# The Fatty Acids and Glycerides of Cow's Milk Fat

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**Milk fats contain a variety of individual fatty acids ranging in carbon chain length from 4 to 26 and of various degrees of unsaturation from monoenes to pentaenes. The unsaturated acids exist in many positional and geometrical isomeric forms. The composition of milk fats is influenced by diet and by the plane of nutrition of the animal. The general distribution patterns of fats have been studied, but no sound conclusions can be drawn about milk fats. Investigative tools are now becoming available whereby both the general distribution pattern and the specific distribution and location in glycerides of individual fatty acids can be determined.**

FATS have always had a special place in the diet of man. They are important because of their high caloric value—2.25 times that of equal weights of carbohydrate or protein—but, perhaps more important are the noncaloric functions. These have been reviewed by Burr and Barnes (*4*) and include such things as vitamin-carrying functions as well as direct vitamin activity by the essential fatty acids. The property of improving the palatability of foods should not be overlooked. When properly distributed among the muscle fibers of meat, fats contribute to tenderness and also have a general lubricating effect for the passage of otherwise dry foods through the mouth. Certain fats such as butter also contribute appetizing flavors to many foods.

Because fats play such an important role in the human diet, it is essential to know their composition, structure, and properties for the understanding of their digestion, absorption, and metabolism as well as their technology and preservation. Research in the chemistry of fats has lagged behind that of other substances of biological importance. Far-reaching advances were made in the chemistry of proteins and carbohydrates in the 19th century, but it was not until well into this century that much interest centered on fats. Within the last 25 to 30 years, however, much progress has been made. This progress has been greatly accelerated in recent years by the great advances in instrumentation analyses. The development and adoption of countercurrent distribution separation, infrared and ultraviolet spectrometries, and gas-liquid partition chromatography as analytical tools make routine a study of complex substances that was impossible or impractical a few years ago.

The milk fats among the major food fats are of interest because of their intended use directly as food for the newly born animal, in contrast to the depot fats which are storage reservoirs. The fat of egg yolk is the only other animal fat intended solely for this purpose. The milk fats contain a great variety of fatty acids and are characterized particularly by their content of the shorter chain fatty acids—butyric, caproic, caprylic, and capric—and of those in the monoethenoid series from decenoic through octadecenoic. Most tables of composition for milk fats contain nine saturated and seven unsaturated fatty acids of sufficient quantitative importance to justify listing, whereas in the major depot food fats, three saturated and two unsaturated acids are the ones usually reported.

## *Fatty Acid Composition*

Milk fat occurs as an emulsion in milk and contained within the complex are a number of lipide materials. These include triglycerides, free fatty acids, possibly mono- and diglycerides, phospholipides, and a heterogeneous group designated as unsaponifiable matter. The fatty acids present in the free and glyceride state are discussed here. They are available from three sources: ingested fat, depot fat, and in the case of ruminants, those resulting from rumen metabolism. Table I shows a comparison of the major fatty acid composition of milk fats from animals of different species. The quantities of each will vary depending on many factors, but the listed values are representative.

The amounts of the three shortest chain acids—$C_4$ to $C_8$—are small in the fats of the nonruminants, but are substantial in those of the ruminants. Is this because of different mechanisms of syntheses or because of different dietary needs of the young?

**Factors Influencing Fatty Acid Composition.** It seems reasonable that the dietary needs of the young are a major factor in determining the inclusion of the main fatty acid constituents. The most convincing evidence is the behavior of young calves, when fed rations containing milk fats and others containing fats from plant sources. The animals do not thrive on these foreign fats.

The values for the amounts of the different fatty acids in cow's milk fat in Table I are representative. However, there may be a considerable quantitative variation depending on several factors (*15*). The character of feed will influence the composition of the fat. Concentrate feeds rich in unsaturated fatty acids tend to produce milk fats with an increased unsaturation. Pasture grasses also increase the unsaturation of the milk fat produced from them. The type of feed also affects the total quantity of fat produced. Rations with little or no roughage produce a milk of substantially lower fat content than those with a liberal amount of roughage. Whether or not the composition of the fat is affected by the roughage content of the ration has not been determined fully, but preliminary observations indicate that along with a decrease in the amount of fat there is a pronounced increase in the degree of unsaturation, particularly in the polyethenoid acids.

The plane of nutrition has a significant effect on milk fat composition. Underfeeding results in a decrease in lower molecular weight volatile fatty acids and an increase in unsaturated acids. Other factors such as environmental temperature and stage of lactation have been reported to influence the

**Table I. Major Fatty Acid Components of Milk Fats from Different Species**

| Animal Species | Component Acids, Weight %: Saturated | | | | | | | | | Unsaturated | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $C_4$ | $C_6$ | $C_8$ | $C_{10}$ | $C_{12}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{20}$ | $C_{10}$ | $C_{12}$ | $C_{14}$ | $C_{16}$ | $C_{18}$ | $C_{20}$ | Polyethenoid |
| Nonruminants | | | | | | | | | | | | | | | | |
| Human (*3*) | 0.4 | 0.1 | 0.3 | 2.2 | 5.5 | 8.5 | 23.2 | 6.9 | 1.1 | 0.1 | 0.1 | 0.6 | 3.0 | 36.5 | 7.8 | 3.3 |
| Mare (*12*) | 0.4 | 0.9 | 2.6 | 5.5 | 5.6 | 7.0 | 16.1 | 2.9 | 0.3 | 0.9 | 1.0 | 1.8 | 7.5 | 42.4 | 5.1 | |
| Sow (*5*) | | | 1.3 | | | 1.5 | 26.9 | 6.5 | | | | | 8.3 | 36.7 | 4.2 | 14.6 |
| Ruminants | | | | | | | | | | | | | | | | |
| Cow (*13*) | 3.5 | 1.4 | 1.7 | 2.7 | 4.5 | 14.7 | 30.0 | 10.4 | 1.7 | 0.3 | 0.2 | 1.5 | 5.7 | 18.7 | 1.0 | 2.1 |
| Goat (*12*) | 3.0 | 2.5 | 2.8 | 10.0 | 6.0 | 12.3 | 27.9 | 6.0 | 0.6 | 0.3 | 0.3 | 0.8 | 2.6 | 21.1 | 0.2 | 3.6 |
| Buffalo (*1*) | 5.8 | 0.6 | 0.9 | 1.0 | 1.6 | 9.0 | 35.2 | 15.3 | 0.1 | 0.1 | 0.1 | 0.6 | 3.3 | 20.5 | 4.4 | 1.5 |
| Sheep (*12*) | 2.8 | 2.6 | 2.2 | 4.8 | 3.9 | 9.7 | 23.9 | 12.6 | 1.1 | 0.1 | 0.1 | 0.6 | 2.2 | 26.3 | 1.9 | 5.2 |

composition of milk fat. However, the effects reported cannot be distinguished from those resulting from a lowered plane of nutrition and no clear-cut conclusions can be drawn. Environmental temperatures above 85° F. cause a decrease in the volatile acids and an increase in the degree of unsaturation. Whether or not these changes are the result of temperature per se or of "hyperthermic undernutrition" cannot be stated. The latter seems the more probable.

**Minor Saturated Fatty Acids.** Improved analytical techniques and instruments have made possible the isolation and identification of a large number of fatty acids formerly thought not to exist in milk fat, and of some present in recently identified isomeric forms. The work of Shorland and Hansen (*10*, *21*) has been closely identified with the establishment of the presence of *n*-odd numbered acids, branched chain acids, and *n*-even numbered high molecular weight, saturated acids. Table II shows the reported occurrence of these acids.

The presence of these acids, particularly the odd-numbered acids and those with branched chains, is unexpected in the classical concept of fat composition and is of considerable theoretical interest. However, their significance to the physiologist, the biochemist, the nutritionist, and the technologist is not yet established.

**Table II. Some Minor Saturated Fatty Acids in Cow's Milk Fat**

| n-Even Numbered Acids | Weight % | Branched Chain Acids | Weight % |
|---|---|---|---|
| $C_{22}$ | 0.07 | $C_{13}$ (iso)[a] | 0.05 |
| $C_{24}$ | 0.05 | $C_{13}$ (anteiso)[b] | 0.01 |
| $C_{26}$ | 0.06 | $C_{14}$ (iso) | 0.05 |
| *n-Odd Numbered Acids* | | | |
| $C_{11}$ | Trace | $C_{15}$ (anteiso) | 0.43 |
| $C_{13}$ | 0.03 | $C_{17}$ (iso) | Trace |
| $C_{15}$ | 0.82 | $C_{17}$ (anteiso) | 0.41 |
| $C_{17}$ | Identified | $C_{18}$ | Trace |
| $C_{19}$ | Identified | $C_{18}$ (multibranched) | Trace |
| $C_{21}$ | 0.05 | | |
| $C_{23}$ | 0.06 | | |

[a] The iso acids have the methyl group in the penultimate position.
[b] The anteiso acids have the methyl group in the antepenultimate position.

**Table III. Polyethenoid Fatty Acids of Milk Fat**

(Average values, weight %)

| Conjugated: Diene | Conjugated: Triene | Conjugated: Tetraene | Nonconjugated: Diene | Nonconjugated: Triene | Nonconjugated: Tetraene | Nonconjugated: Pentaene |
|---|---|---|---|---|---|---|
| 0.89[a] | 0.02[a] | 0.003[a] | 1.45[a] | 0.83[a] | 0.35[a] | 0.02[b] |

[a] (*22*). [b] (*20*).

**The Unsaturated Acids.** The kinds of unsaturated fatty acids present illustrate the compositional and the configurational complexity of milk fat. Table I shows the major groupings usually considered, but within these groupings are a large number of different individual acids, different with respect to the location of the double bonds and to their geometric configuration.

Smith, Freeman, and Jack (*22*) have confirmed earlier reports that the lower homologs of oleic acid occur in milk fat. The location of the double bond in these lower homologs generally has been reported only in the 9-10 position. However, Gupta, Hilditch, and Paul (*8*) and others have similarly confirmed the occurrence of vaccenic acid (*trans*-11-octadecenoic acid). The monoethenoid acids were all thought to be exclusively in the cis form until Smith *et al.* (*22*) showed the presence of trans acids. Backderf (*2*) found also a *trans*-16-octadecenoic acid. Thus, the monoethenoid acids exhibit both positional and geometric isomerism.

The polyethenoid acids, at one time thought to be mainly isomers of linoleic acid—*cis-trans*-9, 12- and *trans-cis*-9, 12-octadecadienoic acids—have been shown to be much more complicated. The length of the carbon chain of these acids extends to at least 22 carbons (*20*) —docosapentaenoic acid—with both conjugated and nonconjugated forms and all possible combinations of geometric isomers. Table III shows average values for the amounts of the polyethenoid acids in terms of their degree of unsaturation and their conjugation.

## Glyceride Composition

Milk fat contains a large number and a great variety of fatty acids.

**Distribution Patterns.** Two main proposals have been made to account for the distribution of fatty acids in the glyceride molecule. These are "even distribution" and "random distribution." Hilditch (*11*) has been the foremost advocate of the even distribution theory. According to this scheme, a fatty acid will not repeat itself in a glyceride until it exceeds 30 to 35% of the total acids and will not form a simple triglyceride until it exceeds 65% of the total acids. Longenecker and others (*6*, *7*, *16*, *17*), on the other hand, proposed that the fatty acids are distributed indiscriminately throughout the molecule, according to the mathematical rules of randomness.

Hilditch claims that the rule of even distribution holds for fats from plant sources, but requires modification for animal fats. He believes that even in the case of animal fats, including milk fat, which diverge somewhat from the general pattern the unsaturation is evenly distributed. Some investigations by the author (*14*) a few years ago, based on permanganate oxidation of the un-

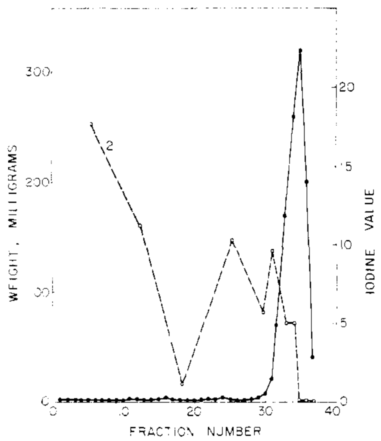


Figure 1. Countercurrent distribution of +4° C. precipitate

1. Weight of sample
2. Iodine value of sample

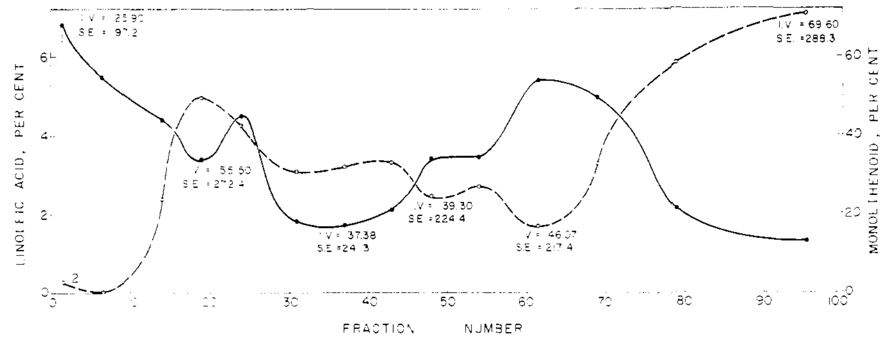


Figure 2. Countercurrent distribution separation of mono- and polyethenoid acids in −53° C. filtrate

1. Polyethenoid acids
2. Monoethenoid acids

**Table IV. Distribution of Fatty Acid Types in −53° C. Filtrate Fraction**

| Sap. Equiv. | $I_2$ Value | Average Length Carbon Chain (approx.) | Mole % | | |
|---|---|---|---|---|---|
| | | | Satd. Fatty acids | Poly-ethenoid | Mono-ethenoid |
| 288.3 | 69.60 | 18 | 28.6 | 2.4 | 69.0 |
| 272.4 | 55.50 | 17 | 40.9 | 5.1 | 54.0 |
| 241.3 | 37.38 | 15 | 70.5 | 3.0 | 26.5 |
| 224.4 | 39.30 | 14 | 75.9 | 5.3 | 18.8 |
| 217.4 | 46.07 | 13 | 78.1 | 9.5 | 12.4 |
| 197.2 | 25.90 | 12 | 91.1 | 7.1 | 1.8 |

saturated acids, supported Hilditch's thesis for this group of acids in milk fat.

The theory of random distribution also has been modified by most proponents and it is generally proposed, based on analytical data obtained, that the strict random pattern does not hold rigidly. As the investigations become more extensive and the analytical tools more refined, it becomes increasingly evident that some mechanism for selectivity is operative in the synthesis of most natural fats.

**Countercurrent Distribution Separation of Milk Fat.** Data obtained in the author's laboratory (*9*) using countercurrent distribution separation on fractions obtained by solvent precipitation provide evidence for some degree of selectivity in the synthesis of milk fat. The fractions chosen as most interesting for detailed study were the most saturated fraction precipitating at +4° C. and the most liquid fraction appearing as the −53° C. filtrate.

The countercurrent distribution of the +4° C. precipitate and the iodine values of the individual samples are shown in Figure 1. There is a considerable quantity of glycerides in this fraction that has an iodine value of practically zero. These completely saturated glycerides were converted to methyl esters and then analyzed by gas chromatography. The molar percentage of the esters were: methyl laurate, 0.2; methyl myristate, 3.6; methyl palmitate, 80.4; and methyl stearate, 15.7. This amount of methyl palmitate, 80.4 mole %, requires, therefore, that some of the glycerides from which the esters were prepared be tripalmitin.

The countercurrent distribution of the −53° C. filtrate shows an interesting separation of the monoethenoid and the polyethenoid acids (Figure 2). Early fractions are high in polyethenoid acids and low in monoethenoid; intermediate fractions are intermediate in amounts of each, while latter fractions are high in monoethenoid acids and low in polyethenoid. Study of the saponification equivalents of the samples in relation to the degree of unsaturation shows a definite relationship (Table IV) wherein the longer chain saturated acids are associated in the glyceride with the monoethenoid acids and a less definite relationship, but nonetheless a tendency for the low molecular weight acids to be associated with the polyethenoid.

Thus it can be said with some confidence that there is some specificity in the structure of milk fat glycerides.

**Enzymatic Lipolysis Studies.** Perhaps of greater importance than the general distribution pattern of the fatty acids is the specific location in the glyceride molecule of the different types and of the individual acids. Savary *et al.*(*19*) and others have developed a technique using controlled enzymatic lipolysis to split off the terminal fatty acids and leave β-monoglycerides. This technique has been applied to a number of food fats, with the result that it has been established that in most fats the unsaturated acids are mainly in the β, or internal, position, whereas in pig body fat the unsaturated acids are in the α or external position. Only one report has been published about the application of this technique to milk fat. Patton and associates (*18*) have suggested that the unsaturated fatty acids are in the external position corresponding in this respect to pig body fat.

When the total composition and the exact configuration of this important food are known, it will aid greatly in the better understanding of the physiology of milk synthesis, the biochemistry of its digestion and metabolism, and in the technology of processing.

### Literature Cited


(1) Achaya, K. T., Banerjee, B. N., *Biochem. J.* **40,** 664 (1946).
(2) Backderf, R. H., Ph.D. thesis, Ohio State University, Columbus, Ohio, 1956.
(3) Baldwin, A. R., Longenecker, H. E., *J. Biol. Chem.* **155,** 407 (1944).
(4) Burr, G. O., Barnes, R. H., *Physiol. Revs.* **23,** 256 (1943).
(5) de la Mare, P. B., Shorland, F. B., *Nature* **153,** 38 (1944).
(6) Deuel, H. J., "Lipids," Vol. **1,** p. 224, Interscience, New York, 1951.
(7) Doerschuk, A. P., Daubert, B. F., *J. Am. Oil Chemists' Soc.* **25,** 425 (1948).
(8) Gupta, S. S., Hilditch, T. P., Paul, S., Shrivastava, R. K., *J. Chem. Soc.*, **1950,** 3484.
(9) Haab, W., Smith, L. M., Jack, E. L., *J. Dairy Sci.* **42,** 454 (1959).
(10) Hansen, R. P., Shorland, F. B., Cooke, N. J., *J. Dairy Research* **26,** 190 (1959).
(11) Hilditch, T. P., *Ann. Rev. Biochem.* **22,** 125 (1953).
(12) Hilditch, T. P., Jasperson, H., *Biochem. J.* **38,** 443 (1944).
(13) Jack, E. L., Henderson, J. L., *J. Dairy Sci.* **28,** 65 (1945).

(14) Jack, E. L., Henderson, J. L., Hinshaw, E. B., *J. Biol. Chem.* **162,** 119 (1946).
(15) Jack, E. L., Smith, L. M., *J. Dairy Sci.* **39,** 1 (1956).
(16) Kartha, A. R. S., *J. Am. Oil Chemists' Soc.* **31,** 85 (1954).
(17) Longenecker, H. E., *Chem. Revs.* **29,** 201 (1941).
(18) Patton, Stuart, Evans, Laura, McCarthy, R. P., *J. Dairy Sci.* **43,** 95 (1960).
(19) Savary, P., Flanzy, J., Desnuelle, P., *Biochim. et Biophys. Acta* **24,** 414 (1957).
(20) Scott, W. E., Herb, S. F., Magidman, Paul, Riemenschneider, R. W., J. AGR. FOOD CHEM. **7,** 125 (1959).
(21) Shorland, F. B., Hansen, R. P., *Dairy Sci. Abstr.* **19,** 168 (1957).
(22) Smith, L. M., Freeman, N. K., Jack, E. L., *J. Dairy Sci.* **37,** 399 (1954).

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**RICE PROTEIN SUPPLEMENTATION**

# Further Studies on the Nutritional Improvement of Rice

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**The proteins of whole and milled rice can be effectively supplemented by those of whole milk solids, lactalbumin, and defatted whole egg. Data are presented on the content of amino acids (essential and nonessential), vitamins, and other constituents of milk solids, and defatted whole egg. Lactalbumin was tested for total amino acid content. The results of these experiments show the extent to which rice rations can benefit from addition of small amounts of milk solids, lactalbumin, and whole egg. This is of importance in those areas of the world where much rice is consumed and where these high protein foods are economically available.**

IN AN ATTEMPT to improve rice diets further, studies were made with whole milk powder, nonfat dried milk (skimmilk), lactalbumin, and nonfat whole egg powder as supplements to whole rice and to white milled rice. An investigation was started on the effect of replacing whole rice proteins with those of milk solids and powdered whole egg; 1, 3, and 5% of these solids replaced equivalent amounts of proteins in milled and whole rice rations which were fed to albino rats for 70 days. Another series of experiments was made on the effect of adding small amounts of these solids to rations containing rice as the only source of protein. This paper also reports studies on the content of all members of the vitamin B complex, amino acids (including nonessentials), calcium, phosphorus, and iron, and on the growth value of the proteins of samples of commercial dry milk solids, lactalbumin, and whole egg powder.

### *Experimental Procedure and Results*

Commercial samples of dry milk solids (spray-dried whole milk, nonfat dry milk solids, or skimmilk), lactalbumin, and fat-extracted whole egg powder were used for the determination of vitamins, minerals, amino acids, and supplementary and growth values of the proteins.

Supplementary values were determined in studies using albino rats as experimental animals fed milled white rice rations containing 5.5% protein and whole rice rations containing 6.38% protein. Milled and whole rice furnished the only source of protein in these rations and at levels to incorporate the necessary protein. The per cent of protein content of the white milled rice was 6.45, of whole brown rice 7.25, of dried whole milk 25.0, of fat-extracted whole egg 65.8, of lactalbumin 80.7, and of skimmilk 36.2. The composition of the rest of the rations was 4% of salt mixture No. 1 (*6*), 4% of hydrogenated vegetable shortening, 2% of cellu flour, 2% of cod liver oil, and 1% of wheat germ oil as sources of vitamins A, D, and E, and the rest as glucose (cerelose).

In the protein replacement experiments, the other solids were added at three levels—1, 3, and 5%; the proteins of these solids were added at the expense of the rice proteins, leaving the total protein in the ration the same. In the protein addition experiments, the solids were added also to the basal ration at these levels of 1, 3, and 5% at the expense of cerelose, and the protein content was slightly increased.

The following crystalline components of the vitamin B complex were administered daily to each animal separately from the rations with double doses on Saturday: 25 γ each of thiamine, riboflavin, pyridoxin, and niacin; 150 γ of calcium pantothenate; 3 mg. of *p*-aminobenzoic acid; 6 mg. of choline chloride; and 1 mg. of inositol. The animals, 28 to 32 days old when started on the experiment and weighing about 50 grams each, were divided in two groups, each having equal numbers of both sexes; they were fed for 70 days. Each animal was weighed weekly and accurate weekly records for food consumption were kept. From these data the protein efficiency ratios were calculated and expressed as gains in body weight per gram of protein intake.

In the study (*3*) of the supplementary value of the proteins of whole milk solids for those of white milled rice, an equivalent amount of the proteins of rice was replaced by the proteins of 1, 3, and 5% whole milk powder, leaving the protein level at 5.55. In experiments reported here, 1, 3, and 5% of whole milk were added, which increased the protein level to 5.86, 6.24, and 6.62%, respectively (Table I, *A*, ration 9 listed).

The protein content of the various foods was determined from nitrogen