sufficient stability to be safe at all seasons of the year. There have also been many occasions in the past few years where it has paid to mix shortenings for reasons of economy, and the use of more than one shortening in a cracker dough is a fairly common practice at present.

Dr. A. H. Gill

The American Oil Chemists' Society has lost a distinguished and valued member in the death on November 11, 1936, of Dr. Augustus Herman Gill, professor emeritus of technical analysis, Massachusetts Institute of Technology. Dr. Gill leaves a widow, a son Paul H. Gill, and a daughter, Mrs. C. McK. Welling.

Dr. Gill was always interested in oil chemistry and analysis, his manual, "Oil Analysis," having appeared in 14 or 15 editions, and became a member of the Society some years ago. He has never been able to attend our annual meetings, but did attend our fall meetings when they were first started in New York. He has been ever ready to cooperate in every way possible, and his advice and counsel as a member of the Olive Oil Committee have been very valuable.

As a former professor and teacher, the writer will miss him greatly; he was always a courteous, patient teacher, and a Christian gentleman.

—H. P. TREVITHICK.

THE IDENTIFICATION OF MINOR COMPONENT FATTY ACIDS IN FATS AND OILS*

By J. B. BROWN

From the Laboratory of Physiological Chemistry of the Ohio State University, Columbus, Ohio

A DECADE ago chemists in the field of fats and oils concerned themselves primarily with relatively superficial analysis. Certain physical and chemical constants were determined. If the fat were a new one, these data would serve for future reference. If the fat had been previously described, the data would serve as a yardstick by which the fat could be compared with the one previously described. With many of the common fats definite limits were established for these constants; these served in identifying and in detecting adulteration.

Of course there were many more detailed investigations of the component fatty acids of fats and oils. The methods used permitted only gross generalization and approximate calculations of the amounts of the principal fatty acids.

In the past few years, there has been a decided tendency to a far more detailed investigation, not only of the fatty acids which make up the fat but also of the state of combination in which they exist originally in the fat. Fats and oils which have been examined critically by applying more modern methods of chemical investigation have been found to contain new and interesting fatty acids. Although many of these acids occur only in very small amounts, certain important properties of the fat in question such as stability, flavor and nutritional value have been found to reside in these so-called minor component acids. An excellent example of this is the investigation which has been in progress in our own laboratory on butter fat. Bosworth and I in 1933 (1) reported at least six new fatty acids in this fat in addition to the eleven which had been previously reported. While these acids occur in very small amounts, nevertheless they are certainly of importance to the nutritional value of butter fat. Incidentally the discovery of new acids in a fat serves to decidedly complicate its chemistry.

In addition to investigating the fatty acids of fats and oils the chemist is now directing his attention to the state in which these acids occur in the original fat or oil. This new interest is largely due to Hilditch and his co-workers in England (2) who have furnished us with a new attack on glyceride structure. By Hilditch's method of permanganate oxidation in acetone some idea at least may be obtained of the types of glycerides which occur. Other methods of attack on this problem are needed because none at present available is adequate to give a clear picture of the hundreds of compounds which are possible and which may be present. The problem would have been comparatively simple if nature had made up these fats and oils as mixtures of simple triglycerides, but we know that there is a general rule that the fatty acids are spread over as many molecules of glycerides as possible.

The purpose of this discussion is to discuss briefly methods that are available and which are now being used to identify fatty acids in fatty acid mixtures. Although the emphasis is to be on the minor component acids, that is, those which are present in small amount, yet naturally the methods are of quite general value. The problem of separating fatty acid mixtures is a complex and difficult one. It is simplified in many cases by relative simplicity of composition. Most of the terrestrial animal fats and oils consist primarily of palmitic, stearic and oleic acids. Naturally it is much easier to investigate such fats than it is to investigate coconut oil, butter fat or a fish oil which are exceedingly complex. The great difficulty lies in the fact that we are dealing with mixtures of organic compounds which are very similar in nature so that almost no clear-cut separations are possible. As you well know, differences in these acids may be grouped roughly into two classes; length of the carbon chain and degree of unsaturation.

The first step in a detailed examination of a fatty acid mixture is to separate the constituent fatty acids so far as possible according to boiling point. To accomplish this it is necessary to resort to fractional distillation at reduced pressure and under the most favorable conditions. The fatty acids should never be employed for this purpose due to their tendency to interact with anhydride formation or, in the case of the unsaturated acids, to react between the double bond and the carboxyl group. Neither of these is important with single brief distillations, but in a fractionation covering many

*A paper presented at the Fall Meeting, A.O.C.S., Chicago, October 8-9, 1936.

hours and repeated several times significant loss and alteration of material may result. The double bond-carboxyl reaction takes place so readily at high temperatures in the case of the highly unsaturated acids of fish oils that they can not be distilled without severe change. In past researches the methyl esters have been employed almost invariably for this fractionation, because they are easy to prepare, are reasonably stable, and their boiling points are appreciably lower than those of the fatty acids. Conversion of an oil into methyl esters is simple. The oil is refluxed for a day with an equal weight of dry methyl alcohol containing 1-2% of dry HCl gas. The resultant esters are washed with water, the last traces of which are immediately removed by warming under reduced pressure. Due to the conditions of the reaction and the properties of the reacting substances the yield is excellent, and there is a minimum of decomposition. The writer has repeatedly distilled methyl esters of iodine number 360 with almost no decomposition. The resultant esters are fractionated several times at 15 mm. or at a lower pressure. It is necessary to employ an efficient column and a constant pressure regulator. The fractions thus obtained are examined one by one for compounds contained therein, applying both general and special methods. As you know, separation into carbon series by fractional distillation is by no means complete. A fraction designated by boiling point as C_{16}, for example, will certainly contain C_{14} and C_{18} acids and small amounts of acids from other series. Obviously, an efficient fractionation greatly simplifies the problem.

In other laboratories an alternate procedure has been adopted as a first step in examining a fatty acid mixture; it involves separation of the fatty acids into saturated and unsaturated acids by the lead soap-ether or lead soap-alcohol methods. The resultant products are then separately esterified and distilled (3) or, in some cases, the unsaturated esters may be reduced and then distilled (4). Either procedure is subject to certain advantages and disadvantages. In view of the fact that separation into saturated and unsaturated acids is never quantitative, we prefer in our laboratory to employ direct distillation of the original methyl esters—more particularly when the object of the work is to identify acids present in very small amount.

In the time available it will be possible to review only the more important and more generally applicable methods of investigating the composition of the ester fractions. The boiling points of esters of saturated and unsaturated acids of a given series are very close so that quite generally it may be said that further separation by distillation is not practical. Fractions are selected by boiling point and molecular weight which are believed to contain a certain series of acids, C_{16}, C_{18}, etc., for example. The fatty acids are prepared from the esters of this fraction and separated into saturated and unsaturated acids by one of the two methods mentioned above. Iodine number and mean molecular weight determinations give general information. The acids are then studied in detail.

Isolation and Identification of Saturated Acids

Saturated acids may be specifically identified only by isolation of the acid and determination of its constants. Specific chemical tests are not available. With the higher acids repeated crystallization from acetone or methyl alcohol is a valuable method of purification. Constant melting points after many crystallizations, application of mixed melting points with a specimen of the pure acid, if available, and correct mean molecular weight serve in positive identification. In some instances cumulative evidence, based on mean molecular weight and iodine number, may be sufficient to infer, at least, the presence of an acid. Positive identification of small amounts of myristic acid in the presence of palmitic would be difficult but the evidence of boiling point of the fraction combined with a mean molecular weight determination on the saturated acids of that fraction might strongly *infer* the presence of an acid of lower molecular weight than palmitic (5). In our investigation of butter fat maximum fractions showed clearly that C_6, C_8 and C_{10} saturated acids were present. Serious doubts existed as to the presence of lauric acid (C_{12}), because the C_{12} fraction was a minimum.

Isolation and Identification of Unsaturated Acids

Due to the reactivity of the double bond several distinct types of methods other than the usual proximate determinations are available for further separation and identification of the unsaturated acids. These include:

(1) Separation as Soaps
(2 Separation by direct Crystallization of Acids
(3) Bromination
(4) Oxidation and Reduction

The first three of these may be used for actually accomplishing further separation.

(1) *Separation as Soaps*

The two most commonly employed soap separations are the barium soap benzene method (6) and the lithium soap-acetone method of Tsujimoto (7). The former is based on the solubility of barium soaps of acids with more than one double bond in benzene containing small amounts of water and the insolubility of the soaps of saturated acids and of oleic acid. Actually due to mutual solubility of the two groups and to formation of mixed soaps of acids of both groups the separation is very unsatisfactory. It may be used as a means of concentration of linolic acid and the more unsaturated acids but never gives a pure product. Bosworth and Helz (8) used this method along with other methods to concentrate and finally isolate hydroxy-palmitic acid in butter fat. Tsujimoto's method was used by him to separate directly the highly unsaturated fatty acids of fish oils from other acids. Lithium soaps of acids with four and more double bonds are soluble in 95% acetone. We have found another application of lithium soaps; crystallizing the lithium soaps of cottonseed oil from n-butyl alcohol affords a concentration of lithium linoleate (9). There are numerous examples in the literature of the use of other metal soaps. Soaps of monovalent metals should be employed to avoid mixed soap formation.

(2) *Separations by direct Crystallization of Acids or Esters*

The greatest physical difference between the saturated and unsaturated fatty acids is their solubility in the various organic solvents. Stearic acid is quite insoluble in cold alcohol while oleic acid is miscible in all proportions. In this connection we have recently carried out in our laboratory an extended series of experiments (9) designed to separate linoleic acid from oleic in cottonseed and corn oils. Our results have shown clearly that by use of low temperatures nearly pure linoleic acid may be prepared from corn oil by direct crystallization of the fatty acids from acetone or

methyl alcohol at temperatures from —20 to —80° C. We have also succeeded in obtaining pure oleic acid from olive oil by similar methods (10). This work we are continuing in the hope of extending these results to others of the common unsaturated fatty acids. I can not emphasize this principle of crystallization too strongly as a means of concentration and purification. May I illustrate: In human fat linoleic acid may be considered to be a minor component acid. A specimen of this fat was converted into fatty acids. These were dissolved in acetone and held overnight at —20°. The crystals of saturated acids were removed by filtration. The unsaturated acids in the filtrate were recovered and distilled under reduced pressure. It is seen from the data below (Table I) that the iodine number (98) is appreciably above that of oleic acid.

TABLE I.—DATA OF HUMAN FAT

	Iodine Number	Sap. No.	% Linoleic
Fat	72.2	193.4	..
	Mean Mol. Wt.		
Fatty Acids	80.2	273.9	..
Unsaturated Acids.	98.2	280.3	9
Crystals —65°	82.6	282.5	..
Filtrate	120.0	284.0	30

After re-dissolving the unsaturated acids in acetone and cooling to —65°, most of the oleic acid crystallized out. The iodine number of the acids in the filtrate at this temperature was 120. Assuming the excess iodine number to be due to linoleic acid, the acids of the filtrate now contained 30% of this acid. By simple crystallization, therefore, a concentrate of a minor component acid was obtained.

(3) *Bromination*

When unsaturated fatty acids are brominated in cold organic solvents each double bond is replaced almost instantly by 1-2 dibromo groupings. These offer an excellent means of chemical identification as well as separation. The original fatty acids are easily regenerated by treatment with zinc dust. Linoleic and linolenic acids yield products of definite melting point, tetrabromo-stearic acid being soluble in ether and insoluble in petroleum ether and hexabromo-stearic acid insoluble in both. Separation of the two compounds is relatively simple. There is no test more conclusive for linolenic acid than the hexabromide test; for linoleic acid than the tetrabromide test. Analysis of the product for melting point and bromine content serve to further identify. I have no data on the delicacy of these tests; in case only very small amounts of the acids are present, an attempt to concentrate the acid in question should be made.

When arachidonic acid, $C_{20}H_{32}O_2$, is brominated in cold ether, it yields an insoluble octabromide which does not melt without decomposition, but when the methyl ester is employed, a bromide melting at 228-30° is obtained. Mainly by employing this test Eckstein identified traces of arachidonic acid in human fat (11); Ellis and Isbell (12) and Brown and Deck (13) found it in lard; Brown found it in large amounts in certain glandular lipids (14) (15). Other highly unsaturated acids have been identified in animal fats (16). Small amounts of octadecatetrenoic acid (formerly called clupanodonic) were shown to be present in menhaden oil (17).

The lower mono-unsaturated fatty acids yield ester bromides which may be distilled under reduced pressure. An interesting instance when this property has been employed in an actual separation is the isolation of traces of decenoic acid from butter fat by Bosworth and Brown (1) (also reported by Grün and Wirth) (18). We had available the C_{10} fraction of butter fat methyl esters. This consisted of about nine parts of capric acid and one part of decenoic acid. The usual methods of separating saturated and unsaturated acids failed, the lead soaps, for example, being equally soluble in ether. Addition of bromine, however, formed from the methyl decenoate methyl dibromo-caprate, boiling sixty degrees higher than methyl caprate. Distillation then effected an easy separation. The methyl decenoate was regenerated by reduction with zinc. A similar procedure was employed in isolating tetradecenoic acid. Ester bromides of C_{16} and higher fatty acids decompose with heat making the method worthless for these acids.

The bromination method has decided limitations. The first of these is the question of bromide isomerism. Pure arachidonic acid gives only about one-fourth the predicted amount of ether-insoluble octobromo-arachidic acid. Linolenic acid gives about one-third and linoleic acid about one-half the predicted insoluble bromides. While the method is excellent for qualitative detection, therefore, it is useless as a means of quantitative estimation, unless these yields are taken into consideration (cf. Ault and Brown) (19). When these yields are taken into account we have the best means now available for quantitative estimation. Another limitation of the bromination method lies in the question of isomerism in the fatty acids themselves. So far as our information now goes, the tetrabromide test is specific only for ordinary linoleic acid and probably is worthless for other numerous possible isomers of this acid. For example, we were unable to identify ordinary linoleic acid in butter fat by application of the tetrabromide method to the appropriate ester fraction. Bosworth (20) however did find linoleic acid in human milk fat. Hilditch (21) at first claimed this acid to be present in butter fat on the basis of detailed examination of the higher fractions, certain excess iodine numbers being ascribed to the presence of this acid. In later work (22), however, he believes an octadecadienoic acid other than ordinary linoleic is responsible for his data. In spite of these limitations, the bromination test is of tremendous value in the investigation of a fat or oil. After all, until recently, this method has furnished practically the only procedure by which linoleic, linolenic and the many highly unsaturated fatty acids of fish oils have been not only identified as being present but have been actually prepared in the pure state.

(4) *Oxidation and Reduction*

Since oxidation and reduction permanently alter the unsaturated acids these procedures serve to characterize or prove the structure of an acid rather than as a means of separation.

Oxidation provides three kinds of information. By cold oxidation with dilute permanganate the double bonds are saturated by two hydroxyl groups (23). These dihydroxy acids are usually high melting compounds which are of assistance in identification. Mottram (24) has employed oxidation of unsaturated acids with an excess of permanganate as a means of quantitative estimation of small amounts of saturated acids in unsaturated acids. The latter are destroyed by the oxidation, the residual unattacked saturated acids being isolated and weighed. Oxidation, or rather ozonization, has been used in determining the structure of unsaturated acids. These add ozone readily at the double bond; upon mild hydrolysis the ozonide is decomposed into aldehydes or acids of low molecular weight. The structure of the original acid is worked out from a study of these resultant products.

Reduction of fats and fatty acids,

as is well known, is of tremendous commercial importance; it is also a valuable tool in the research laboratory. It is easily accomplished by treatment with hydrogen at ordinary temperature in the presence of active platinum black (25). Unsaturated fatty acids, usually low melting compounds, are converted into higher melting crystalline compounds which may be crystallized and purified. The carbon chain of the reduced acid is identical with that of the unsaturated acid. Anderson (26) has used reduction along with the lead soap method to isolate and identify new saturated liquid acids with branched carbon chains. The unsaturated acids of certain fractions of tubercle bacilli lipids were reduced. The reduced acids were again subjected to the liquid lead soap separation; lead soaps of saturated acids remained in solution in ether, tuberculostearic and phthioic acids. Detailed investigation of other lipids will disclose, no doubt, the presence of similar acids.

In concluding this brief review I would criticize workers in this field as having been too much satisfied with gross generalizations as to the composition of fatty acid mixtures; they have depended too much on the dogma that all naturally occurring fatty acids have an even carbon chain and a straight carbon chain and that most fats and oils contain relatively few fatty acids. Careful investigation of even the most common of these fats and oils will disclose, I believe, fatty acids, hitherto unsuspected of being present. At the same time I would compliment these workers who with few specific tests and fewer specific methods of separation but with tedious detailed and comprehensive application of these methods have given us the great amount of information about fats and oils we now possess.

Bibliography

1. A. W. Bosworth and J. B. Brown. J. Biol. Chem., **103**, 115 (1933).
2. G. Collin and T. P. Hilditch. J. Soc. Chem. Ind., **47**, 261T (1928).
   G. Collin and T. P. Hilditch. Biochem. J., **23**, 1273 (1929).
3. T. P. Hilditch, E. C. Jones and A. J. Rhead. Biochem. J., **28**, 786 (1934).
4. E. Klenk. Zts. f. Physiol. Chem., **200**, 51 (1931).
5. W. C. Ault and J. B. Brown. J. Biol. Chem., **107**, 607 (1934).
6. J. Lewkowitsch. Technology of Oils, Fats and Waxes, Vol. 1, 183, 191 (1921).
7. M. Tsujimoto. J. Chem. Ind., Japan, **23**, 107 (1920).
8. A. W. Bosworth and G. E. Helz. J. Biol. Chem., **112**, 489 (1936).
9. J. B. Brown and G. G. Stoner. (Unpublished results, this laboratory).
10. J. B. Brown and G. Y. Shinowara. (Unpublished results, this laboratory).
11. H. C. Eckstein. J. Biol. Chem., **64**, 797 (1925).
12. N. R. Ellis and H. S. Isbell. Ibid. **69**, 219, 239 (1926).
13. J. B. Brown and E. M. Deck. J. Am. Chem. Soc., **52**, 1135 (1930).
14. J. B. Brown. J. Biol. Chem., **83**, 777 (1929).
15. J. B. Brown. J. Biol. Chem., **80**, 455 (1928).
16. J. B. Brown and C. C. Sheldon. J. Am. Chem. Soc., **56**, 2149 (1934).
17. J. B. Brown and G. G. Beal. Ibid. **45**, 1289 (1923).
18. A. Grun and T. Wirth. Ber. Chem. Ges., **55**, 2197 (1922).
19. W. C. Ault and J. B. Brown. J. Biol. Chem., **107**, 615 (1934).
20. A. W. Bosworth, ibid. **106**, 235 (1934).
21. T. P. Hilditch and E. C. Jones. Analyst, **54**, 75 (1929).
    T. P. Hilditch. Biochem. J., **28**, 779 (1934).
22. T. G. Green and T. P. Hilditch. Biochem. J., **23**, 1564 (1935).
23. A. Lapworth and E. N. Mottram. J. Chem. Soc., **127**, 1628 (1925).
24. S. H. Bertram. Z. Untersuch. Lebensm., **55**, 179 (1928).
25. R. Adams and R. L. Shriner. J. Am. Chem. Soc., **45**, 217 (1923).
26. R. J. Anderson. J. Biol. Chem., **74**, 537 (1927).
    R. J. Anderson. Ibid, **83**, 169 (1929).

REPORT OF THE UNIFORM METHODS AND PLANNING COMMITTEE AMERICAN OIL CHEMISTS' SOCIETY FALL MEETING, 1936

THE Soap Committee in their report have made certain recommendations which were submitted by mail to the members of the Uniform Methods and Planning Committee.

They suggested a slight modification in the method for Free Alkali Determination, this method to be modified to read as follows:

"Heat the filtrate from the above nearly to boiling, add 0.5 cc. of a 1 per cent solution of phenolphthalein, and titrate with standard acid or alkali solution, and calculate the alkalinity to sodium hydroxide (or potassium hydroxide) or acidity to oleic acid."

The Uniform Methods and Planning Committee have approved this modification. A motion for its adoption was made and seconded and passed by the Society.

In the method for determining Moisture in Paste Soaps Containing Glycerine, a section "c" was added. The entire method will then read as follows:

"*MOISTURE.* The oven method given below is generally applicable to all soaps. Experience has shown, however, that certain exceptions to this method must be made if accurate results are desired. These exceptions include:

a. For soaps containing appreciable amounts of sodium silicate the distillation method is preferred.

b. Soaps of linseed and other oxidizing oils absorb oxygen and if the oven method is used may gain in weight near the end of the test. Therefore, either an inert atmosphere or vacuum oven should be used. The distillation method is also applicable to these types of soaps.

c. Soaps containing appreciable amounts of glycerine, such as cold made and semi-boiled (including paste soaps), usually give high results by the oven method. The distillation method is preferred for most accurate results on these types of soaps."

The Uniform Methods and Planning Committee approve this addition to the method as printed. The motion for its adoption was made and seconded and passed by the Society.

The Soap Committee points out that there was an omission in the first printing of the Modified Wolff Method for Rosin. They suggest that it read as follows under "Second Esterification":

"Cool and dissolve the residue in 20 cc. of absolute ethyl alcohol and then proceed as above under 'First Esterification.' Add 30 cc. neutral alcohol (94 per cent or higher) and titrate rosin or rosin soap as desired, using phenolphthalein as indicator."

The Uniform Methods and Planning Committee approve this addition to the method as printed. A motion for its adoption was made and seconded and passed by the Society.

The reports of the Color Committee and the Committee on Sulphonated Oils were not received in time to submit to the Uniform Methods and Planning Committee and will have to be held over for approval at the Spring meeting.

E. B. Freyer
H. P. Trevithick
M. L. Sheely
P. E. Ronzone
J. J. Vollertsen, Chairman.