The Flavor Potential of Milk Fat. A Review of Its Chemical Nature and Biochemical Origin[1, 2]

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Abstract

Actual and potential flavors are among the most important attributes of milk fat. In some dairy products (milk, cream, ice cream, uncultured butter) the aim is to retain the faint pleasant flavor associated with the fat of fresh milk. However in cooking, baking, and many processed food applications the object is to generate buttery, creamy, cheesy, and caramel-like flavor qualities from milk fat. Although the ultimate chemical characterization of all these flavors has not been achieved, the existing information is reviewed in this paper. Some recent findings concerning the biological variability and significance of the lactone and ketone flavor potential of milk fat are also presented. A scheme which outlines means for more effectively utilizing the flavor capabilities of milk fat is included.

Introduction

WITH THE ADVENT of modern micro-analytical techniques there has been a rapid and productive expansion in research concerning flavor chemistry, and dairy products have been perhaps the most thoroughly analyzed (1–6). These investigations were approached with the aim of either elucidating the chemical quality of desirable flavors or defining and characterizing the compounds responsible for the development of off-flavors in dairy products.

Food flavor is a complex of at least three factors, viz., aroma, taste, and texture, perceived principally by the olfactory, gustatory, and tactile senses respectively. Other mental impressions such as color, sound, and previous experiences may modify one's definition of a flavor. Flavor is generally judged as the total physiological response, and for practical purposes this is calibrated against physicochemical analyses. The flavors of dairy products are diverse, and what is the sine qua non of one product may be highly distasteful in another, e.g., the methyl ketones of blue cheese are undesirable in fresh cream, or the low-molecular-weight fatty acids so essential for cheddar flavor (7) spoil the flavor of fresh beverage milk. Paradoxically fresh milk is rather devoid of flavor, and its slight olfactory character is attributed to such low-molecular-weight compounds as acetone, acetaldehyde, methyl sulfide, traces of C_4–C_{10} fatty acids, methyl ketones, and lactones. The major flavor sensation of milk is ascribed to its tactile effect and its pleasant in the mouth feel, mainly because of its emulsion and colloidal structure.

The flavor potential of milk, especially milk fat, is prodigious because of the ability of milk fat in various products, especially those normally characterized by mild flavor (fluid milk, cream, butter) to produce off-flavors. Many of the compounds have been identified, and it is evident that milk fat can produce a multitude of flavor compounds, classified as oxidatively and non-oxidatively generated. Microbial metabolism (cheese ripening) can also produce an array of flavor compounds from milk fat, but these are not discussed herein.

Oxidative Flavors

Some of the descriptive terms applied to oxidized off-flavors found in milk fat are shown in Table I. These are products of the autoxidation of the unsaturated fatty acids, mainly oleic, linoleic, and linolenic associated with phospholipids. Triglycerides and cholesterol esters may also be involved but to what extent is unknown (8,9). Some of these substances may have a direct dietary origin (50). These compounds have an extremely high flavor potency and are organoleptically detectable at very low concentrations (Table II). Despite the similarity in the qualitative carbonyl content of these off-flavor categories, the actual perceptible flavor differences are pronounced because of variation in the quantity and proportions of active components and groups of components and the interaction of these, i.e., additive, synergistic, antagonistic effects.

The preponderance of certain groups of carbonyls impart specific flavors. Forss (10) found that the quantity of carbonyls in "painty" butterfat was much greater than in "fishy" though the qualitative composition was rather similar. Day (11) has significantly shown that these compounds, even at subthreshold concentrations, compositely give rise to a characteristic oxidized flavor, i.e., an additive effect, particularly among the aldehydes. Octenone alone is organoleptically perceived as metallic, but in the presence of some aldehydes this assumes an oxidized flavor (12). Meijboom (13) also studied the flavor properties of these aldehydes and indicated that they have a lower taste than odor threshold. In contrast to Day's findings he reported some antagonistic effects between certain flavorful aldehydes, e.g., decadienal and (*trans*-2-nonenal) could completely mask

TABLE I

Some Descriptive Flavors and Associated Compounds Identified in Oxidized Milk Fat

Flavor[a]	Compounds
Oxidized	oct-2-ene-3-one, octanal, hept-2-enal, 2,4-heptadienal, n-alkanols (C_2–C_9)
Cardboard, tallowy	n-octanol, n-alkanals (C_9, C_{11}), alk-2-enals (C_6, C_9), 2, 4-dienals (C_7, C_{10}), 2, 6-dienal (C_9)
Oily	n-alkanals (C_5, C_6, C_7), hex-2-enal, 2, 4-dienals (C_5, C_{10})
Painty	n-alkanals (C_5–C_{10}), alk-2-enals (C_5–C_{10}), 2,4-dienal (C_7),2-alkanone (C7)
Fishy	n-alkanals (C_5–C_{10}), alk-2-enals (C_5–C_{10}), 2,4-dienal (C_7), 2-alkanones (C_3–C_{11}), oct-1-ene-3-one
Grassy	alk-2-enal (C_6), 2,6-dienal (C_9)
Metallic	oct-1-en-3-one
Beany	alkanals, non-2-enal
Mushroom	oct-1-en-3-ol
Cucumber	2,6-dienal (C_9)
Nutmeg	octadienal; 2,4-dienals
Creamy	4-cis-heptenal
Fruity	n-alkanals (C_5, C_6, C_8, C_{10})

[a] Compiled from References 4, 8, 9, 10, 11, 12, 16, 51, 53.

[1] Authorized for publication on December 1, 1966 as Paper No. 3198 in the Journal Series of the Pennsylvania Agricultural Experiment Station.
[2] Presented at the AOCS Meeting, Philadelphia, October 1966.

TABLE II

Flavor Threshold Values (FTV) of Autoxidation Products from Milk Fat

Compounds	FTV in milk (3.8%) (ppm)		Reference
Ethanal	1.20		(50)
Propanal	0.43		(11)
Butanal	0.19		(11)
Pentanal	0.13		(11)
Hexanal	0.05		(11)
Heptanal	0.12		(11)
Octanal	0.46		(11)
Nonanal	0.22		(11)
Decanal	0.24		(11)
n-hex-2-enal	0.067		(11)
n-hex-*trans*-2-enal	0.10	(skim)	(51)
n-hept-*cis*-4-enal	0.0015	(butterfat)	(16)
n-hept-2-enal	0.077		(11)
n-non-2-enal	0.0042		(11)
n-deca-2-enal	0.092		(11)
n-hepta-2, 4-dienal	0.049		(11)
n-nona-2, 6-dienal	0.01	(oil)	(12)
n-deca-2, 4-dienal	0.0005	(water)	(52)
oct-1-ene-3-ol	0.01	(skim)	(53)
oct-1-ene-3-ol	0.01	(skim)	(53)

the perception of *cis*-3-hexenal flavor even when the concentration of the latter was far above its flavor threshold value (FTV). The presence of double bonds, their number and stereochemical configuration, and the length of the hydrocarbon chain also influence the flavor quality and intensity.

Finally the physical nature of the medium in which these compounds are dispersed affects their perceptible threshold concentration (14). Generally flavor potential is much stronger in an aqueous (lipophobic) than in an oil (lipophilic) medium. This also depends on the polarity of the particular flavor compound. Thus substances of low polarity (long-chain hydrocarbons) have low FTV in aqueous media whereas more polar substances have lowest FTV in lipophilic media. In dairy products a much lower FTV is obtained by using liquid milk rather than butter oil as a dispersing medium, e.g., oct-1-en-3-ol has FTV of 1 part in 10^8 parts of skim milk, and 1 in 10^7 parts of butter oil; lactones have FTV's of 1–2 ppm in milk and 5 ppm in butter oil (15).

Most of these products of lipid autoxidation, at detectable concentrations, are undesirable because of their adverse effect on flavor although one characterized exception is *cis*-4-heptenal. This substance imparts a creamy flavor to some dairy products (butter, cream fudge) at an average level of 1.5 parts per billion and is commercially used as a flavoring agent. It arises from the autoxidation of isolinoleic acid (16).

Nonoxidative Flavors

The triglyceride fraction of milk, which composes approximately 98% of the fat elaborated and secreted by the mammary gland, is the source of characterized flavors, which are potentiated by nonoxidative mechanisms. These involve hydrolysis of enol-ether linkages to yield long-chain aldehydes (17), which impart a waxy candle-like flavor to milk lipids and hydrolysis of acyl bonds to liberate normal, β-keto, gamma, and delta hydroxy fatty acids. The keto acids may be subsequently decarboxylated to yield methyl ketones, and the hydroxy acids can spontaneously lactonize to yield lactones.

Hydrolysis of acylated glycerides is generally undesirable on account of the ensuing rancidity of the liberated volatile fatty acids, C_4 through C_{12} (18). In certain dairy products (fresh cream butter, cheese, milk chocolate) and in some confectionery items the presence of limited amounts of unesterified low-molecular-weight fatty acids is required for optimum flavor. At low levels these are undoubtedly important in the characteristic flavor of milk and cream.

The methyl ketones and lactones occurring in heated milk fat are highly flavorful compounds and, though undesirable except in trace quantities in beverage milk, they are important constituents in the subtle flavor of butter and the pleasant flavors associated with pastries and confectioneries which are prepared with butter shortenings. These two series of trace flavor compounds which are commercially important present a unique problem involving their biochemical origin on one hand and the elucidation of their function in milk lipid synthesis and secretion on the other.

Since these substances are the products of endogenous bovine metabolism, the flavor potential of milk fat is subject to biological variation and should vary with the physiological state of the animal producing it. The authors are now studying the parameters which may influence the production of these flavor compounds, i.e., diet, breed, season, and stage of lactation. They are also investigating the precise metabolic origin of these flavor precursors and the factors which stimulate or depress their production. It is anticipated that the knowledge gained from these studies will enable the food technologists of the future to utilize milk fat so as to maximize its rich flavor capabilities.

Methyl Ketones

Parks and Patton (19) first reported the existence of a homologous series of saturated normal methyl ketones with odd-numbered carbon atoms (C_3–C_{15}) in low temperature steam distillate of dry whole milk. Tharp and Patton (20) identified the C_3–C_{15} n-2-alkanones in steam distillates and unsaponifiable substances from milk fat; they also demonstrated the occurrence of keto alkanoic acids in milk fat. Wong and Patton (21) isolated the C_4 through C_7 methyl ketones from the cold distillate of milk and cream and suggested that these originated by decarboxylation of β-keto acids. Winter et al. (22) showed that heptanone and nonanone were normal flavor constituents of butter flavor. These occur in all products that contain heated milk fat, also in concentrated stored dairy products. The concentration of methyl ketones in various samples of butterfat is presented in Table III. Both the individual and total concentrations vary widely between each sample. Schwartz et al. (23) reported that the total quantity of these methyl ketones can range from 0.4 μ moles to 1.75 μ moles per gram of butterfat.

To ascertain the actual precursor of these 2-alkanones Van der Ven et al. (24) used Girard's T reagent. This forms adducts with carbonyl compounds to facilitate their separation and, on heating,

TABLE III

Concentration of Alk-2-ones in Different Samples of Butter Oil (ppm)

Carbon No.	Sample				
	A (54)[a]	B (5)	C (25)	D (24)	E (26)
C_3	42.0	.....	...	2.8	13.9
C_5	13.0	....	6.0	12.3	20.6
C_7	25.0	12.0	16.3	8.0	37.8
C_9	16.0	9.0	9.5	10.0	17.8
C_{11}	14.0	13.0	12.1	18.2	20.4
C_{13}	24.0	22.0	14.0	17.6	44.5
C_{15}	46.0	42.0	36.4	....	65.2
Total	180.0	98.0	94.3	68.9	220.2

A—0.88 μmoles/g butterfat (54)
C—0.60 μmoles/g butterfat (25)
E—1.75 μmoles/g butterfat (26)
F—1.20 μmoles/g butterfat (23)

[a] Reference number.

these adducts form pyrazolones. They isolated a homologous series of six pyrazolones from butterfat, which corresponded to the six even-numbered carbon β-keto fatty acids C_6 to C_{16}. These workers asserted that these β-keto acids were the ketone precursors and existed in milk fat esterified to a glyceride, which totally amounted to 0.03% of butterfat. Pursuing this study further, Parks et al. (25) actually isolated the β-keto acid containing glycerides. These contained two other fatty acids normally found in butterfat except that palmitic acid was usually a little higher. They estimated that β-ketoglyceride amounted to 0.045% of butterfat. The structure of this glyceride is presented in Figure 1, which shows the mechanism of the formation of methyl ketones from their precursors. Langler and Day (26) determined that, by heating milk fat for three hours at 140C in the presence of trace amounts of water (.0031%), the maximum quantities of methyl ketones were generated.

With 1-^{14}C acetate as tracer, the endogenous biochemical origin of these methyl ketone precursors was investigated in the bovine by Lawrence and Hawke (27). They concluded that the β-keto acids arise biosynthetically by acetate condensation and that both the methyl ketone precursors and the normal fatty acids originate from the same acetate pool in the mammary gland. They suggested that these β-keto acids are apparently unfinished fatty acids which, during synthesis, become dissociated from their acyl carrier protein (ACP) prior to completion of the normal biosynthetic steps. These free β-keto acids are then incorporated into a glyceride without further reduction; or the β-keto acyl-ACP intermediate may be incorporated into a forming glyceride as a normal process and then escape the normal reductive steps because of rapid removal of the ketoglyceride from the site of synthesis (Figure 2). The biochemical conditions governing the quantity of keto acids synthesized are unknown, and it is particularly puzzling that no correlation exists between the relative amounts of methyl ketones and the amounts of the corresponding saturated fatty acids. Octanoic acid is low in milk fat, yet the corresponding β-keto octanoic (2-heptanone precursor) acid is relatively abundant. It may be speculated that certain β-keto acyl groups dissociate from their synthesizing enzymes more easily than others, or the free β-keto acids may differ in their affinity for the forming glycerides.

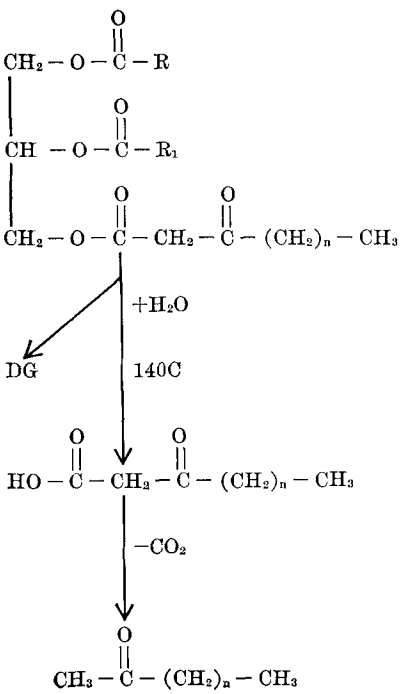

FIG. 1. Mechanism of formation of alk-2-ones.

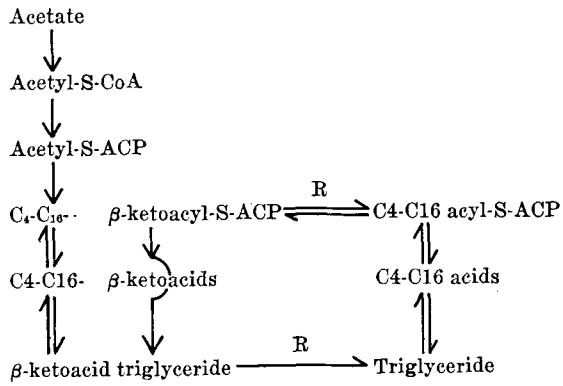

FIG. 2. Proposed mechanism, showing origin of ketoglyceride.

Langler and Day (26) evaluated the flavor properties of these compounds in milk (Table IV). They found that flavor potency varied with carbon-chain length and showed that heptanone had the lowest FTV. Subthreshold concentrations of the individual ketones interacted in a synergistic manner and imparted a perceptible flavor to milk. The total methyl ketone potential of normal beverage milk was above the minimum FTV and, when maximally developed, a distinct flavor deterioration of the milk ensued. Thus a slight generation of these methyl ketones is organoleptically detectable, and the pleasant flavor attributes of many dairy products (milk, cream, butter) probably depend on the optimum production of these ketones whereas excessive generation of those compounds impart abnormal and hence undesirable flavors.

Lactones

Keeney and Doan (28) were the first workers to intimate that aliphatic lactones occurred in milk fat and imparted a coconut flavor thereto. In 1956 Keeney and Patton (29) identified delta decalactone (δ-C_{10}) in milk fat, and Tharp and Patton (20) isolated the δ-C_{10} δC_{12} lactones from the steam distilltae of butterfat. These workers were concerned with the involvement of these lactones in the off-flavor development of dry whole milk. Commercial interests were obviously concerned with the possible use of lactones as flavoring agents and were busily engaged in research during this time, as evidenced by two patent applications (30) claiming that both gamma (γ) and delta (δ) lactones imparted a buttery flavor to margarine. In 1962 Boldingh and Taylor (5) summarized the findings of 15 years of research on lactones. They reported the occurrence of a homologous series of γ- and δ-lactones in butterfat.

TABLE IV
Average Flavor Thresholds (AFT) of Alk-2-ones (ppm) (26)

Methyl ketone	AFT	Mixture relative concn. at AFT	Maximum potential of 4% milk fat
C_4	79.50	0.13	0.52
C_5	8.38	0.20	0.80
C_7	0.70	0.38	1.52
C_9	3.48	0.18	0.72
C_{11}	15.50	0.20	0.80
C_{13}	18.43	0.46	1.84
Total	126.00	1.50	6.20

TABLE V

Amounts of γ- and δ-Aliphatic Lactones Isolated from Butterfat (ppm)[a]

Carbon No.	δ-Lactones	γ-Lactones
C_8	2.0	t
C_7	(0.2)[b]	....
C_8	2.6	0.5
C_9	(0.4)[b]	0.2
C_{10}	15.0	1.2
C_{11}	0.7	0.5
C_{12}	35.0	1.6
C_{13}	1.5	0.5
C_{14}	34.0	1.4
C_{15}	6.4	1.3
C_{16}	23.2	1.3
C_{18}	(2.3)[b]	....
2,3 dimethyl-2,4-nonadien-4-olide		0.5

[a] Compiled from References 5, 20, 31, 32, 34, 47, 55.
[b] Semiquantitative.

Using the isotope dilution technique, Jurriens and Oele (31) quantified these lactones by column and gas chromatography. We have identified some additional ones in the homologous series (32). Table V summarizes the present situation, showing that the total lactone potential of milk fat is approximately 110 ppm. The FTV of these lactones is 1–2 ppm in skim milk and 5 ppm in butterfat. Hence a mere trace of these odoriferous (coconut-like) compounds in beverage milk is optimal whereas 5–10 ppm are desirable in butter. Activation of the total lactone potential of butterfat results in distasteful dairy products whereas in confectioneries and high-quality candies it is a major source of their unique and titillating flavor characteristic.

Freshly secreted milk contains an almost negligible quantity of free lactones, and its lactone potential is realized by heating or acidifying. This observation implies that the lactone precursor is present in bound form, and Patton (34) suggested that the lactone precursors were the corresponding γ- and δ-hydroxy alkanoic acids esterified in a glyceride molecule. This has been confirmed by infrared absorption spectroscopy, column, thin-layer, and gas-liquid chromatography (31–33). A chromatographic scan of the delta lactones, isolated by silicic acid chromatography, is shown in Figure 3, and the molar ratios are presented in Table VI. Treatment of the hydroxy acid containing glycerides with pancreatic lipase indicated that the γ- and δ-hydroxy acids were esterified to the α-position of the glycerides (32). These glycerides contained approximately 50% palmitic, 18% myristic, 16% oleic, and 8% stearic with trace amounts of shorter-chain fatty acids.

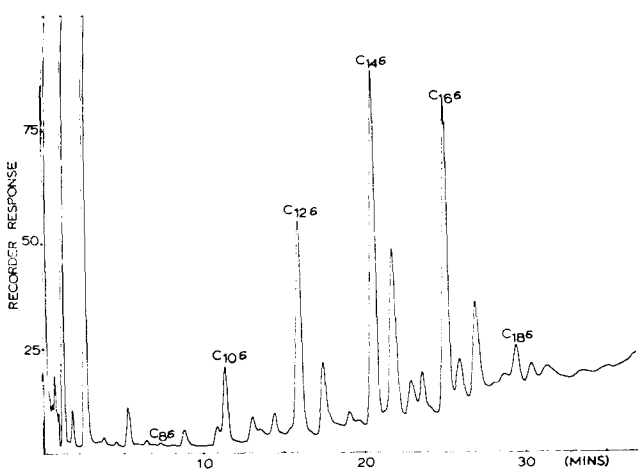

FIG. 3. A chromatogram of the lactone-rich fraction, isolated from butterfat by silicic acid column chromatography. These delta lactones were separated on a diethylene glycol adipate column (10%), temperature-programmed from 150C to 180C, using a Barber-Coleman model 5000 gas chromatograph with hydrogen flame detector.

TABLE VI

Quantities of δ-Lactones Isolated from Butterfat by Column Chromatography

Lactone	Mole %
δ-C_8	0.15
δ-C_{10}	8.14
δ-C_{12}	25.54
δ-C_{14}	35.43
δ-C_{16}	26.86
δ-C_{18}	3.49

The mechanism of the formation of lactones from the precursor glycerides is presented in Figure 4. Wyatt and Day (35) reported that trace amounts of water were necessary; Dimick and Walker (36) showed that the maximum yield of lactones was obtained by heating butterfat at 190C for five hours under conditions of vacuum-steam distillation.

The precise biological origin of these γ- and δ-hydroxy acids is under investigation. The hydroxy acid-containing glycerides are quite similar to other high-molecular-weight glycerides secreted by the mammary gland, but the exact source of hydroxy acids is not known. These acids are ubiquitous in the animal kingdom. Dimick et al. (37) reported their occurrence in the milks of the cow, goat, sheep, pig, and human being, also in adipose tissue from the cow, sheep, and pig and in bovine serum lipoproteins. They are also present in the liver of the goat (38). These findings indicate that the hydroxy acids occur in both monogastric and polygastric animals. Hence it may be assumed that the bovine rumen and/or mammary gland are not the exclusive sources of these lactone precursors. They appear to be the products of normal lipid metabolism and seem to occur in greatest abundance in lipid-anabolizing tissues, as epitomized by the mammary gland.

Van der Ven et al. (39) isolated the corresponding homologous series of γ- and δ-keto alkanoic from butterfat at a concentration of 2.5 ppm. He suggested that possibly these produce the corresponding hy-

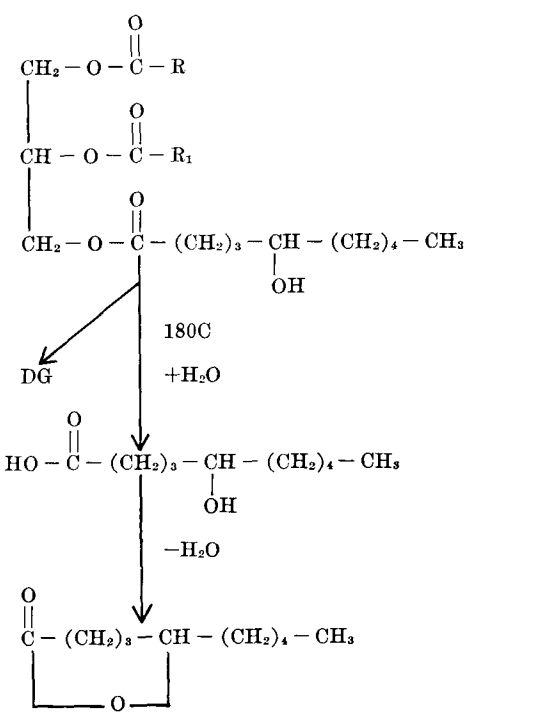

FIG. 4. Mechanism of lactone formation.

droxy acids on enzymatic reduction. The requisite enzymes were actually isolated from the bovine liver. Hsu et al. (40), in their studies of fatty acid synthetases of liver, have consistently found keto-containing compounds as side-products (β,δ-diketohexanoic acid), thus indicating that the fatty acid synthetase is not fully efficient and side-products are produced perhaps fortuitously; or, more likely, another minor biosynthetic pathway may be operative. In view of the constant ratio between the quantities of lactones, the latter scheme would appear more rational. The fatty acid synthesizing system of the bovine mammary gland (41) is similar to that of the liver, and it is conceivable that in such an intensely synthesizing organ the quantity of side-products and intermediates dissociating from enzymes might be relatively high.

Limited evidence is available to demonstrate the origin of lactones by degradative mechanisms. Japanese workers (42) have reported the formation of γ-C10, γC12, and δ-C11 aliphatic lactones by microbial oxidation of long-chain hydroxy fatty acids. Apparently the presence of an hydroxyl group at the γ- or δ-carbon impairs further β-oxidation. The potential of the vast flora and fauna of the rumen must also be considered. Recently Katz and Keeney (43) have isolated at least nine isomers each, of keto and hydroxy stearic acid from the bovine rumen. These have also been found in milk and depot fat (44). Intestinal micro-organisms can oxidize these acids (45), and it is theoretically possible to derive a series of γ- and δ-lactone precursors via this pathway though it is doubtful that this mechanism makes a significant contribution to the large quantities found in milk fat.

The physiological factors which influence quantitative production of lactones are presently being studied, viz., diet, stage of lactation, season, and breed. The feeding of a concentrated grain diet containing heated corn, a diet that depresses the fat content of milk, also caused a 25% decrease in the content of δC_{10} and δC_{12} lactones (46) (Table VII). This observation indicates a positive connection between milk lipid synthesis and the prevalence of lactones. Virtanen's work (47), using purified defined diets, corroborates this implication. Empirically these rich, low-roughage diets reduce the amounts of acetate available to the mammary gland for fatty acid synthesis (48) and, apparently, for lactone precursor synthesis.

Data concerning summer and winter dietary effects reveal that a high-roughage diet (winter type of diet) causes an increase in the lactone content of bovine milk fat. Similarly, goats receiving a starvation diet of hay and water produced milk fat richer in lactones than normal samples (49). These observations further implicate acetate as the probable direct metabolic precursor of hydroxy acid biosynthesis.

Maximizing the Flavor Potential of Butterfat

The full physical, chemical, and flavor potential of butterfat is rarely completely exploited by the food industry. Since the chemical composition of this fat is now well elucidated, its mode of utilization might be altered to maximize its many aesthetic and practical properties. An outline of the suggested procedures for maximizing the uses of butterfat is presented in Figure 5. It proposes the use of butterfat as a raw material from which desirable constituents can be isolated and custom-used to meet the requirements of various food products. The initial stages of this procedure have been patented (56). Adoption of this scheme would provide an easily available source of the elusive "buttery" flavor concentrate and provide a stable deodorized fat eminently suitable for use in dry whole milk or dry ice cream; or it could be further exploited as a source of volatile fatty acids and emulsifiers. This proposal connotates flexibility in the use of butterfat, thus providing a more competitive food product commercially.

TABLE VII

Concentrations of δ-C_{10} and δ-C_{12} Lactones (ppm) in Milk Fat from Cows on Normal and Fat-Depressing Diet

Animal	A		B		C	
Diet	N[a]	H	N	H	N	H
δ-C_{10}	12.6	9.3	16.0	11.7	12.1	9.2
δ-C_{12}	25.5	20.0	27.9	21.9	20.8	16.1

[a] N = Normal; H = Fat-depressing diet (heated corn).

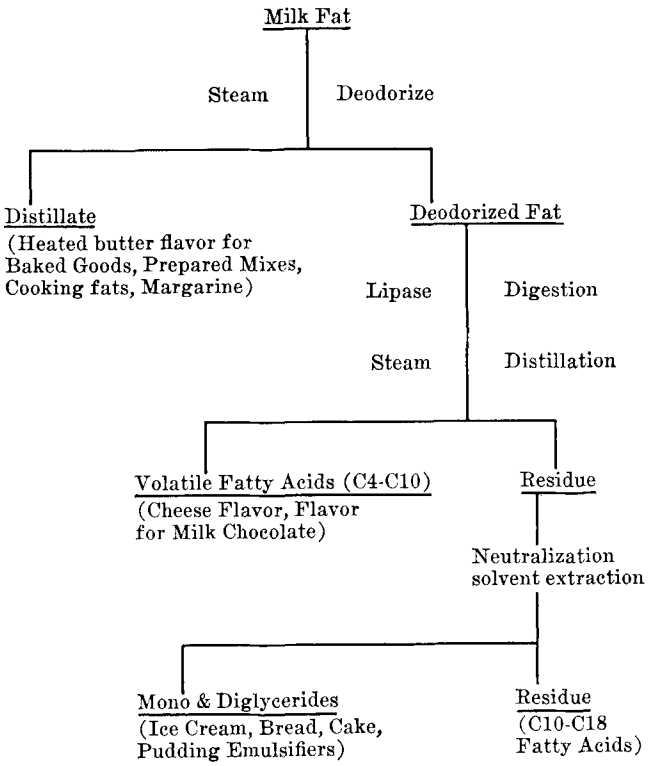

FIG. 5. A schematic total utilization of milk fat.

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