COMPOSITION AND STRUCTURAL CHARACTERISTICS OF GLYCERIDES IN RELATION TO CLASSIFICATION AND ENVIRONMENT[1]

HERBERT E. LONGENECKER

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania

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I. INTRODUCTION

The steadily increasing volume of analyses of natural fats during the past two decades has emphasized two things: (*a*) that plants and animals classed together by biologists on the basis of their structural peculiarities frequently elaborate similar kinds and proportions of fatty acids combined as triglycerides, and (*b*) that the most intricate structures and most complex fatty acid mixtures are found in fats of the simplest forms of plant and animal life. A gradual simplification is observed both in structure and number of component acids on ascending the evolutionary scale of development. Hilditch (97) has commented on these facts and has suggested that biochemical changes in fats have accompanied evolutionary development (126). The available information, much of which is either derived or inspired from Hilditch's laboratory, has recently been assembled in a monograph (100) which clearly expresses the various interrelationships. This monograph should be consulted for the extensive details of composition of some one hundred fats of aquatic origin, four hundred and twenty from plant species, and about eighty from land animals. It is possible to give here only a brief survey of the vast amount of available information.

A complete knowledge of the composition of natural fats in terms of

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constituent acids and glycerides is desirable for the intelligent use of the many available products (96). Detailed quantitative information regarding the individual component acids provides a means of sorting the fats for most purposes. Such data are not always adequate, however, for the correct prediction of the physical or chemical behavior of the fat. The basis for the physical dissimilarity of cocoa butter and mutton tallow, despite their similar fatty acid composition, lies in the nature of the glycerides found in the two fats (55, 58, 120, 121). Other examples demonstrating physical anomalies may be selected in comparing partially hydrogenated vegetable oils with naturally occurring glyceride mixtures of nearly identical acid composition (112). Recently, in connection with studies on cottonseed oil, Hilditch and Maddison (128) again pointed out that the analyses of component acids may be interpreted in several ways in deducing the probable component glycerides.

In most cases, the qualitative identification and quantitative measurement of fatty acid composition is possible. However, the close similarities in physical properties of the glycerides makes their separation and quantitative analysis exceedingly difficult. Only in the case of relatively few fats have the component glycerides been studied, and even then the compositions have been expressed for the most part in general terms rather than as specific configurations. A beginning has been made towards the understanding of structural peculiarities of natural fats and of changes effected during their hydrogenation to successive stages of saturation. Interestingly enough, the study of partially hydrogenated products has been a major aid in the elucidation of the structure of glycerides originally present in an oil. Much more work is needed, however, to provide a completely satisfactory picture of either the original structures present in natural fats or those which arise from some treatment, such as hydrogenation, selective adsorption, selective extraction, molecular distillation, or fractional crystallization.

While our present knowledge of the exact constitution of glycerides is far from complete, certain broad generalizations have emerged. The construction of glycerides seems to take place along lines which are similar for natural fats from a wide variety of plant or animal species. Glyceride molecules are fashioned largely independently of either the particular acids present or any biological relationship. A maximum formation of mixed triglycerides with several fatty acids, rather than simple triglycerides containing only one acid, is the regular observation.

II. COMPONENT ACIDS OF NATURAL FATS

A study of the organization of animal or vegetable fats, which are in the main triglycerides, is logically approached by referring first to the

qualitative and quantitative nature of the component fatty acids. Comparatively simple and satisfactory methods are available for the quantitative determination of fatty acids by the ester fractionation procedure (8, 99, 195, 269). A mixture of acids obtained from a given fat is first separated into two groups on the basis of the solubility of their lead salts in alcohol (101, 141, 263). The soluble portion contains most of the unsaturated acids, together with saturated acids of lower molecular weight (C_6 to C_{12}) and some myristic and palmitic acids; the insoluble portion is made up mainly of saturated acids but also has some oleic acid and, if they were present in the original sample, elaidic, isoöleic (e. g., petroselinic acid or partially hydrogenated linoleic acid), eleostearic, erucic, or hydroxy acids. A similar separation may be effected by the fractional crystallization of the original acid mixtures (36). Each group of acids is then converted to neutral methyl esters for fractional distillation. From qualitative identifications of the acids present and analytical data such as saponification equivalent, iodine value, and thiocyanogen number for the various fractions, the composition of each fraction and eventually of the entire mixture may be readily calculated (92, 97, 197).

A. Fats of aquatic origin

An exceptional variety of acids, predominantly unsaturated, distinguishes aquatic fats from those of higher plant and animal species. Only 15 to 20 per cent of saturated acids are found; these are mainly palmitic acid and small amounts of stearic, myristic, lauric, and caprylic acids (223). The unsaturated acids, on the other hand, vary in amount and extent of unsaturation from C_{16} to C_{24}. Not all of the unsaturated acids have been identified with certainty, and analyses are customarily expressed in terms of the mean unsaturation of groups of unsaturated acids of the same carbon content. For example, $C_{18(-3H)}$ is used to indicate a mixture of C_{18} acids whose mean unsaturation corresponds to 1.5 double bonds. The determination of the fatty acid compositions of such complex fats has not been accomplished within the most desired limits of accuracy (92). Nevertheless, the differences which appear for the compositions of fats from different species are in many cases three to ten times the possible experimental error, making it decidedly unlikely that such observations are fortuitous.

For example, gross differences exist between the fats of sea- and fresh-water algae or plankton. The sea-water or marine fats have, in general, the more complex mixtures with high proportions of C_{18}, C_{20}, and C_{22} unsaturated acids. Fats of fresh-water algae contain larger proportions of C_{16} and C_{18} unsaturated acids, with little C_{20} and practically no C_{22} acids (90, 103, 205). In both cases there has been found an extremely high average unsaturation for acids of the C_{20} and C_{22} series, indicating the

presence of three to six ethylenic linkages. The compositions of the fats of many larger forms of water life show these same general features.

The fats of some species show special features in composition, and in many fish the liver serves apparently as a main depot for fat storage, in contrast to the situation in higher animal species. In several cases (some Elasmobranch families and the *Physeteridae*, or sperm whales) fatty acids are linked with glycerol as ethers rather than esters or are in combination with other alcohols than glycerol (6, 107, 124, 125, 206, 258, 259, 270). The unsaturation of the acids is usually low.

The dolphin and porpoise family (*Delphinidae*) presents one of the most interesting of extremes in fatty acid composition. The jaw, head, and blubber fats of this family of marine animals have substantial proportions (30 to 45 per cent) of the branched-chain, uneven-numbered carbon chain acid, isovaleric acid (49, 81, 203, 271). This acid is absent from the liver, lungs, and heart (203).

Considerable study has been given whale blubber fat because of its technical significance (7, 152, 262). A higher degree of unsaturation in Arctic whale oil, as compared with Antarctic oils, has been reported. Whale oil is typical of the marine type of fats in composition, with sizeable quantities of C_{20} and C_{22} unsaturated acids.

Salmon body fats show an interesting progressive change from the fresh-water type of component fatty acid mixtures to the marine type, as the fish develop from purely fresh-water animals (two to three years old) into adult fish and swim out to sea. Lovern (204) has studied these changes at several stages in the salmon's life cycle.

B. Fats of land animals

A decided simplification in fatty acid composition accompanies the increased specialization of higher orders in the scale of evolutionary development. The change in the type of fat is not an abrupt one. Amphibia and reptiles retain considerable proportions of unsaturated acids (83, 132, 135, 181, 183). There are, however, notable decreases in the percentages of C_{16}, C_{20}, and C_{22} unsaturated acids from those found in aquatic fats. These decreases are compensated chiefly by increases in C_{18} acids which are somewhat more unsaturated than oleic acid.

A single detailed analysis (208) of the fat from an invertebrate, the earthworm, indicates the desirability of studying other invertebrates. Acids ranging from C_{10} to C_{22} with 70 per cent of highly unsaturated acids were found.

A few quantitative determinations of the composition of bird depot fats (114, 150) have been made. Only small amounts of C_{20} and C_{22} acids are found in hen fats and, in general, the composition is more closely related

to that of fats of the higher species of land animals than to aquatic fats. A characteristic feature is the occurrence of 27 to 30 per cent (molar) of palmitic acid with only small amounts of stearic acid (*ca.* 5 per cent).

It is important to bear in mind that animal depot fats may arise either by synthesis from non-lipid sources in the diet or, when rations contain relatively large proportions of fat, by direct assimilation of the ingested fatty acids. The extent to which either or both processes operate in the formation of animal depot fats determines to a considerable extent the degree to which such fat is characteristic of the animal.

Thus the composition of the body fat of sea birds, the food of which is mainly fish, probably reflects the nature of their diet rather than a relation to biological species (187). Lovern (207) has analyzed the depot fats of several sea birds and has found a considerable increase in the proportions of C_{20} and C_{22} unsaturated acids at the expense of C_{18} unsaturated acids and palmitic acid. These fats resemble the marine type of aquatic fats. Lovern suggests two possible explanations for this divergence from the broad rule that the type of fat may be correlated with phylogenetic relationships: (*a*) that sea birds have no specific requirements and any type of depot fat will serve equally well, or (*b*) that their specific requirements have been produced or modified to suit the animal's normal diet.

Most quantitative studies of the effect of dietary and other conditions on animal body fat have been made on the rat and the pig. In order to evaluate the extent to which food fat influences the body fat, it is necessary to know the composition of fat formed exclusively, or almost entirely, from dietary sources other than fat. On low fat diets, these animals synthesize body fats which have several points of interest (16, 18, 25, 36, 48, 60, 73, 74, 75, 122, 146, 183, 196, 199, 200, 240). As is characteristic of fats of most higher animals, the fats of both the rat and the pig contain 25 to 30 per cent of palmitic acid. Stearic acid is found in small proportions in rat fat (*ca.* 5 per cent) but to a somewhat greater extent in pig fats (*ca.* 10 to 15 per cent). While oleic acid is predominant among the unsaturated acids in both cases (45 to 50 per cent), the quantity of the C_{16} monounsaturated acid,—palmitoleic acid ($\Delta^{9,10}$-hexadecenoic acid),—is relatively high in rat fats formed from non-fatty sources. Small amounts (*ca.* 1 per cent) of more highly unsaturated acids are found. The effect of including fat in the diet has been shown to result in considerable alterations in the composition of the fat without any apparent disturbance to the animals, who seem to be quite capable of tolerating well the gross changes in type of body fat. In the rat, the direct deposition (233, 234) of food fatty acids always results in a considerable reduction in the proportion of palmitoleic acid (to *ca.* 5 per cent), no change in stearic acid, and complete changes for those acids present only in small amounts in the

TABLE 1

Fatty acid composition of various animal fats (in weight percentages)

ANIMAL	SATURATED ACIDS			UNSATURATED ACIDS					
	C_{14}	C_{16}	C_{18}	C_{14} (−2H)	C_{16} (−2H)	C_{18} Oleic	C_{18} Linoleic	C_{20}	C_{22}
Liver oils:									
Cod (91)	2	14	1	2	10	26 (−3.3H)		25 (−5.5H)	20 (−7.4H)
Halibut (202)	3.9	15.1	0.5		18.7	34.4(−2.0H)		13.8(−5.5H)	13.6(−7.6H)
Fresh-water fish (202):									
Pike	4.7	13.2	0.5	0.8	20.8	38.4(−2.8H)		15.3(−7.5H)	6.3(−7.5H)
Perch	3.5	12.5	2.0	1.1	19.4	40.5(−3.2H)		13.8(−6.8H)	7.1(−9.2H)
Whale (152)	6.3	18.2	2.4	3.7	13.3	38.4(−2.6H)		11.4(−5.6H)	6.3(−9.0H)
Salmon (204):									
Young	3.4[a]	17.7	3.3	3.1	21.7[b]	30.0(−3.8H)		12.9(−8.3H)	9.9(−10.2H)
Adult	2.7	14.3	5.0	0.7	7.9	32.2(−3.2H)		19.4(−6.7H)	17.8(−9.4H)
Frog (181)	4	11	3		15	52 (−2.5H)		15 (−6H)	
Lizard (135)	4	29	10		12	40 (−2.7H)		5 (−5.5H)	
Earthworm (208)	12[c]	8	12	7[d]		20 (−3.9H)		21 (−6.8H)	18 (−?H)[e]
Chicken (114)	0.1	25.6	7.0		7.0	38.4	21.3	0.6(−8H)	
Rat (196):									
Low fat diet	3.1	26.7	4.0	0.9	15.6	47.2	2.2	0.3(−8H)	
5 % fat diet	1.8	24.4	4.4	0.3	4.8	44.3	18.6	1.4(−8H)	
High corn oil diet	0.7	12.2	3.1		6.9	44.8	32.3		
High coconut oil diet	44.5[f]	19.9	4.0	2.0[g]	4.4	23.8	1.4		
Pig (back fat):									
Low fat diet (122)	0.8	25.9	12.2	0.2	2.0	48.1	7.8	3.0(−8H)	
Low fat diet (74):									
Alone	1.7	25.5	13.7			50.2	8.9		
+4% cottonseed oil	1.1	25.0	21.1			39.5	13.3		
+12% cottonseed oil	1.1	13.8	26.5			31.8	26.8		

Beef tallow (123)	3.3	24.9	24.9	0.4	2.4	41.8	1.8	0.5(−8H)	
Mutton tallow (58)	4.6	24.6	30.5			36.0	4.3		
Goat (67)	5.6[h]	25.5	28.1			38.4		2.4(−8H)	
Organ glycerides:									
Beef liver (146)	1.4	32.5	5.0	1.5	11.7	40.3(−3.0H)		7.4(−6.9H)	
Pig liver (146)	0.1	25.8	10.5		9.2	44.1(−2.4H)		9.2(−6.8H)	1.1(−6.8H)
Beef heart (182)		22	20		12	45			
Beef adrenal phospholipids (13)	1.2	23.8	13.1[i]			40.2		22.2(−8H)	

[a] Including 0.7 per cent C_{12}.
[b] (−2.3H).
[c] Including 6 per cent C_{12}.
[d] (−2.7H).
[e] Including 8 per cent C_{24}.
[f] Including 27.2 per cent C_{12}.
[g] Including 0.6 per cent C_{12}.
[h] Including 3.5 per cent C_{12}.
[i] Including 2.0 per cent C_{20}.

"synthetic" fat, such as saturated acids of low molecular weight, like lauric and myristic, or highly unsaturated acids such as linoleic, linolenic, or arachidonic (48, 196). Pig depot fats respond somewhat differently to food fatty acids.

For the next higher forms of land animals, the recorded data are indeed meagre, but they show again some additional simplification in fatty acid composition. In beef and mutton tallow, for example, three acids predominate,—palmitic, stearic, and oleic; they are present in the proportions 25 to 30, 20 to 25, and 40 to 45, respectively (15). It is of interest that little perceptible effect on the body fat of steers was produced by feeding liberal allowances of oils containing highly unsaturated acids (255).

The progressive changes in composition observed in passing from the simpler to the more complex biological species are indicated in table 1.

C. Vegetable fats

For the most part, the component acids of vegetable fats are not so complex as many animal fats (table 2). The acids which predominate are palmitic, oleic, and linoleic. Certain distinctive acids are found in special cases, usually only in the seed fats from the same or related plant families. The relationship between the composition of the seed fat and the botanical grouping is considered here for several component fatty acids (*cf.* Hilditch (100) for a more detailed discussion). A few representative analyses of common and unusual vegetable fats (out of hundreds available) are shown in table 2.

It is apparent that the highest amounts of linolenic acid are found in seeds from large trees (pine (1), walnut) and certain herbaceous plants (perilla, linseed, hemp), together with large amounts of linoleic acid and some oleic acid. Several families other than the ones represented by these trees and shrubs have been found to contain linolenic acid but in variable amounts in different species. Thus blackberry (193), rubber (86, 158), and Stillingia (170) seed fats contain from 20 to 25 per cent of linolenic acid. In related species of these families (*Rosaceae* and *Euphorbiaceae*), however, linoleic and linolenic acids are largely replaced by exceptional acids such as licanic acid (oiticica nut, 74 to 80 per cent (176, 209, 216)), eleostearic acid (tung seed, 74 to 95 per cent (176, 201, 215, 217, 218, 241, 263)), or ricinoleic acid (castor oil, 88 to 92 per cent (224, 244)). Linoleic acid is the predominant acid in the above group of "drying" oils. It is also found extensively in the absence of linolenic acid in a series of oils which are either "semi-" or "non-drying" (*cf.* table 2 and references 19, 163–166, 168, 228, 237).

In all these cases, the saturated acids rarely form more than 10 per cent of the total component acids. Several plant families, e.g., *Malvaceae*

(cottonseed), *Solanaceae* (tobacco, tomato, etc.), *Anacardiaceae* (cashew, pistachio, etc.), and *Bombaceae* (kapok (169)) have characteristically a

TABLE 2

Fatty acid composition of several vegetable fats (in weight percentages)

VEGETABLE FAT	SATURATED ACIDS			UNSATURATED ACIDS		
	Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Pericarp fats:						
Palm oil (61)	2.7	42.5	3.4	40.9	10.5	
Piqui-a (143)	1.5	45.1	1.8	49.6	2.0	
Stillingia tallow (140)	1.4	48.4	0.9	46.0	3.3	
Olive oil (153)	0.5	10.0	3.4*	77.5	8.6	
Seed (kernel) fats:						
Perilla (174)		6.7		10.7	33.6	49.0
Linseed (113)	0.2	5.4	4.1*	9.6	42.6	38.1
Stillingia seed (170)		4.7	1.9	8.1	59.4	25.9
Cedar nut (156)				32.5–35.8	31.1–34.2	16.6–27.8
Hemp (86)		5.8	1.7	6.7	68.8	15.9
Hemp (155)		24.1	11.7	45.7	18.5	
Walnut (86)		7.0	1.1	19.1	65.9	6.9
Grape (167)		6.3	3.1*	33.5	54.6	2.4
Safflower (162)		4.2	2.0*	26.3	67.4	0.1
Horse chestnut (177)		4.4	3.6	67.1	22.7	2.2
Thorn apple (108)		3.3	5.1	17.1	74.5	
Poppy (71)		4.8	2.9	30.1	62.2	
Sunflower (21)		3.5	2.9	34.1	58.5	
Sesame (110)		9.1	5.1*	45.4	40.4	
Tobacco (231)		3.3	5.1	17.1	74.5	
Tomato (89)		5.7	10.7*	45.4	38.2	
Grapefruit (161)		20.3	7.6	20.7	51.4	
Cottonseed (142)	2.0	19.6	3.4	24.6	50.4	
Coffee (235)		28.2	12.7	17.3	35.8	
Brazil nut (236)		14.3	2.7	58.3	22.8	
Cashew nut (155)		4.1	6.0	68.2	21.7	
Pistachio nut (65)	0.6	8.2	1.6	69.6	20.0	
Mowrah butter (109)	0.6	7.6	1.4*	83.3	7.4	
Margosa (137)		14.9	15.7*	61.9	7.5	
Shea butter (84)		5.7	41.0	49.0	4.3	
Phulwara butter (42)		56.6	3.6	36.0	3.8	
Cocoa butter (151)		24.4	35.4	38.1	2.1	
Borneo tallow (139)	1.4	21.5	39.0	38.1		

* Indicates the inclusion of a small amount (0.5 per cent) of arachidic acid.

slightly higher saturated acid content (15 to 25 per cent, largely palmitic acid), and in this respect they resemble seed fats of grains such as barley (252), rice (157), rye (245), wheat (159, 249), and corn (20, 196). The

appearance of stearic acid as a major component acid is observed in some tropical families for which the seed fats of several genera have been thoroughly studied (*cf.* table 2 and reference 145). It is interesting to note that only small amounts of linoleic acid are present when the content of stearic acid is high. While this might be suggestive of some kind of biological interrelation between the C_{18} acids or their immediate precursors, Hilditch (100) prefers to regard the presence of such large amounts of stearic acid as a specific characteristic of the seed fat in a given plant family.

Saturated acids other than palmitic and stearic occur in the seed fats of only a few specific families. Lauric acid is the only major saturated acid found in the *Lauraceae* (cinnamon (226), laurel oil (56, 57)); myristic acid similarly characterizes the *Myristicaceae* (nutmeg (56, 57)). The two acids, lauric and myristic, occur together in large proportions as the chief saturated acids in several other families (43, 57, 87, 243). Both the seed and fruit coat fats of the *Palmae* are characterized by their high amounts of lauric acid (45 to 50 per cent) along with substantial amounts of myristic acid (15 to 20 per cent) and 5 to 10 per cent each of caprylic, capric, palmitic, and stearic acids (e.g., coconut oil (9, 27, 55, 196, 222, 253) and palm oil (10, 55, 61, 102, 118, 119, 171)).

Saturated acids of higher molecular weight,—arachidic, behenic, and lignoceric,—are present almost exclusively in the seed fats of *Leguminosae* and *Sapindaceae*, although not all the species studied have been alike in this respect. Arachidic acid occurs as 20 per cent of the mixed acids of kusum oil (66), and there are 5 to 7 per cent of C_{20-24} saturated acids in peanut oil (110, 113, 154, 160, 172, 195). Soybean oil (68) has only minor amounts of these acids.

The occurrence of unusual unsaturated acids in particular plant families has been referred to above in connection with linolenic, licanic, and eleostearic acids. Several other unsaturated acids characterize different plant families. Petroselinic ($\Delta^{6,7}$-octadecenoic) acid, for example, is common to the seed fats of umbelliferous plants such as parsley (115, 268), parsnip, fennel, celery, carrot, caraway, coriander (50), and others (117, 225, 242, 261). Similarly, erucic acid is elaborated in most Cruciferous plants (e.g., rape seed (134, 144, 246), wallflower seed (116), and jamba seed (178, 247)) and in the seed fats of the nasturtium (80, 129, 248). The cyclopenteno acids,—hydnocarpic, chaulmoogric, and gorlic,—have been found only in the seed fats of the *Flacourtiaceae* (54).

III. COMPONENT GLYCERIDES OF NATURAL FATS

The structure of glycerides found in natural fats presents a complex problem in isomerism. In no fat from a natural source is there just one

fatty acid. Even the simplest fats have several different acids which vary in length of carbon chain, degree of unsaturation, and configuration, and there is the possibility of locating each fatty acid at any one or all of the three carbon atoms of glycerol. The possibility of combination with the optical antipodes of glycerol to produce optically active glycerides adds an additional complication which, perhaps fortunately, has not yet been found in natural fats.

The number of possible glycerides increases geometrically with each additional fatty acid. If n = the number of component acids, then the theoretical number of combinations which would result from a maximum even distribution of the acids in the three positions of glycerol is n^3. The actual number of molecules distinguishable chemically is $\frac{1}{2}(n^3 + n^2)$.

TABLE 3

The numerical relationship between component fatty acids and glycerides

NUMBER OF FATTY ACIDS (n)	THEORETICAL COMBINATIONS (n^3)	NUMBER OF GLYCERIDES $\frac{1}{2}(n^3 + n^2)$
1	1	1
2	8	6
3	27	18
4	64	40
5	125	75
6	216	126
7	343	196
8	512	288
9	729	405
10	1000	550

Thus in the case of just two fatty acids, O and P, there will be eight (2^3) theoretically possible combinations with the trihydric glyceryl radical, CH_2CHCH_2 (G), as follows:

(i)	G (O O O)	(v)	G (O P P)
(ii)	G (O O P)	(vi)	G (P O P)
(iii)	G (O P O)	(vii)	G (P P O)
(iv)	G (P O O)	(viii)	G (P P P)

It will be observed that (ii) and (iv) and (v) and (vii) are identical chemical molecules, because the end carbon atoms of glycerol are identical. Therefore, the actual number of individual glycerides which may be obtained by any permutation of two acids is six. The theoretical combinations and actual number of glycerides obtainable for more complex acid mixtures are given in table 3.

Little more than an approach has yet been made towards the ultimate

goal of analyzing natural glyceride mixtures for the number of specific configurations indicated in table 3. Certain procedures have allowed the estimation, within limits, of the proportions of groups of component glycerides, e.g., trisaturated, disaturated–monounsaturated, monosaturated–diunsaturated, and triunsaturated, without regard to specific structures. The techniques which have proven most fruitful have aimed at separating the general structural types present in a fat into either pure fractions or essentially simpler mixtures containing a predominant amount of a given type.

Distillation in high vacuum was employed very early for the purpose of separating partially the glycerides of laurel kernel fat and nutmeg butter (189). Krafft (188, 190–192) and others (29, 30, 32, 45) used this procedure extensively to obtain glycerides of low molecular weight, e.g., trilaurin, trimyristin, lauromyristins, etc. The inability to distill glycerides of higher molecular weight limited the use of this procedure until recently, when interest was aroused in molecular distillations (44, 62, 63, 77, 95, 180, 227, 272). While the latter technique enables a distillation of high molecular weight materials without decomposition, it has not proved entirely satisfactory for the fractionation of glycerides (95, 227, 229).

Fractional crystallization offers another possible means of separating the glycerides into simpler mixtures. The early workers who chose this means had little success other than to demonstrate clearly for the first time the general occurrence of mixed rather than simple triglycerides. Amberger, Bömer, Klimont, and others (2–5, 28–35, 184–186) pursued the method assiduously for many years. In many instances they were able to effect sufficient separation to obtain qualitative identification of specific mixed triglycerides. Nothing approximating quantitative treatment resulted, however. The same is true of the fractional crystallization of brominated unsaturated fats such as linseed oil (70–72), soybean oil (93, 250), and a series of marine oils (251). Brominated mixed glycerides of oleic, linoleic, linolenic, and C_{20-22} unsaturated acids were isolated. No reference substances of the bromo derivatives reported have yet been synthesized to confirm the proposed structures and, in view of the uncertain solubilities of such derivatives (22), it would be unwarranted to expect such information to express quantitative relations. Daubert and King (59) have made an extensive study of the compounds reported in many of these investigations to determine the correlation of the isolated products with known reference structures.

The first significant quantitative method in the study of glyceride structures was developed by Hilditch and Lea (120) to measure the trisaturated glycerides. The method was based on Armstrong and Hilditch's preparation (11) of saturated esters from a mixture with unsaturated esters by

oxidation of the latter with potassium permanganate in acetone and subsequent removal of the acidic scission products from the unchanged saturated esters (*cf.* also 23). With suitable precautions, the glycerides composed entirely of saturated acids may be determined. This determination, coupled with studies of fatty acid composition, provides a means for obtaining a first approximation of general glyceride structure. When the molar proportions of the saturated acids found in the fully saturated glycerides are simply subtracted from the proportions found for the whole

TABLE 4

Proportions of general glyceride structures in various animal and vegetable fats (in molar percentages)

SOURCE OF FAT	TRISATURATED GLYCERIDES	DISATURATED–MONOUNSATURATED GLYCERIDES	MONOSATURATED–DIUNSATURATED GLYCERIDES	TRIUNSATURATED GLYCERIDES
Animal fats:				
Beef tallow (14)	25.8	33.7–53.9	40.5–0	0–20.3
Mutton tallow (58)	26	30 –52	44 –0	0–22
Goat (male) (67)	29.2	30.9–50.9	39.9–0	0–19.9
Goat (female) (64)	30.8	29.2–49.2	40.0–0	0–14.9
Butter (147)	33.7	36.5–51.4	29.8–0	0–14.9
Vegetable fats:				
Pericarp fats:				
Palm oil (17, 118)	3.5–7	16.5–29	66–58	0–14
Sterculia (148)	1	14	85	0
Piqui-a (143)	2	42	56	0
Stillingia tallow (140)	27.6	61–65	11.4–0	0–6
Seed fats:				
Coconut (55)	84	12	4	
Palm kernel (55)	63	26	11	
Cocoa butter (121)	2.5	77	16	4
Borneo tallow (139)	4.5	85	6.5	4
Shea butter (145)	2.3	30.5–65	69.5–0	0–35
Allanblackia (145)	1.5	63.5–82	36.5–0	0–18
Taban Merah (148)	2	77	21	0
Cottonseed (113)		15	60	24

original fat, the composition of the mixed saturated–unsaturated glycerides results. The latter must be mono-, di-, or tri-unsaturated. Limiting values for each group may be readily calculated from the molar proportions of saturated and unsaturated acids in the saturated–unsaturated glycerides (table 4).

An interesting relationship between the percentage of saturated fatty acids and the trisaturated glycerides has been observed as a characteristic feature of many fats. In seed fats, trisaturated glycerides do not usually

appear until the proportion of saturated acids reaches *ca.* 60 per cent. When saturated acids are present in approximately these amounts, it has been further observed that the ratio of saturated to unsaturated acids (called the "association ratio") in the non-fully saturated glycerides is 1.2 to 1.4. The proportion of saturated acids indicated by this ratio (*ca.* 60 per cent) is not exceeded even when the saturated acids in the whole fat are unusually high as, e.g., in coconut oil (94 per cent). Such findings, obtained repeatedly, indicate the general pattern of glyceride structures in the seed fats. Hilditch (100) uses these facts in support of what has come to be called a "rule of even distribution" of fatty acids throughout the glycerides of seed fats. Animal fats and some plant fats are considered by Hilditch as exceptions to this generalization, because they have a much greater amount of trisaturated glycerides for a given ratio of saturated to unsaturated acids in the whole fat. Recent studies (198), based on the most probable random distribution of any given fatty acids as glycerides (221), have indicated, however, that the animal fats are really more "evenly distributed" than are seed fats.

A further operation which has proved useful in studying glyceride structures (98, 102, 104, 110, 113, 149, 153) is the hydrogenation of fat samples, either partially or completely, with the subsequent determination of the amount and composition of the trisaturated glycerides in each hydrogenated product. Hydrogenation raises the trisaturated glycerides as the proportion of saturated acids is increased, as would be anticipated from the above remarks. The "agitation" hydrogenation process, which provides continual intimate contact of catalyst and reactants, is stepwise according to Hilditch *et al.* (41, 76, 110, 112). The disaturated–monounsaturated glycerides are completely saturated before any monosaturated–diunsaturated or triunsaturated molecules are fully hydrogenated. Different results are obtained by the "drip" process, in which a stationary catalyst tends to hydrogenate completely any molecules it contacts (110, 142). In completely hydrogenated vegetable oils, tristearin, palmitodistearin, and dipalmitostearin are the least soluble glycerides usually found. These may be fractionally crystallized and the approximate content of tristearin calculated from either the equivalent weights of the crystalline fractions or the setting points of mixtures of palmitic and stearic acids in the fractions. When such determinations have been made (e.g., 128) the amount of tri-C_{18} glycerides (which are given by the tristearin figures) was close to the minimum calculated for the limiting values derived from data on component acids.

Recently a series of publications from Hilditch's laboratory (12, 42, 43, 84, 109, 127, 128, 130, 131, 133, 136–38, 151, 219) has indicated that a combination of several of the above procedures provides more informa-

tion regarding the component glycerides than had been available previously. Fractional crystallization of the whole fat in acetone solution is employed first to separate the fat into simpler mixtures of glycerides. Trisaturated glycerides are then determined on the whole fat and, in some cases, on the individual portions which have been crystallized. Each

TABLE 5

Glycerides of vegetable and animal fats

P = palmitic; S = stearic; U = unsaturated acids (oleic + linoleic)

FAT	TRISATURATED GLYCERIDES				DISATURATED-MONOUNSATURATED GLYCERIDES			MONOSATURATED-DIUNSATURATED GLYCERIDES		TRIUNSATURATED GLYCERIDES
	P_3	P_2S	PS_2	S_3	P_2U	S_2U	PSU	PU_2	SU_2	U_3
Mowrah (109)		1.2			0.9		26.9	41.3	29.7	
Phulwara butter (42)	7.9				62.4		7.2	22.5		
Shea butter (84)		3.0	1.5			34.4		11.3	45.3	4.5
Borneo tallow (42)	1.4	1.9	1.3	0.7	7.6	39.8	30.7	3.3	13.3	
Kokum butter (266)				1.5	1.6	57.9	14.8	1.8	21.4	1.0
Hodgsonia capniocarpar seed fat (130)	2.1	0.6			33.1		27.3	24.1		12.8
Margosa oil (131)		←0.6→			5.0		12.3	26.0	33.6	22.5
Allanblackia floribunda kernel fat (219)			1.3			76.0	5.4	Trace	15.4	1.9
Allanblackia parviflora kernel fat (219)			1.3			60.0	9.3	Trace	26.1	3.3
Baku kernel fat (12)			1.3			26.3	7.5	6.0	46.7	12.2
Palm oil (Cameroons) (127)	5.1	3.3			42.7		10.8	31.5		6.6
Palm oil (Bassa) (127)	3.0	2.7			30.5		9.9	41.5		12.4
Sapota seed oil (264)							7.8	59.4*	27.8	5.0
Ox depot fat (136)	3.4	7.8	5.8	0.4	14.7	2.3	32.0	22.7	10.9	Trace
Pig, outer back fat (138)	0.6	2.4	2.4		5.4		34.0	45.4	←9.8→	
Pig, perinephric fat (138)	0.1	4.3	4.7		9.0		39.2	35.2	←7.5→	
Cow depot fat (133)	3.0	22.6	10.0		18.2		35.4	9.7	←0.8→	
Cow depot fat (133)		16.5	11.8		11.0	1.8	41.6	14.2	←3.1→	

* Included in this figure is 22.8 per cent myristodiolein.

portion is further analyzed for (*a*) quantitative distribution of fatty acids by ester fractionation and (*b*) content of tri-C_{18} glycerides by measuring the tristearin present after complete hydrogenation. From these data and the general order of solubility of known individual glycerides, it is possible to deduce the approximate glyceride composition of the original fat. The results of some of these determinations are assembled in table 5.

In addition to the determinations shown in table 5, other contributions have appeared from Hilditch's laboratory which cite the successful use of this method for solid fats (12, 43, 88, 137).

One of the limitations in these studies has been the inability to distinguish between various general types of tri-C_{18} glycerides, such as stearodioleins, stearoöleolinoleins, stearolinoleins, oleolinoleins, triolein, and trilinolein, all of which are ultimately determined in the form of tristearin after complete hydrogenation. This fact makes the figures for stearodiunsaturated and triunsaturated glycerides in table 5 less certain than, for instance, the values for the trisaturated glycerides. The greatest difficulties arise in the technologically important liquid fats (both "drying" and "non-drying" oils) in which the component acids are predominantly C_{18} and unsaturated, and, consequently, the content of trisaturated glycerides is negligible.

In order to differentiate the glyceride structures in the more unsaturated liquid fats, the latter have been converted to more solid fats by geometrical isomerization (elaidinization), using the oxides of nitrogen or sulfur (85, 113, 105, 106) or selenium (24, 88, 154). Mixed oleoglycerides are transformed by this process into mixed elaidoglycerides. The latter were studied directly in the earlier work and were resolved, more recently, into fractions of different solubility in acetone. Determination of the saturated, elaidic, oleic, and linoleic acids affords data from which the proportions of the mixed glycerides present may be approximately deduced for the elaidinized fat. The order of accuracy of quantitative conclusions drawn from results obtained in this manner is definitely lower than that which may be obtained for (solid) fats with an originally higher content of saturated acids. The polymerization of linoleic acid has been considered in these studies, but not its geometrical isomerization (173, 214).

Recently, two promising methods for treating the mixed saturated–unsaturated glycerides have been proposed. The device of low-temperature crystallization, used so effectively by Brown and his associates for the purification of unsaturated acids (36–40, 238, 239), gave Hilditch and Maddison (128) a sufficient separation of cottonseed oil to enable them to draw quantitative conclusions, within limits, as to the types of glycerides present, from considerations of the component acid analyses and the ratio of oleic to linoleic acid in each fraction. Vidyarthi and Mallya (265) have also contributed to the component glyceride analysis of similar liquid fats by a systematic examination of (debrominated) products obtained from fractions of brominated glycerides separated from suitable solvents,—a procedure which was also suggested, but not reported upon in detail, by Gunde and Hilditch (88). A limitation of the latter method which has

not received due consideration is the known incomplete and variable separation of unsaturated components as bromine addition products (22, 37, 82, 111, 214, 230, 232).

In the continuation of attempts to find methods for the preliminary resolution of complex glyceride mixtures two techniques, important commercially but not yet used in connection with problems of glyceride structure, seem promising on the basis of preliminary results. Solvent extraction of such oils as linseed, soybean, fish, perilla, and cottonseed for the selective removal of glycerides of different degrees or types of unsaturation is described by Freeman (78). Certain types of non-reactive, organic, polar solvents, notably those which are not completely miscible with the glycerides at low temperatures, are used preferentially to dissolve the unsaturated glycerides. Selective chromatographic adsorption (273) appears to provide somewhat similar separations of both fatty acids and glyceride mixtures (and also sterols and phospholipids) (47, 175, 179, 213, 256, 257). Especially in consideration of Kaufmann's work (175) does this procedure seem valuable for separations of glycerides. There are not yet sufficient data available describing either the relative adsorbability of adsorbents or the action of various solvents.

Little can be said with assurance of the specific configurations present in natural fats. The determination of configuration patterns is the next great task. There have been several instances (17, 79, 94, 112, 113, 128, 134, 145, 148, 151, 185) of the isolation of derivatives of glycerides from several natural fats in which the β-position of the glycerol molecule was occupied by the acid present in lesser quantities. However, the evidence is too scanty for more than a tentative working conception that this finding may appear generally. Such selective production of one isomeric form of a triglyceride may mean the operation of specific biological process.

It seems important to indicate that the determination of glyceride configurations is preferably based upon physical rather than chemical properties. For this reason, the extensive studies on the synthesis of glycerides of known configuration (59) and the studies of their physical properties,—such as melting and transition points and crystal structures (46, 53, 210–212), spectrographic absorption characteristics (69, 220), heats of combustion and specific heats (51, 52), dielectric properties (26, 194), and activation energies (267),—are the more valuable.

Such, in brief, is the present state of our knowledge of the structural relations of natural fats. That considerable progress has been made during the past quarter of a century is self-evident. In view of the strategic significance of certain types of natural fats, it is not unreasonable to anticipate a quickened pace in similar investigations. The broad outlines have

been defined in detail in many instances. It is, however, entirely possible that modifications of the existing conceptions may be necessary, as a result of further research.

REFERENCES

(1) ADAMS, M., AND HOLMES, A.: Ind. Eng. Chem. **5**, 285 (1913).
(2) AMBERGER, C.: Z. Untersuch. Nahr. u. Genussm. **26**, 65 (1913); **35**, 313 (1918); **40**, 192 (1920).
(3) AMBERGER, C., AND BAUCH, J.: Z. Untersuch. Nahr. u. Genussm. **48**, 371 (1924).
(4) AMBERGER, C., AND BROMIG, K.: Z. Untersuch. Nahr. u. Genussm. **42**, 193 (1921).
(5) AMBERGER, C., AND WIESEHAHN, A.: Z. Untersuch. Nahr. u. Genussm. **46**, 176 (1923).
(6) ANDRÉ, É., AND BLOCH, A.: Compt. rend. **195**, 627 (1932).
(7) ARMSTRONG, E. F., AND ALLAN, J.: J. Soc. Chem. Ind. **43**, 216T (1924).
(8) ARMSTRONG, E. F., ALLAN, J., AND MOORE, C. W.: J. Soc. Chem. Ind. **44**, 61T (1925); **44**, 143T (1925).
(9) ARMSTRONG, E. F., ALLAN, J., AND MOORE, C. W.: J. Soc. Chem. Ind. **44**, 63T (1925).
(10) ARMSTRONG, E. F., ALLAN, J., AND MOORE, C. W.: J. Soc. Chem. Ind. **44**, 143T (1925).
(11) ARMSTRONG, E. F., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **44**, 180T (1925).
(12) ATHERTON, D., AND MEARA, M. L.: J. Soc. Chem. Ind. **58**, 353 (1939); **59**, 97 (1940).
(13) AULT, W. C., AND BROWN, J. B.: J. Biol. Chem. **107**, 607 (1934).
(14) BANKS, A., AND HILDITCH, T. P.: Biochem. J. **25**, 1168 (1931).
(15) BANKS, A., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **51**, 111T (1932).
(16) BANKS, A., AND HILDITCH, T. P.: Biochem. J. **26**, 298 (1932).
(17) BANKS, A., DEAN, H. R., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **54**, 77T (1935).
(18) BANKS, A., HILDITCH, T. P., AND JONES, E. C.: Biochem. J. **27**, 1375 (1933).
(19) BAUGHMAN, W. F., AND JAMIESON, G. S.: J. Am. Chem. Soc. **42**, 152 (1920).
(20) BAUGHMAN, W. F., AND JAMIESON, G. S.: J. Am. Chem. Soc. **43**, 2696 (1921).
(21) BAUGHMAN, W. F., AND JAMIESON, G. S.: J. Am. Chem. Soc. **44**, 2952 (1922).
(22) BEDFORD, A.: "Über die ungesättigten Säuren des Leinols," Dissertation, Halle, 1906.
(23) BERTRAM, S. H.: Z. Untersuch. Lebensm. **55**, 179 (1928).
(24) BERTRAM, S. H.: Chem. Weekblad **33**, 3 (1936); Öle, Fette, Wachse, Seife, Kosmetik **1938**, No. 7, 1.
(25) BHATTACHARYA, R., AND HILDITCH, T. P.: Biochem. J. **25**, 1954 (1931).
(26) BHIDE, B. V., AND BHIDE, R. D.: Current Sci. **7**, 16 (1938); J. Univ. Bombay **7**, 97 (1938); **8**, 220 (1939).
(27) BIROSEL, D. M., MILLAR, F., NESSIA, E., AND TAGORDA, F.: Univ. Philippines Nat. and Appl. Sci. Bull. **7**, 39 (1939).
(28) BÖMER, A.: Z. Untersuch. Nahr. u. Genussm. **17**, 353 (1909); **25**, 321 (1913).
(29) BÖMER, A., AND BAUMANN, J.: Z. Untersuch. Nahr. u. Genussm. **40**, 97 (1920).
(30) BÖMER, A., AND EBACH, H.: Z. Untersuch. Lebensm. **55**, 501 (1928).
(31) BÖMER, A., AND ENGEL, H.: Z. Untersuch. Lebensm. **57**, 113 (1929).
(32) BÖMER, A., AND HÜTTIG, H.: Z. Untersuch. Lebensm. **75**, 1 (1938).
(33) BÖMER, A., AND MERTEN, H.: Z. Untersuch Nahr. u. Genussm. **43**, 101 (1922).

(34) BÖMER, A., SCHEMM, A., AND HEIMSOTH, F.: Z. Untersuch. Nahr. u. Genussm. **14,** 90 (1907).
(35) BÖMER, A., AND SCHNEIDER, K.: Z. Untersuch. Nahr. u. Genussm. **47,** 61 (1924).
(36) BROWN, J. B.: J. Biol. Chem. **90,** 133 (1931); Chem. Rev. **29,** 333 (1931).
(37) BROWN, J. B., AND FRANKEL, J.: J. Am. Chem. Soc. **60,** 54 (1938).
(38) BROWN, J. B., AND GREEN, N. D.: J. Am. Chem. Soc. **62,** 738 (1940).
(39) BROWN, J. B., AND SHINOWARA, G. V.: J. Am. Chem. Soc. **59,** 6 (1937).
(40) BROWN, J. B., AND STONER, G. G.: J. Am. Chem. Soc. **59,** 3 (1937).
(41) BUSHELL, W. J., AND HILDITCH, T. P.: J. Chem. Soc. **1937,** 1767.
(42) BUSHELL, W. J., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **57,** 48T, 447T (1938).
(43) BUSHELL, W. J., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **58,** 24 (1939).
(44) BURCH, C. R., AND VAN DIJCK, W. J. D.: J. Soc. Chem. Ind. **58,** 39T (1939).
(45) CALDWELL, K. S., AND HURTLEY, W. H.: J. Chem. Soc. **95,** 853 (1909).
(46) CARTER, M. G. R., AND MALKIN, T.: J. Chem. Soc. **1939,** 577, 1518.
(47) CASSIDY, H. G.: J. Chem. Education **16,** 88 (1939); J. Am. Chem. Soc. **62,** 3073, 3076 (1940).
(48) CHANNON, H. J., JENKINS, G. N., AND SMITH, J. A. B.: Biochem. J. **31,** 41 (1937).
(49) CHEVREUL, M.: *Recherches chimiques sur les corps gras*, p. 115. (1823).
(50) CHRISTIAN, B. C., AND HILDITCH, T. P.: Biochem. J. **23,** 327 (1929).
(51) CLARKE, T. H., CRAIG, R. S., AND STEGEMAN, G.: Private communication.
(52) CLARKE, T. H., AND STEGEMAN, G.: J. Am. Chem. Soc. **62,** 1815 (1940).
(53) CLARKSON, C. E., AND MALKIN, T.: J. Chem. Soc. **1934,** 666.
(54) COLE, H. I., AND CARDOSO, H. T.: J. Am. Chem. Soc. **60,** 614, 617 (1938).
(55) COLLIN, G., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **47,** 261T (1928).
(56) COLLIN, G., AND HILDITCH, T. P.: Biochem. J. **23,** 1273 (1929).
(57) COLLIN, G., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **49,** 138T (1930).
(58) COLLIN, G., HILDITCH, T. P., AND LEA, C. H.: J. Soc. Chem. Ind. **48,** 46T (1929).
(59) DAUBERT, B. F., AND KING, C. G.: Chem. Rev. **29,** 269 (1941).
(60) DEAN, H. K., AND HILDITCH, T. P.: Biochem. J. **27,** 1950 (1933).
(61) DEAN, H. K., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **52,** 165T (1933).
(62) DETWILER, S. B., JR.: Oil & Soap **17,** 241 (1940).
(63) DETWILER, S. B., JR., AND MARKLEY, K. S.: Oil & Soap **16,** 2 (1939); Ind. Eng. Chem., Anal. Ed. **12,** 348 (1940).
(64) DHINGRA, D. R., AND HANEEF, M.: J. Soc. Chem. Ind. **58,** 292 (1939).
(65) DHINGRA, D. R., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **50,** 9T (1931).
(66) DHINGRA, D. R., HILDITCH, T. P., AND VICKERY, J. R.: J. Soc. Chem. Ind. **48,** 281T (1929).
(67) DHINGRA, D. R., AND SHARMA, D. N.: J. Soc. Chem. Ind. **57,** 369 (1938).
(68) DOLLEAR, E. G., KRAUCZUNAS, P., AND MARKLEY, K. S.: Oil & Soap **15,** 263 (1938); **17,** 120 (1940).
(69) DORTA, G., AND REGGIANI, M.: Atti. congr. intern. chim., Rome **4,** 277 (1938).
(70) EIBNER, A., AND BRASEL, F.: Chem. Umschau Fette, Öle, Wachse Harze **35,** 157 (1928).
(71) EIBNER, A., AND WIBELITZ, B.: Chem. Umschau Fette, Öle, Wachse Harze **31,** 109, 121 (1924).
(72) EIBNER, A., WIDENMAYER, L., AND SCHILD, E.: Chem. Umschau Fette, Öle, Wachse Harze **34,** 312 (1927).
(73) ELLIS, N. R., AND ISBELL, H. S.: J. Biol. Chem. **69,** 239 (1926).
(74) ELLIS, N. R., ROTHWELL, C. S., AND POOL, W. O.: J. Biol. Chem. **92,** 385 (1931).
(75) ELLIS, N. R., AND ZELLER, J. H.: J. Biol. Chem. **89,** 185 (1930).
(76) ESCOURROU, R.: Bull. soc. chim. **6,** 360 (1939).

(77) FAWCETT, E. W. M.: J. Chem. Ind. Trans. and Comm. **1939,** 49.
(78) FREEMAN, S. E.: U. S. patent 2,200,390-1 (May 14, 1940).
(79) FRITZWEILER, R.: Z. Untersuch. Nahr. u. Genussm. **5,** 1164 (1902).
(80) GADAMER, J. G.: Arch. Pharm. **237,** 471 (1899).
(81) GILL, A. H., AND TUCKER, C. N.: Oil & Fat Industries **7,** 101 (1930).
(82) GREEN, T. G., AND HILDITCH, T. P.: Biochem. J. **29,** 1552 (1935).
(83) GREEN, T. G., AND HILDITCH, T. P.: Biochem. J. **32,** 681 (1938).
(84) GREEN, T. G., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **57,** 49T (1938).
(85) GRIFFITHS, H. N., AND HILDITCH, T. P.: J. Chem. Soc. **1932,** 2317.
(86) GRIFFITHS, H. N., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **53,** 75T (1934).
(87) GUNDE, B. G., AND HILDITCH, T. P.: J. Chem. Soc. **1939,** 1015.
(88) GUNDE, B. G., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **59,** 47T (1940).
(89) GUPTE, M. P., AND DUTT, S.: J. Indian Chem. Soc. **13,** 613 (1936).
(90) HARPER, D. A.: Reference 100, p. 24.
(91) HARPER, D. A., AND HILDITCH, T. P.: J. Soc. Chem. Ind. **56,** 322T (1937).
(92) HARPER, D. A., HILDITCH, T. P., AND TERELESKI, J. T.: J. Soc. Chem. Ind. **56,** 310T (1937).
(93) HASHI, K.: J. Soc. Chem. Ind. Japan **30,** 849, 856 (1927); **31,** 117 (1928).
(94) HEISE, R.: Tropenpflanzer **1,** 10 (1897).
(95) HICKMAN, K. C. D.: Ind. Eng. Chem. **29,** 968, 1107 (1937); U. S. patent 2,126,466 (August 9, 1937); British patent 485,549 (May 18, 1937); Ind. Eng. Chem. **32,** 1451 (1940).
(96) HILDITCH, T. P.: J. Soc. Chem. Ind. **49,** 210 (1930); Chem. Trade J. **86,** 287 (1930); Oil & Fat Industries **7,** 339, 347 (1930); J. Oil Colour Chem. Assoc. **13,** 209 (1930); Food **4,** 350 (1935).
(97) HILDITCH, T. P.: Proc. Roy. Soc. (London) **B103,** 111 (1928); Allgem. Oel- u. Fett-Ztg. **27,** 93, 111, 155, 219 (1930); J. Soc. Chem. Ind. **52,** 169 (1933); Proc. Pacific Sci. Congr., 5th Congr., Can., 1933, **5,** 3647 (1934); Fettchem. Umschau **42,** 105 (1935); Chemistry & Industry **1935,** 139, 163, 184; in Schönfeld's *Chemie und Technologie der Fette und Fettprodukte,* 2nd edition, edited by G. Hefter, Vol. I, pp. 60, 149, J. Springer, Vienna (1937); Congr. intern. tech. chim. ind. agr. (Scheveningen) **5, II,** 367 (1937).
(98) HILDITCH, T. P.: J. Chem. Soc. **1932,** 805; Sci. J. Roy. Coll. Sci. **5,** 63 (1935).
(99) HILDITCH, T. P.: Biochem. J. **28,** 779 (1934).
(100) HILDITCH, T. P.: *The Chemical Constitution of Natural Fats.* John Wiley and Sons, Inc., New York (1940).
(101) HILDITCH, T. P.: Chem. Products **3,** 78 (1940).
(102) HILDITCH, T. P., BANKS, A., AND DEAN, H. K.: J. Soc. Chem. Ind. **54,** 77T (1935).
(103) HILDITCH, T. P., COLLIN, G., DRUMMOND, J. C., AND GUNTHER, E. R.: J. Exptl. Biol. **11,** 198 (1934).
(104) HILDITCH, T. P., DHINGRA, D. R., AND RHEAD, A. J.: J. Soc. Chem. Ind. **51,** 195T (1932).
(105) HILDITCH, T. P., AND GRIFFITHS, H. N.: Analyst **59,** 312 (1934).
(106) HILDITCH, T. P., AND GRIFFITHS, H. N.: J. Soc. Chem. Ind. **53,** 75T (1934).
(107) HILDITCH, T. P., AND HOULBROOK, A.: Analyst **53,** 246 (1928).
(108) HILDITCH, T. P., AND ICHAPORIA, M. B.: J. Soc. Chem. Ind. **55,** 189T (1936).
(109) HILDITCH, T. P., AND ICHAPORIA, M. B.: J. Soc. Chem. Ind. **57,** 44T (1938).
(110) HILDITCH, T. P., ICHAPORIA, M. B., AND JASPERSON, H.: J. Soc. Chem. Ind. **57,** 363 (1938).
(111) HILDITCH, T. P., AND JASPERSON, H.: J. Soc. Chem. Ind. **58,** 233 (1939).

(112) HILDITCH, T. P., AND JONES, E. C.: J. Chem. Soc. **1932**, 805.
(113) HILDITCH, T. P., AND JONES, E. C.: J. Soc. Chem. Ind. **53**, 13T (1934).
(114) HILDITCH, T. P., JONES, E. C., AND RHEAD, A. J.: Biochem. J. **28**, 786 (1934).
(115) HILDITCH, T. P., AND JONES, E. E.: J. Soc. Chem. Ind. **46**, 174T (1927).
(116) HILDITCH, T. P., AND JONES, E. E.: J. Soc. Chem. Ind. **46**, 467T (1927).
(117) HILDITCH, T. P., AND JONES, E. E.: Biochem. J. **22**, 326 (1928).
(118) HILDITCH, T. P., AND JONES, E. E.: J. Soc. Chem. Ind. **49**, 363T, 369T (1930).
(119) HILDITCH, T. P., AND JONES, E. E.: J. Soc. Chem. Ind. **50**, 171T (1931).
(120) HILDITCH, T. P., AND LEA, C. H.: J. Chem. Soc. **1927**, 3106.
(121) HILDITCH, T. P., AND LEA, C. H.: J. Soc. Chem. Ind. **48**, 41T (1929).
(122) HILDITCH, T. P., LEA, C. H., AND PEDELTY, W. H.: Biochem. J. **33**, 493 (1939).
(123) HILDITCH, T. P., AND LONGENECKER, H. E.: Biochem. J. **31**, 1805 (1937).
(124) HILDITCH, T. P., AND LOVERN, J. A.: J. Soc. Chem. Ind. **47**, 105T (1928).
(125) HILDITCH, T. P., AND LOVERN, J. A.: J. Soc. Chem. Ind. **48**, 359T, 365T (1929).
(126) HILDITCH, T. P., AND LOVERN, J. A.: Nature **137**, 478 (1936).
(127) HILDITCH, T. P., AND MADDISON, L.: J. Soc. Chem. Ind. **59**, 67T (1940).
(128) HILDITCH, T. P., AND MADDISON, L.: J. Soc. Chem. Ind. **59**, 162T (1940).
(129) HILDITCH, T. P., AND MEARA, M. L.: J. Chem. Soc. **1938**, 1608.
(130) HILDITCH, T. P., MEARA, M. L., AND PEDELTY, W. H.: J. Soc. Chem. Ind. **58**, 26T (1939).
(131) HILDITCH, T. P., AND MURTI, K. S.: J. Soc. Chem. Ind. **58**, 310T (1939).
(132) HILDITCH, T. P., AND MURTI, K. S.: J. Soc. Chem. Ind. **58**, 351T (1939).
(133) HILDITCH, T. P., AND MURTI, K. S.: Biochem. J. **34**, 1301 (1940).
(134) HILDITCH, T. P., AND PAUL, H.: J. Soc. Chem. Ind. **54**, 331T (1935).
(135) HILDITCH, T. P., AND PAUL, H.: Biochem. J. **31**, 227 (1937).
(136) HILDITCH, T. P., AND PAUL, S.: Biochem. J. **32**, 1775 (1938).
(137) HILDITCH, T. P., PAUL, S., GUNDE, B. G., AND MADDISON, L.: J. Soc. Chem. Ind. **59**, 138 (1940).
(138) HILDITCH, T. P., AND PEDELTY, W. H.: Biochem. J. **34**, 971 (1940).
(139) HILDITCH, T. P., AND PREISTMAN, J.: J. Soc. Chem. Ind. **49**, 197T (1930).
(140) HILDITCH, T. P., AND PREISTMAN, J.: J. Soc. Chem. Ind. **49**, 397T (1930).
(141) HILDITCH, T. P., AND PREISTMAN, J.: Analyst **56**, 354 (1931).
(142) HILDITCH, T. P., AND RHEAD, A. J.: J. Soc. Chem. Ind. **51**, 198T (1932).
(143) HILDITCH, T. P., AND RIGG, J. G.: J. Soc. Chem. Ind. **54**, 109T (1935).
(144) HILDITCH, T. P., RILEY, T., AND VIDYARTHI, N. L.: J. Soc. Chem. Ind. **46**, 457, 462 (1927).
(145) HILDITCH, T. P., AND SALETORE, S. A.: J. Soc. Chem. Ind. **50**, 468 (1931); **52**, 101 (1933).
(146) HILDITCH, T. P., AND SHORLAND, F. B.: Biochem. J. **31**, 1499 (1937).
(147) HILDITCH, T. P., AND SLEIGHTHOLME, J.: Biochem. J. **25**, 507 (1931).
(148) HILDITCH, T. P., AND STAINSBY, W. J.: J. Soc. Chem. Ind. **53**, 197T (1934).
(149) HILDITCH, T. P., AND STAINSBY, W. J.: Biochem. J. **29**, 90 (1935).
(150) HILDITCH, T. P., AND STAINSBY, W. J.: Biochem. J. **29**, 594 (1935).
(151) HILDITCH, T. P., AND STAINSBY, W. J.: J. Soc. Chem. Ind. **55**, 95T (1936).
(152) HILDITCH, T. P., AND TERELESKI, J. T.: J. Soc. Chem. Ind. **56**, 315T (1937).
(153) HILDITCH, T. P., AND THOMPSON, H. M.: J. Soc. Chem. Ind. **56**, 434T (1937).
(154) HILDITCH, T. P., AND VIDYARTHI, N. L.: J. Soc. Chem. Ind. **46**, 172T (1927).
(155) ICHAPORIA, M. B.: Dissertation, Liverpool, 1937.
(156) IVANOV, S. L., AND RESNIKOVA, S. B.: Schriften zentral. biochem. Forsch. Inst. Nahr. u. Genussmittelind (U. S. S. R.) **3**, 239 (1933).
(157) JAMIESON, G. S.: Oil & Fat Industries **3**, 256 (1926).

(158) JAMIESON, G. S., AND BAUGHMAN, W. F.: Oil & Fat Industries **7**, 419, 437 (1930).
(159) JAMIESON, G. S., AND BAUGHMAN, W. F.: Oil & Soap **9**, 136 (1932).
(160) JAMIESON, G. S., BAUGHMAN, W. F., AND BRAUNS, D. H.: J. Am. Chem. Soc. **43**, 1372 (1921).
(161) JAMIESON, G. S., BAUGHMAN, W. F., AND GERTLER, S. I.: Oil & Fat Industries **7**, 181 (1930).
(162) JAMIESON, G. E., AND GERTLER, S. I.: Oil & Fat Industries **6**, No. 4, 11 (1929).
(163) JAMIESON, G. S., AND GERTLER, S. I.: Oil & Fat Industries **7**, 371 (1930).
(164) JAMIESON, G. S., AND MCKINNEY, R. S.: Oil & Fat Industries **6**, 21 (1929).
(165) JAMIESON, G. S., AND MCKINNEY, R. S.: Oil & Soap **10**, 147 (1933).
(166) JAMIESON, G. S., AND MCKINNEY, R. S.: Oil & Soap **11**, 193 (1934).
(167) JAMIESON, G. S., AND MCKINNEY, R. S.: Oil & Soap **12**, 241 (1935).
(168) JAMIESON, G. S., AND MCKINNEY, R. S.: Oil & Soap **13**, 202 (1936).
(169) JAMIESON, G. S., AND MCKINNEY, R. S.: Oil & Soap **13**, 233 (1936).
(170) JAMIESON, G. S., AND MCKINNEY, R. S.: Oil & Soap **15**, 295 (1938).
(171) JAMIESON, G. S., AND ROSE, W. G.: Oil & Soap **17**, 144 (1940).
(172) JANTZEN, E., AND TIEDCKE, C.: J. prakt. Chem. **127**, 277 (1930).
(173) KASS, J. P., AND BURR, G. O.: J. Am. Chem. Soc. **61**, 1062 (1937).
(174) KAUFMANN, H. P.: Allgem. Oel- u. Fett-Ztg. **27**, 39 (1930).
(175) KAUFMANN, H. P.: Fette u. Seifen **46**, 268 (1939); **47**, 460 (1940); Angew. Chem. **53**, 98 (1940).
(176) KAUFMANN, H. P., AND BALTES, J.: Ber. **69**, 2676 (1936).
(177) KAUFMANN, H. P., AND BALTES, J.: Fette u. Seifen **45**, 175 (1938).
(178) KAUFMANN, H. P., AND FIEDLER, H.: Fette u. Seifen **45**, 299 (1938).
(179) KAUFMANN, H. P., AND SCHMIDT, O.: Fette u. Seifen **47**, 294 (1940).
(180) KAUFMANN, H. P., AND WOLF, W.: Fette u. Seifen **47**, 252 (1940).
(181) KLENK, E.: Z. physiol. Chem. **221**, 67, 259, 264 (1933).
(182) KLENK, E., AND DITT, F.: Z. physiol. Chem. **226**, 213 (1934).
(183) KLENK, E., DITT, F., AND DIEBALD, W.: Z. physiol. Chem. **232**, 54 (1935).
(184) KLIMONT, J.: Ber. **34**, 2636 (1901); Monatsh. **23**, 51 (1902); **24**, 408 (1903); **25**, 929 (1904); **26**, 563 (1905); **33**, 441 (1912); Z. Untersuch. Nahr. u. Genussm. **12**, 359 (1906).
(185) KLIMONT, J.: Monatsh. **25**, 931 (1904).
(186) KLIMONT, J., AND MEISELS, E.: Monatsh. **30**, 341 (1909).
(187) KOYAMA, R.: J. Soc. Chem. Ind. Japan **31**, 140, 211, 238, 259, 298 (1928).
(188) KRAFFT, F.: Ber. **10**, 2034 (1877); **13**, 1413 (1880).
(189) KRAFFT, F.: Ber. **36**, 4339 (1903).
(190) KRAFFT, F., AND DYES, W. A.: Ber. **28**, 2583 (1895).
(191) KRAFFT, F., AND NOERDLINGER, H.: Ber. **22**, 816 (1889).
(192) KRAFFT, F., AND WEILANDT, H.: Ber. **29**, 1316 (1896).
(193) KRZIZAN, R.: Chem. Rev. Fett- u. Harz-Ind. **15**, 7, 29 (1908).
(194) LEWIS, G. L.: J. Am. Chem. Soc. **62**, 1529 (1940).
(195) LONGENECKER, H. E.: J. Soc. Chem. Ind. **56**, 199T (1937).
(196) LONGENECKER, H. E.: J. Biol. Chem. **128**, 645 (1939); **129**, 13 (1939); **130**, 167 (1939).
(197) LONGENECKER, H. E.: Oil & Soap **17**, 53 (1940).
(198) LONGENECKER, H. E.: Unpublished observations.
(199) LONGENECKER, H. E., GAVIN, G., AND MCHENRY, E. W.: J. Biol. Chem. **134**, 693 (1940).
(200) LONGENECKER, H. E., AND HILDITCH, T. P.: Biochem. J. **32**, 784 (1938).
(201) LOON, J. VAN: Farben-Ztg. **35**, 1767 (1930).

(202) LOVERN, J. A.: Biochem. J. **26,** 1978 (1932).
(203) LOVERN, J. A.: Biochem. J. **28,** 394 (1934).
(204) LOVERN, J. A.: Biochem. J. **28,** 1955, 1961 (1934); **30,** 20 (1936).
(205) LOVERN, J. A.: Biochem. J. **29,** 847 (1935); **30,** 387 (1936).
(206) LOVERN, J. A.: Biochem. J. **31,** 755 (1937).
(207) LOVERN, J. A.: Biochem. J. **32,** 2142 (1938).
(208) LOVERN, J. A.: Biochem. J. **34,** 709 (1940).
(209) MACHADO, A.: Rev. soc. brasil. quím. **7,** 73 (1938).
(210) MALKIN, T., AND MEARA, M. L.: J. Chem. Soc. **1939,** 103, 1141.
(211) MALKIN, T., AND SHURBAGY, M. R. EL: J. Chem. Soc. **1936,** 1628.
(212) MALKIN, T., SHURBAGY, M. R. EL, AND MEARA, M. L.: J. Chem. Soc. **1937,** 1409.
(213) MANUNTA, C.: Helv. Chim. Acta **22,** 1156 (1939).
(214) MCCUTCHEON, J. W.: Can. J. Reaearch **18,** 231 (1940).
(215) MCKINNEY, R. S., AND JAMIESON, G. S.: Oil & Soap **12,** 92 (1935).
(216) MCKINNEY, R. S., AND JAMIESON, G. S.: Oil & Soap **13,** 10 (1936).
(217) MCKINNEY, R. S., AND JAMIESON, G. S.: Oil & Soap **14,** 2 (1937).
(218) MCKINNEY, R. S., AND JAMIESON, G. S.: Oil & Soap **15,** 30 (1938).
(219) MEARA, M. L., AND ZAKY, Y. A. H.: J. Soc. Chem. Ind. **59,** 25 (1940).
(220) MILLER, E. S., AND BURR, G. O.: Chem. Rev. **29,** 419 (1941).
(221) MONTROLL, E.: Private communication.
(222) NOBORI, H.: J. Soc. Chem. Ind. Japan **43,** 59B (1940).
(223) NOBORI, H.: J. Soc. Chem. Ind. Japan **43,** 340B (1940).
(224) PANJUTIN, P., AND RAPOPORT, M.: Chem. Umschau Fette, Öle, Wachse Harze **37,** 130 (1930).
(225) PIGULEVSKIĬ, G., AND SIMONOWA, N.: J. Gen. Chem. (U. S. S. R) **9,** 1928 (1939).
(226) PUNTAMBEKAR, S. V.: J. Indian Chem. Soc. **15,** 19 (1938).
(227) RAWLINGS, H. W.: Oil & Soap **16,** 231 (1939).
(228) RIEBSOMER, J. L., AND NESTY, G. A.: J. Am. Chem. Soc. **56,** 1784 (1934).
(229) RIEMENSCHNEIDER, R. W., SWIFT, C. E., AND SANDO, C. E.: Oil & Soap **17,** 145 (1940).
(230) RIEMENSCHNEIDER, R. W., WHEELER, D. H., AND SANDO, C. E.: J. Biol. Chem. **127,** 391 (1939).
(231) ROBERTS, W. L., AND SCHUETTE, H. A.: J. Am. Chem. Soc. **13,** 613 (1936).
(232) ROLLETT, R.: Z. physiol. Chem. **62,** 410, 424 (1909).
(233) SCHOENHEIMER, R.: Harvey Lectures, 1936–37.
(234) SCHOENHEIMER, R., AND RITTENBERG, D.: J. Biol. Chem. **111,** 163, 169, 175 (1935); **113,** 505 (1936); **114,** 381 (1936); **117,** 485 (1937); **120,** 155, 503 (1937); **121,** 235 (1937).
(235) SCHUETTE, H. A., COWLEY, M. A., AND CHANG, C. V.: J. Am. Chem. Soc. **56,** 2085 (1934).
(236) SCHUETTE, H. A., THOMAS, R. W., AND DUTLEY, M. V.: J. Am. Chem. Soc. **52,** 4114 (1930).
(237) SCHUETTE, H. A., AND ZEHNPFENNIG, R. G.: Oil & Soap **14,** 269 (1937).
(238) SHINOWARA, G. Y., AND BROWN, J. B.: J. Am. Chem. Soc. **60,** 2734 (1938).
(239) SHINOWARA, G. Y., AND BROWN, J. B.: J. Biol. Chem. **134,** 331 (1940).
(240) SPADOLA, J. M., AND ELLIS, N. R.: J. Biol. Chem. **113,** 205 (1936).
(241) STEGER, A., AND LOON, J. VAN: J. Soc. Chem. Ind. **47,** 361T (1928).
(242) STEGER, A., AND LOON, J. VAN: Rec. trav. chim. **47,** 471 (1928).
(243) STEGER, A., AND LOON, J. VAN: Chemistry & Industry **13,** 1095 (1935).
(244) STEGER, A., LOON, J. VAN, AND SMELT, C.: J. Soc. Chem. Ind. **55,** 41T (1936).

(245) STOUT, A. W., AND SCHUETTE, H. A.: J. Am. Chem. Soc. **54**, 3298 (1932).
(246) SUDBOROUGH, J. J., WATSON, H. E., AYYAR, P. R., AND DAMLE, N. R.: J. Indian Inst. Sci. **9A**, 26 (1926).
(247) SUDBOROUGH, J. J., WATSON, H. E., AYYAR, P. R., AND MIRCHONDANI, T. J.: J. Indian Inst. Sci. **9A**, 65 (1926).
(248) SUDBOROUGH, J. J., WATSON, H. E., AYYAR, P. R., AND DAMLE, N. R.: J. Indian Inst. Sci. **9A**, 65 (1926).
(249) SULLIVAN, B., AND BAILEY, C. H.: J. Am. Chem. Soc. **58**, 383, 390 (1936).
(250) SUZUKI, B., AND YOKOYAMA, Y.: Proc. Imp. Acad. (Tokyo) **3**, 526, 529, 531 (1927).
(251) SUZUKI, B., AND MASUDA, Y.: Proc. Imp. Acad. (Tokyo) **3**, 351 (1927); **4**, 165 (1928); **5**, 268 (1929).
(252) TÄUFEL, K., AND RUSCH, M.: Z. Untersuch. Lebensm. **57**, 422 (1929).
(253) TAYLOR, E. R., AND CLARKE, H. T.: J. Am. Chem. Soc. **49**, 2829 (1937).
(254) THALER, H.: Z. Untersuch. Lebensm. **75**, 130 (1938).
(255) THOMAS, B. H., CUTHBERTON, C. C., AND BEARD, F.: Proc. Am. Soc. Animal Production **27**, 193 (1934).
(256) TRAPPE, W.: Biochem. Z. **305**, 150 (1940).
(257) TRAPPE, W.: Biochem. Z. **306**, 316 (1940).
(258) TSUJIMOTO, M.: Chem. Umschau Fette, Öle, Wachse Harze **39**, 50 (1932).
(259) TSUJIMOTO, M.: J. Soc. Chem. Ind. **51**, 317T (1932).
(260) TSUJIMOTO, M.: J. Soc. Chem. Ind. Japan **36**, 676B (1933).
(261) TSUJIMOTO, M., AND KOYANAGI, H.: Bull. Chem. Soc. (Japan) **8**, 161 (1933).
(262) TVERAAEN, I.: *Cf.* reference 100, p. 50.
(263) TWITCHELL, E.: Ind. Eng. Chem. **13**, 806 (1921).
(264) VIDYARTHI, N. L., AND MALLYA, M. V.: J. Indian Chem. Soc. **16**, 443 (1939).
(265) VIDYARTHI, N. L., AND MALLYA, M. V.: J. Indian Chem. Soc. **17**, 87 (1940).
(266) VIDYARTHI, N. L., AND RAO, C. J. D.: J. Indian Chem. Soc. **16**, 437 (1939).
(267) VOLAROVICH, M. P., AND RAVICH, G. B.: Compt. rend. acad. sci. U. S. S. R. **23**, 252 (1939).
(268) VONGERICHTEN, E., AND KOHLER, A.: Ber. **42**, 1638 (1909).
(269) WEITKAMP, A. W., AND BRUNSTRUM, L. C.: Oil & Soap **18**, 47 (1941).
(270) WANG, T. H., AND KAN, C. H.: J. Chinese Chem. Soc. **4**, 393 (1936).
(271) WILLIAMS, N. V., AND MASLOV, N. Y.: Schriften zentral. Forsch. Inst. Lebensmittelchem. (U. S. S. R.) **4**, 150 (1935).
(272) WITTKA, F.: Angew. Chem. **53**, 557 (1940).
(273) ZECHMEISTER, L., AND CHOLNOKY, O.: *Die chromatographische Adsorptionsmethoden*. J. Springer, Vienna (1938).