# T. P. HILDITCH SYMPOSIUM ON ANALYSIS OF NATURAL FAT TRIGLYCERIDES

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## The Study of Natural Fat Triglycerides— Retrospect and Prospect

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THE SOCIETY has named the Symposium at the Houston meeting the "T. P. Hilditch Symposium on Analysis of Natural Fat Triglycerides." For me this is a very great honor and, moreover, a mark of esteem on the part of my American colleagues which is very precious; I value greatly the privilege of being thus associated with the symposium and regret only that I cannot be with you to meet my fellow-members of the American Oil Chemists' Society at their 1965 Spring Convention.

President Stillman kindly suggested a short talk at the opening of the symposium on a "topic of my choice": it may be appropriate to sketch a brief historical survey of the way knowledge of natural glyceride structure has developed, and to add a few tentative notes on future possibilities. This survey must perforce be very general and cannot refer in detail to many of the contributions which have been made; moreover, it represents for the most part only my personal view of the subject.

Intimate study of natural triglyceride composition is essentially a twentieth-century achievement; but one likes to recall that Berthelot (1860) noted, as soon as the trihydric nature of glycerol was realised, that the triglyceride molecule might include more than one kind of fatty acid ("mixed" glycerides), and that in 1902 Guth already spoke of natural fats as "mixtures of mixed glycerides." In the early years of this century several workers attempted to separate individual glycerides from a number of vegetable and animal fats by crystallisation from solvents, but only in one or two instances were more than very minor amounts of any simple triglyceride (e.g., trilaurin, tripalmitin or tristearin) detected, in spite of some hundreds of crystallisations being involved in some cases. This qualitative evidence therefore supported the view that the great majority of natural triglycerides were "mixed," i.e., contained at least two different fatty acids.

Quantitative studies of glyceride composition may be said to date from about 1927, when a method of determination of trisaturated glycerides in a fat was devised, depending upon the conversion of unsaturated groups to acidic (azaleo) glycerides which could be separated from the unchanged trisaturated glycerides. This was the only "chemical" method of attack available for nearly thirty years; it was supplemented by systematic crystallisation of fats from acetone (or ether) at temperatures from about −60C upwards. A considerable number of natural fats were studied in these ways by the writer and his associates, and some general conclusions resulted.

In seed fats and in a number of animal fats it was concluded that there is a "pronounced tendency

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to even distribution of the fatty acids throughout the glycerides,'' irrespective of the particular fatty acids present in different fats: trisaturated glycerides did not appear in quantity unless saturated acids formed 60–65% of the total fatty acids in a fat (isolated exceptions to this statement occurred, e.g., trilaurin in laurel kernel oil). In the fats of ruminant animals (which unfortunately but perhaps naturally have so far been studied more intensively than those of non-ruminants) the proportions of trisaturated glycerides (palmitostearins) were observed to be much greater than in other (e.g., seed) fats with comparable proportions of saturated and unsaturated acids. We now know that ruminant animals were practising ''fat-hydrogenation'' long before the technological process was thought of, and this probably accounts for their particular type of glyceride composition.

The low-temperature crystallisation procedure for segregating natural fats into simpler groups of glycerides can be considered adequate for fats which do not contain excessive proportions of unsaturated acids, but it has become evident that it is incapable of resolving mixtures of preponderatingly unsaturated composition (such as trilinolein, oleodilinolein and dioleolinolein). Thus it failed to indicate the presence of somewhat high proportions of trilinolenin in linseed oil or of trilinolein in safflower seed oil, revealed when Dutton and co-workers applied counter-current distribution between two immiscible solvents to these and other fatty oils (1956–1961).

At length a new means of *chemical* attack on glyceride structure came to light with the observation (1952) that pancreatic lipase selectively hydrolyses acyl groups attached to the primary (1- and 3-) hydroxyl groups of the glycerol molecule largely before those attached to the central (2-) hydroxyl groups are removed. In the next few years Desnuelle and Savary, Mattson and co-workers, Coleman, Barrett et al. and others applied this procedure to many seed fats, and it was at once evident that a very high proportion (usually 96–99%) of the acyl groups attached at the central (2-) positions consisted of oleic and/or linoleic acid. Saturated acids and other unsaturated acids (e.g., erucic), with any excess of oleic or linoleic beyond that necessary to combine with all the 2-glyceryl positions, were almost wholly attached to the outer (1-, 3-) glyceryl groups. In the relatively few animal fats so far similarly examined the position appears less positive: the proportions of saturated (mainly palmitic) acid combined at the central glyceryl group were greater than in seed fats, ranging in a number of species from 20–35% of the total acids in this position, and in one group of pig fats forming 70–80%.

During this period, also, great advances were made in the application of new chromatographic techniques, and in glyceride studies the use of thin layer chromatography, especially with silica gel impregnated with a silver salt as adsorbent, is yielding exceptionally promising results. Moreover, the use of gas-liquid chromatography in the determination of fatty acids has led to immense reduction in time, and in facilitating the use of very small amounts of experimental material. Nevertheless, and without disparaging the great assistance of the physical methods of separation, it may fairly be said that the two procedures which have given most insight into glyceride composition are those involving chemical alteration of the glyceride molecules—the now somewhat antique determination of trisaturated glycerides and the new selective enzyme hydrolysis which has revealed unsuspected selectivity in the attachment of acyl groups to glycerol.

I will digress here to mention a matter which at first glance has little connection with the problems of natural triglyceride structure: Kartha (1964) has observed the complete absence of any mono- or diglycerides at any stage in the fats of ripening coconuts. As he puts it, a glycerol molecule adsorbed on a lipase enzyme is not desorbed until all its three hydroxyl groups have been esterified with fatty acids. If this should prove to hold generally, it must be a basic factor in our ultimate understanding of the biosynthesis of seed glycerides.

*Computation of Triglycerides from Component Fatty Acid Compositions.* Many attempts have been made to find means whereby the component glycerides of a natural fat may be evaluated from its component acid composition, and concurrently to ascertain whether observed glyceride compositions accord with ''even'' or ''random'' distribution; complete approach to either rigid ''even'' or wholly ''random'' types is never observed, but some authors hold the view that the distribution of glycerides takes a ''partial random'' or a ''restricted random'' course. Much argument and not a few papers were produced in the course of this discussion, much of which may now seem rather pointless, since at the time the essential factor of selective attachment of specific fatty acids to the central and outer glyceryl hydroxyl groups had not been ascertained. When this was established, fresh modes of computation based upon the primary assumptions that oleic and linoleic acids are selectively attached at the 2-glyceryl position, and that any excess of these acids, some other unsaturated acids and all saturated acids are attached indiscriminately or random-wise at the 1- and 3-positions were made by Vander Wal (1960) and by Gunstone (1962). The results of such calculations accord well with experimental figures obtained with the more recent techniques and the findings of these two authors (themselves contributors to the symposium) suggest ''that the older conflicting views can now be brought together in a single distribution pattern covering the whole range of vegetable fats'' (Gunstone). But finality has not been approached, and very much remains to be done before our knowledge of natural triglycerides can be considered adequate, much less complete.

*In What Directions Are Further Glyceride Studies Most Needful?* One must not trespass on ground to be covered by others later in the symposium, but some general outline of one's personal opinion may be pardoned: the main need is for more *facts,* and over a much *wider range* of natural fats than has so far been studied. A comparison with the analogous field of *component fatty acid* determinations is illuminating here.

The component fatty acids of some 900 vegetable and 500 animal fats have so far been determined. This is still only a tiny cross-section of the total number of known species in nature, but it has served to define completely the marked extent to which related biological species produce in their fats broadly and often closely similar specific mixtures of fatty acids, at the same time illustrating the sporadic occurrence of unusual acids (e.g., epoxyoleic, ricinoleic, cyclopropenoid, conjugated di- and tri-ene) in a number of individual families or individual genera.

The *component glycerides* of only about *100 seed fats and 20 animal fats* have so far been studied in any detail. The animal fats are largely those of ruminants and the most pressing need is for a survey of the glycerides in many fats of the non-ruminant mammalia and also of birds, reptiles and fish. To a somewhat less extent this lack of factual evidence also applies to the vegetable fats. No satisfactory picture of natural triglyceride constitution will be reached until the range of specimens examined at least approaches that for which the fatty acid compositions are already known. The reason for this paucity is evident: glyceride studies have so far been made mainly on fats of industrial or medicinal interest. Whilst understandable, this affords a very one-sided approach to knowledge of the wider range of natural fats. If, as has hitherto obtained, academic workers show little interest in natural triglycerides, it can only be hoped that some government or industrial institutions may be moved to see the need for fully comprehensive survey of the field irrespective of primarily utilitarian interests. After all, with the techniques presently available, a component glyceride study will occupy much less time than the older component acid analysis before GLC was at hand.

Besides the urgent need for studies over a much wider range of animal fats, it would be interesting to ascertain whether $C_{18}$ polyethenoid acids such as elaeostearic, or even linolenic, cease to exhibit the same preferential attachment for the central glyceryl position as do oleic and linoleic in seed fats; whilst the general build of the 2-acyl groups in animal fats needs a good deal of further clarification and experimental study.

Above all, whilst the great assistance of selective enzyme hydrolysis, thin-layer chromatography and other recently devised procedures will no doubt be bettered by further advances in technique, the discovery of still more novel methods should be sought, especially to see if any new chemical method of attack still remains a possibility.

I wish, before concluding, to set on record my special thanks to two good friends: to Dr. Waldo C. Ault, who most kindly agreed to receive on my behalf your Award and to read this communication for me; and to Dr. F. D. Gunstone, for the great generosity with which he referred to me in the course of his prefatory remarks to the Symposium.

I would also like to repeat my deep appreciation of the manner in which my name has been linked with this Symposium, and to express the wish that it may fulfil all the hopes of its convenors and participants. May its deliberations be constructive, lively, provocative and a stimulant to all toilers in this sector of lipid chemistry.

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# The Triglyceride Composition of *Myrica carolinensis* Fruit Coat Fat (Bayberry Tallow)[1]

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## Abstract

The triglycerides of *Myrica carolinensis* fruit coat fat (bayberry tallow) contain only three fatty acids: myristic (21.5 mole %), palmitic (77.5%), and stearic (1.0%). The component triglycerides of this simple fat were determined by gas-liquid chromatography and pancreatic lipase hydrolysis. Triglycerides with carbon numbers 42, 44, 46, 48, and 50 were found. Lipase hydrolysis showed a preferential but not exclusive esterification of myristic acid at the 2-position. The triglyceride composition calculated from the combined experimental results did not conform to a random or 1,3-random-2-random distribution pattern. Regional differences in fatty acid and triglyceride composition within the fruit coat were also observed.

## Introduction

BAYBERRY TALLOW (also known as myrtle, candleberry, or capeberry wax) is a hard, solid fat with a gray-green color and a spicy fragrance. It has a small commercial importance as an ingredient of fancy candles. The fat comes from the fruit coat of several species of *Myrica,* a small tree which grows wild in America, Europe, and Africa. Youngken (1) has published a comprehensive monograph describing this genus.

The physical and chemical properties of bayberry tallow have been described by numerous workers (2-9). Several recent investigators have determined the fatty acid composition of a commercial product or of the fat from a specific *Myrica species* (3,4,8,10, 11). In all cases, myristic (33–61%) and palmitic (37–52%) acids composed 80–100% of the component fatty acids. Small amounts of lauric, stearic, and oleic acids have also been reported. The triglyceride composition of this fat has not been previously examined.

Since bayberry tallow has such a simple fatty acid composition, it must also have a relatively simple triglyceride composition which could be easily determined by modern analytical methods. This paper describes the application of pancreatic lipase hydrolysis and gas-liquid chromatography (GLC) techniques to the determination of the triglyceride composition of *Myrica carolinensis* fruit coat fat.

## Procedures

### Materials

Preliminary analyses of several commercial samples of bayberry tallow showed widely varying compositions, probably due to different sample origins and to fractional crystallization during processing. GLC results on one commercial product even indicated the presence of substantial amounts of petroleum wax. To insure the authenticity of the sample analyzed here, we obtained the intact *Myrica carolinensis* (also known as *M. pennsylvanica*) fruits from F. W. Schu-

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[1] Presented at the AOCS meeting in Houston, Texas, 1965.