple triglycerides was offered in 1913 by Amberger (2). By means of fractional crystallization and isolation of individual glycerides he was able to separate several mixed glycerides from milk fat.

In 1927 Hilditch and Lea (69) developed an oxidation procedure by which the fully saturated glycerides could be separated from those containing unsaturated components. By using these techniques of fractional crystallization and oxidation it has been possible since that time to separate the glycerides into
four categories, depending on the number of saturated fatty acids in the molecule. These have been characterized as GS₁, GS₂U, GSU₂, and GU₃—where G represents the glyceride component, S the saturated fatty acids present, and U the unsaturated fatty acids present in the glyceride molecules. In 1931 Hilditch and Sleightholme (73) were the first to discuss in detail the glyceride structure of milk fat as established by these procedures. In 1952 Greenbank (46, 47) fractionated milk fat in absolute alcohol and studied the glyceride structure of the different fractions. He concluded that most of the glycerides were GSU₂ and GS₂U.

Relationships have been worked out from the proportions of the different types of fatty acids present in the different groupings in milk fat, and attempts have been made to formulate general rules of occurrence. A molecular, or "association," ratio of saturated to unsaturated acids in the non-fully saturated portion of the fat permits calculation of the proportions of the unsaturated glycerides or indicates the limiting values that must obtain. Banks and Hilditch (6) originally proposed as a result of such considerations that the distribution pattern of fatty acids follows the rule of even, or widest, distribution in fats from the plant kingdom and that fats from animal sources are distributed in a random pattern. However, later results (65) caused Hilditch to suggest that animal fats also follow a modified even-distribution pattern in which the saturated acids are arranged according to random distribution but the unsaturated acids follow the even pattern. According to Hilditch (64) in a pattern of even, or widest, distribution a fatty acid will not repeat itself in a molecule until it forms more than 33 to 35% of the total fatty acids; when it forms 35 to 65% of the total acids, it may be expected to repeat twice in some or all of the molecules; nonmixed glycerides will appear only when the content of a single fatty acid is above 65%.

On the other hand, Longenecker (112) has supported the thesis that all natural fats follow the pattern of random distribution. In random distribution the proportion of occurrence of any specific glyceride is governed by the laws of chance according to the following equation: Molar percentage of any specific glyceride = \( n \times \left[ \frac{(a \times b \times c)}{100^3} \right] \times 100 \), where \( n \) = frequency of the glyceride occurrence in random distribution and \( a, b, \) and \( c \) = molar percentage of the fatty acids in positions 1, 2, and 3 of the glycerol respectively. For example, if the fatty acid mixture contains 63% saturated acids, according to the even distribution theory the amount of fully saturated glycerides would be negligible, whereas with random distribution the molar percentage of fully saturated glycerides would be 25.

The controversy has centered around these two points of view, and various modifications have been proposed to fit the data better. Norris and Mattil (139) supported the viewpoint of random distribution, stating that such a pattern represents a chemical equilibrium, whereas in the even distribution pattern the glycerides are not necessarily in equilibrium with their surroundings, because they must have been formed by a directed rather than a random synthesis. Doerschuck and Daubert (31) proposed a modified random distribution pattern,
whereas Luddy et al. (113) in three of four different fats found no correlation with either random or modified random distribution patterns. Reiser and Dieckert (149) attempted to solve the problem in fat synthesis by studying rats and chicks: Some were on an extremely low-fat diet leading to endogenous fat synthesis; others were on diets providing fats from various sources, including fat from their own species. With rats the fat from endogenous synthesis showed a random pattern but ingested fat conformed to even distribution; the chicks demonstrated a tendency to form simple triglycerides regardless of diet.

Aside from the contributions of Hilditch and associates (64, 65, 67, 70, 71, 73), the reports relating to milk fat are not extensive. Jack et al. (91) presented data on mixed milk fat that conformed closely to the pattern predicted by even distribution. Sommer (166) has commented, however, that whenever fat samples from different sources are mixed the distribution patterns, which might be affected by differences in diet and physiological factors, are also mixed and close adherence to any mathematical relationship should not be expected. Kartha (96) showed data for cow milk fat that he claimed supported the arguments for a restricted random distribution pattern proposed by him. Hilditch (66) in a recent reply to Kartha's paper casts doubt on the validity of some of the experimental data and disagreed with Kartha's analyses of existing data. Hilditch referred to data about to be published, which he says will substantiate further the general postulate of even distribution.

It is apparent that little can be said definitely at present concerning the distribution pattern of fatty acids in milk fat. Considerable space has here been devoted to the general subject to show some of the difficulties involved and the divergent views that have been presented. The complexity of the problem indicates that a complete solution will not soon be achieved with the techniques at hand. Labeling fats in vivo with radioactive isotopes and subsequent isolation and characterization of the resulting individual glycerides suggests itself as a possible approach.

III. PHYSICAL STRUCTURE OF MILK FAT

It is not sufficient to consider solely the chemical composition and distribution pattern of the glycerides; the physical structure must also be studied, both with respect to the geometry and the crystalline habit of the glyceride. Such knowledge is important for its direct bearing on technological problems; it is of increasing importance in its application to the study of composition. Dairy scientists have long been concerned with research on the practical aspects of technology. Numerous studies dealing with cooling procedures, solidification points, and melting points—and sometimes their interrelationships—have appeared in the literature. The majority of these investigators have drawn conclusions based upon analogous behaviors as determined in simple systems.

Relatively few studies of milk fat have dealt directly with fundamental aspects of the problem. Van Dam and Burgers (176) published results of X-ray diffraction studies. However, at that time such studies yielded but little knowl-
edge of either the geometry of the molecule or the crystalline patterns. Jack (88) studied melting rates of fats crystallized by different procedures and related these to possible crystalline patterns. Rishoi (151) reviewed the thermal behavior of milk fat in rapidly cooled cream, and Mulder (133), in studying melting and solidification of milk fat, arrived at the conclusion that it existed in the solid form as mixed crystals. For a more complete understanding of the geometry of glycerides and the crystalline habit of fats, it is necessary to refer to studies made upon relatively simple systems.

It is beyond the scope of this review to discuss in detail all of the material available on this subject. The reader is referred to recent excellent reviews by Lutton (114) and Malkin (116, 117) and to the monograph by Bailey (5). Two proposed general patterns of glyceride structure have been based on knowledge of bond angles and bond distances within the molecule. These are the so-called “tuning fork” structure and, in certain cases, the “chair” structure which are shown in Figure 1 (II, III). The reader is referred to the publications cited above for the arguments supporting these postulates.

Techniques have not yet been developed to determine the positions of specific fatty acids on the glycerol molecule in mixed triglycerides.

X-ray diffraction studies and melting point data are used to examine crystal structure of fats. From X-ray diffraction spacings, three distinctive crystalline forms are generally recognized in glycerides—called α, β', and β, in an ascending order of melting point. Clarkson and Malkin (24, 25) proposed a fourth form, designated as “vitreous.” Although X-ray diffraction data are not recorded for this form, the evidence for its existence seems clear-cut.

It is obvious that the application of these techniques and this knowledge to milk fat will result in a more thorough understanding of many of the problems confronting the dairy scientist. The complexity of milk fat renders direct application difficult, but it is nevertheless desirable. The isolation of less-complex glyceride mixtures, and preferably individual glycerides, should be done because extensive data are available on the characteristics of triglycerides of known constitution. Crystallization, distillation, countercurrent separations, and other techniques of detailed fractionation now available should be brought to bear on the problem.

IV. CHEMICAL REACTIONS OF MILK FAT GLYCERIDES

Milk fat glycerides are subject to all the general chemical reactions of glycerides, but only a few have been studied. Analytical reactions—such as with alkali in determining saponification values and with halogens in determining the degree of unsaturation—are standardized and need not be discussed here. Two reactions that result in deterioration of milk fat in commercial usage—hydrolysis and oxidation—are considered here, but only briefly, inasmuch as extensive pertinent reviews have been published recently (61, 167).

Hydrolysis. Hydrolysis of milk fat involves a reaction between the glycerides and water. It may stop at partial hydrolysis, with the formation of mono- and
diglycerides, or proceed to a complete splitting of the component fatty acids and glycerol. When the reaction is chemically catalyzed in the laboratory, a far greater degree of hydrolysis is obtained than in the enzymatic hydrolysis of fat occurring in biological systems. Generally, in milk fat, the reaction has been studied with respect to the action of the catalysts involved and the resultant fatty acids rather than from the standpoint of the remaining glyceride fragments.

It is well known that hydrolysis of milk fat is catalyzed both by H-ion and by enzymes, and it is the latter that have received the most attention from research workers. Herrington (61) published a comprehensive review on lipase in milk, and the mode of action of these enzyme systems will not be touched upon here. Hillig et al. (75, 76, 77, 78, 79, 80, 81) and others (4, 56) developed methods...
for assaying the fatty acids liberated in deterioration of butter and cream through the action of enzymes and acids. These have been determined as water-soluble acids and water-insoluble acids.

An aspect of lipase activity suitable for consideration here is that of possible specificity of action. Lipolysis in milk has been described as being of two different types, spontaneous and induced. Tarassuk and Jack (172) considered that the differences between spontaneous and induced lipolysis are attributable to differences in the enzyme systems involved. Krukovsky and Sharp (101) and Kelley and Dunkley (102) believed that the specific differences are related to changes in the nature of the surfaces of the fat globules.

The question arises whether lipolytic enzymes catalyze the hydrolysis of fat indiscriminately or whether some are more effective than others in liberating certain fatty acids. It is generally stated that lipases as a class have a low degree of specificity, being able to hydrolyze not only fats but also simple esters. Studies with simpler substrates and lipases from sources other than milk (3) indicate that the ease with which glycerides are split increases with the chain length—perhaps contrary to enzyme systems generally, where activity may decrease with increasing molecular weight (59)—and with unsaturation of fatty acids. Thus there is a fundamental basis for the possibility of some degree of specificity of lipase activity or differences in the relative rates at which hydrolysis of different triglycerides will occur.

Gould (42) and Johnson and Gould (94) determined the chemical characteristics—including acid degree, Reichert-Meissl, Polenske, saponification and iodine values—of fractions obtained from fresh and rancid milk fats, but the data gave no definite indication of selective fat hydrolysis by the lipase system. Gould considered that his results offered some basis for assuming that milk lipase acts on all of the triglycerides present and to about the same extent; but he pointed out that a more specific identification of the fatty acids involved is necessary before final conclusions may be drawn. Some results obtained in this laboratory (39) tend to show that induced lipolysis of homogenized raw cream yielded a higher percentage of volatile acids than spontaneously rancid cream. These latter results are in keeping with reported observations in commercial practice in which marked flavor differences are ascribed to the different types of lipolysis produced under different activating conditions.

The question of lipase specificity in milk should be studied with reasonably pure enzyme fractions and less complex glyceride substrates of known composition.

Oxidation. The voluminous literature pertaining to the oxidative deterioration of lipids has been recently reviewed by Holman (86), Morris (125), and Lea (108). Because of the complexity of the oxidation of natural fats, most of the fundamental concepts are supported by evidence obtained from studies of known mixtures of fatty acids and esters.

Oxidation of fats by molecular oxygen is generally caused by an initial reaction between oxygen and the component fatty acids. Both saturated and unsaturated fatty acids are susceptible to oxidation, but the rate of uptake of oxygen
increases with the degree of unsaturation of the fat and the postulated mechanisms of oxidation for the various types of fatty acids are different. However, it is generally accepted (125) that in the autoxidation of nonconjugated fatty acids a free radical first forms on the α-methylene group, adjacent to a double bond. The free radical absorbs an oxygen molecule, which then accepts hydrogen to form a hydroperoxide. The hydrogen usually comes from another fatty acid or ester, to form another free radical, and thus the chain reaction continues. In the oxidation of monoethenoid acids, which have less active methylene groups, the addition of oxygen occurs initially at the double bond instead of at the α-methylene group, providing the free radicals necessary for the usual α-methylene free radical chain reaction to proceed.

Secondary products are derived in great variety from the decomposition of the peroxides initially formed. Relatively little is known about their structure or the mechanisms by which they are formed, but they include various aldehydes, ketones, and acids. The shorter chain decomposition compounds responsible for oxidized odors and flavors can be detected organoleptically when only a few parts per million are present.

For information concerning the details of the mechanisms of autoxidation, the chemical tests used to measure the extent of oxidative changes occurring in fats, the evaluation of oxidative stability of fats, and the use of antioxidants, the reader is referred to the reviews cited above and to the publications of Bateman (8), Lea (107), Beadle (10), and Kraybill and Dugan (103).

The oxidation of milk fat that occurs in milk and dairy products is complicated by the presence of nonglyceride components, which may differ from triglycerides in susceptibility to oxidation, and by the fact that the reactions frequently take place in an aqueous system. For example, the phospholipid fraction of milk is oxidized more readily than is the glyceride fraction and generally has been considered the major source of oxidized flavors in milk (45, 168, 175). On the other hand, Pont (111), who used the sensitive ferrie-thiocyanate method for the determination of peroxide values, concluded that glyceride oxidation plays a significant part in the development of such flavors. Reviews on oxidized flavor in milk and dairy products—by Brown and Thurston (20), Greenbank (44), Hills (82), Mukherjee (126, 127, 128, 129, 130, 131, 132), Lea (109), and Strobel et al. (167)—emphasize the numerous factors that influence the oxidation of the glycerides and associated lipids.

The influence of fatty acid composition on oxidation of milk fat is difficult to assess because factors such as feed, which produce marked changes in the amounts of various fatty acids, also affect the contents of carotenoids and tocopherols, and possibly of other pro- and anti-oxidants (105). Thomé and Mattssson (174) reported that the oxidation of milk fat, as measured by the increase in peroxide value, takes place in two stages: a more or less lengthy induction period and an active oxidation period. They considered that the induction period is regulated by natural antioxidants in the milk fat, which can vary with feed of the cow. Furthermore, they showed that milk fat with a comparatively high content of conjugated C, and of linoleic acids will have a shorter
induction period and a higher rate of oxidation during the active period than will milk fat with a low content of these acids. Sidwell et al. (159) measured the oxidation of several fats, including milk fat. They plotted peroxide and total carbonyl values of the fat, and TBA* and total carbonyl values of the volatile products, as rate curves. The rate of increase in each case was much lower for milk fat than for lard and for soybean and cottonseed oils. Such differences as these between the oxidative stability of milk fat and of other food fats perhaps can be attributed to differences in the course of autoxidation of milk fat that are related to fatty acid composition and the presence of pro- and anti-oxidants.

Comparatively few conclusive studies have been made on the materials produced during the oxidation of milk fat and on the specific substances responsible for oxidized odors and flavors. Keeney and Doan (99, 100, 101) found that vacuum distillation of oxidized milk fat yielded materials (identified as unsaturated ketones) containing the characteristic odor compounds of oxidized fat. During advanced stages of milk fat oxidation, they were able to isolate flavor fractions typical of earlier stages in the oxidation process. They concluded that changes in flavor during autoxidation were caused by a blending of different flavor compounds. Thomé and Mattsson (174) believed that the substances actually causing the flavor defect were α, β-unsaturated carbonyl compounds, aldehydes, and ketones. Tamsma (170, 171) removed a volatile fraction from oxidized milk fat by deodorization and subsequent solvent-extraction of the distillate. This fraction was found to contain three classes of carbonyl compounds of ketonic character. The nonconjugated unsaturated compounds were responsible for the oxidized flavor, and both the carbonyl group and unsaturation were necessary.

It seems well established that various unsaturated carbonylic compounds contribute to oxidized flavors of milk fat. Furthermore, the deterioration of this fat can be detected organoleptically at very low levels of oxidation as estimated by objective chemical tests (82, 83, 144). The odor and flavor of milk fat change during successive stages of the oxidation process, but the available data indicate that the flavor observed at any one time cannot be attributed to any single compound. Although simple objective chemical tests, such as peroxide value, will continue to be of value in studying the oxidation of milk fat and associated lipids, further work is needed to isolate and identify the compounds associated with oxidized flavor. The trace amounts of such materials, especially during the early stages of oxidative deterioration, and their relative instability make the task difficult, though nevertheless desirable.

Other reactions. Patton et al. (142) reported in dry whole milk and butter oil a lipid-phase deteriorative flavor that they believed to be nonoxidative in nature but to involve lactone formation in glycerides containing 9-decenoic and 9-dodecenoic acids.

The only other chemical reaction that will be discussed in this review is that of inter-esterification. There are, as yet, no published results on such studies with milk fat.

*TBA = 2-thiobarbituric acid.
Baur (9) and Feuge et al. (36) studied inter-esterification of glycerides with triacetin and with acetic anhydride. Formo (38) reviewed ester reactions of fatty materials. Interesting compounds can be produced that have a wide range of properties, including melting point. Principal interest at present lies in the formation of compounds suitable for coatings but various possibilities for food uses are also discussed. Butyrates and other esters may be used, although the properties of resultant inter-esters are not yet reported.

CONCLUSION

The authors realize that there are of necessity many omissions in this paper. They hope there are no serious ones. Except for the topics discussed in detail, only representative papers are cited. Some of these representative papers give typical data, others describe the development of methods and show their application, and still others are topic reviews with extensive bibliographies. To have included all others relating to the subjects would have increased the list of references by three- or fourfold—and needlessly so, since those interested in specific details on these points must necessarily go to the sources.

An attempt has been made to be reasonably critical in examining the matter presented, and, where conflicting views exist, to present both sides with as much analysis as seemed warranted. Particular attention has been given to pointing out where additional, and perhaps different, types of research are indicated, in the hope that workers in the field may be encouraged to investigate some of these problems. If this should be the result, this paper will have been worthwhile.

REFERENCES

(18) BROWN, J. B. The Structure and Chemical Composition of Fats and Oils. *Oil and Soap*, 26: 333. 1941.


(60) Henderson, J. L., and Jack, E. L. The Fractionation of Milk Fat from a Solvent at Low Temperatures. Oil and Soap, 21: 90. 1944.


(100) KEENEY, M., AND DOAN, F. J. Studies on Oxidized Milk Fat. II. Preparation of 2,4-Dinitrophenylhydrazones from the Volatile Material from Oxidized Milk Fat. *J. Dairy Sci.*, 34: 719. 1951.


(141) Okey, Ruth. Personal communication.


CHEMISTRY OF MILK FAT: A REVIEW


(165) SOMMER, H. H. Market Milk and Related Products. 2nd ed., p. 475. Published by the author, Madison, Wis. 1946.


