U greatly preponderates in the same position. Note that one is an animal fat and the other a vegetable fat. Application of the method to the hypothetical fat of known constitution (No. 1) shows that the mathematical process is a valid one. As will be described later, other investigators are in agreement with the results.

It seems reasonable to conclude that for fats such as these, in which C_{16}–C_{18} component acids predominate and in which shorter or longer components are present in relatively small proportions, the process is likely to give a good representation of the actual structure.

Shortly after the foregoing procedure was described in 1959, A. S. Richardson, in private correspondence, described a much simpler method of calculation giving the same results, which presumably he will describe.

Quite recently Youngs (6) has announced the discovery and development of another process for determining the proportions of the glyceride types and isomers, based on separation by means of chromatography of the products of partial oxidation of the unsaturated molecules. Analyses by Youngs confirm the accuracy of Vander Wal's results.

Dutton (7) has described recently the application of the counter-current distribution technique to the analysis of cocoa butter. His article, when published, will contain references to other work by means of the same procedure.

Hammond (8) has employed the temperature gradient technique in research on glyceride structure. His results also agree with those of Vander Wal.

It should be obvious by now that rather than being a helter-skelter, unpredictable, unordered mixture of triglyceride components, the natural fats are highly organized and quite predictable.

THE END is not yet. Another great problem is the explanation of how both plants and animals can produce such complicated but precisely organized substances as fats. This is partly the biochemist's and the enzymologist's job, but the mathematical relationships discussed in this paper must be satisfied by any system of synthesis they evolve. Indeed these individuals may very profitably be guided thereby.

From the point of view of the chemist in industrial fats and oils a knowledge of the structure of fats is as important as a road map to the traveller. Research heretofore has been done without real knowledge of the structure of the substances involved and has therefore been carried on in a state of semiblindness. Now we shall be able to see where we are, what we have to work with, and what we can hope to achieve. The results should be better products, more economically produced, with advantages to all concerned.

REFERENCES

1. Hilditch, T. P., "The Chemical Constitution of Natural Fats," 3rd ed., John Wiley and Sons Inc., New York (1956).
2. Mattson, F. H., and Beck, L. W., J. Biol. Chem., 219, 735 (1956).
3. Mattson, F. H., and Lutton, E. S., J. Biol. Chem., 233, 868 (1958).
4. Kartha, A. R. S., doctoral thesis, University of Madras, July 1949; J. Am. Oil Chemists' Soc., 30, 280 (1953); J. Am. Oil Chemists' Soc., 30, 326 (1953); J. Am. Oil Chemists' Soc., 31, 85 (1954).
5. Vander Wal, R. J., J. Am. Oil Chemists' Soc., 37, 18 (1960).
6. Youngs, C. G., paper presented at the 51st annual meeting, American Oil Chemists' Society, Dallas, Tex., April 4–6, 1960.
7. Dutton, H. J., Scholfield, C. R., and Mounts, T. L., paper presented before the Division of Agricultural and Food Chemistry, American Chemical Society Meeting, Cleveland, O., April 5–14, 1960.
8. Jones, G. V., and Hammond, E. G., J. Am. Oil Chemists' Soc., in press.

Interesterification of Edible Fats

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DURING THE LAST TEN YEARS, rearranged lard has become a very important shortening ingredient. It is prepared from natural lard by redistributing the fatty acid radicals among the triglyceride molecules.

That esters can exchange acid groups has been known for almost one hundred years. In 1865, Friedel and Crafts, of Friedel-Crafts reactions fame, heated a mixture of amyl acetate and ethyl benzoate at 300°C. in a sealed tube. When they analyzed the contents of the tube, they found ethyl acetate and amyl benzoate as well as the original amyl acetate and ethyl benzoate (1). This classical experiment was the first demonstration of interesterification, the exchange of acyl and alkyl groups between esters:

$$RCOOR' + R''COOR''' \rightleftharpoons RCOOR''' + R''COOR'$$
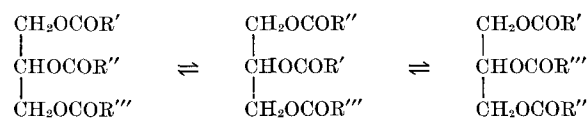
In this discussion the term interesterification will refer only to an exchange of acid or alcohol groups by esters. This is sometimes also called ester interchange or transesterification. Although we are primarily concerned with the interesterification of edible fats, it will be necessary to refer to a number of related ester reactions from time to time. 1. *Esterification* is the formation of an ester and water from an alcohol and acid. 2. *Hydrolysis* is the splitting of an ester by water. Free alcohol and acid are formed. 3. *Saponification* is the splitting of an ester by alkali. Soap and alcohol are formed. 4. *Alcoholysis* is a displacement of the alcohol radical of an ester by another alcohol. 5. *Acidolysis* is a displacement of the acid radical of an ester by another acid.
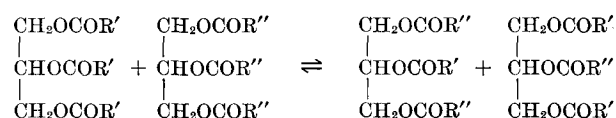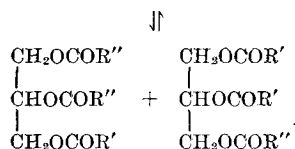
Interesterification of Triglycerides

Fats have three ester linkages per molecule. Therefore the interchange possibilities are many. Not only can the fatty acid groups exchange positions within the molecule, but each fatty acid can also trade places with any one of three fatty acids in another molecule:

Interesterification within Molecules

Interesterification between Molecules

$$\text{JF}$$

$$
\begin{array}{ll}
CH_2OCOR'' & CH_2OCOR' \\
| & | \\
CHOCOR'' \quad + & CHOCOR' \\
| & | \\
CH_2OCOR' & CH_2OCOR''
\end{array}
$$

Interesterification in the liquid phase is often called random interesterification. This differentiates it from directed interesterification which will be discussed later. It is assumed that the fatty acids regroup themselves among the glyceride molecules according to chance. Triglyceride analyses are not very exact, but they do indicate a completely random arrangement at reaction equilibrium (2,3). The glyceride composition of randomly interesterified fats can be calculated with the following statistical formulas if the fatty acid compositions are known(4):

Where A, B, C, etc., are the molar percentages of fatty acids a, b, c, etc.

$$\text{glyceride } a - a - a = 1 \times \frac{A.A.A.}{10,000} \text{ mole } \%$$

$$\text{glyceride } a - a - b = 3 \times \frac{A.A.B.}{10,000} \text{ mole } \%$$

$$\text{glyceride } a - b - c = 6 \times \frac{A.B.C.}{10,000} \text{ mole } \%$$

If, for example, 50 mole % of tristearin were interesterified with 50 mole % of triolein the equilibrium mixture should have the following composition:

$$\text{stearic} - \text{stearic} - \text{stearic} = 1 \times \frac{50.50.50}{10,000} = 12.5\%$$

$$\text{stearic} - \text{stearic} - \text{oleic} = 3 \times \frac{50.50.50}{10,000} = 37.5\%$$

$$\text{stearic} - \text{oleic} - \text{oleic} = 3 \times \frac{50.50.50}{10,000} = 37.5\%$$

$$\text{oleic} - \text{oleic} - \text{oleic} = 1 \times \frac{50.50.50}{10,000} = 12.5\%$$

It will be noted that 75% of the interesterified fat would consist of glycerides which were not present in the original mixture. This of course is an extreme case. Most natural fats are closer to random distribution and therefore undergo less change during interesterification.

Although interesterification has been known for nearly a century, it was not applied to fats until the 1920's. Normann (5,6) and Van Loon (7), two European industrial fats and oils chemists, were the pioneers in this field. Working independently, they interesterified a variety of fat mixtures. Among these were stearins and vegetable oils, beef fat and coconut oil, beef fat and tributyrin to simulate butter, tristearin and triacetin. Their search for catalytic materials was extensive. Among those found to be effective were tin, cadmium, lead, zinc and their compounds, sulfonic acids, and compounds of alkaline earth metals and alkali metals. Included with the latter were the alkali metal alcoholates which are used extensively today.

After Normann disclosed that fats can be interesterified, Grün (8,9) studied the theoretical aspects of the reaction. His analyses of interesterified mixtures showed that all of the theoretically possible compounds, including positional isomers, were formed. Some of his results were in good agreement with the theory of random arrangement.

Interesterificated fats were not an immediate commercial success. Nevertheless the contributions of Normann, Van Loon, and Grün provided an excellent starting point for those who did later research in this field.

Lard Interesterification

Large-scale interesterification is a postwar development primarily concerned with lard. Interesterification had been used to make confectionery fats from mixtures of coconut oil and vegetable fats (10), but this apparently was not a sizeable operation.

To appreciate why lard lends itself so well to interesterification, it is necessary to consider two characteristics of natural lard. One of these is a large, coarse crystal structure which gives it a dull, unattractive appearance. The other is its poor creaming quality, that is, its inability to incorporate enough air into cake batters while these are being mixed. This leads to poor texture and volume in the baked cakes.

It has been known for years that lard glycerides have unique properties. The well-known Bömer test for lard adulteration, devised in 1913, is based on the melting point of the trisaturated glycerides (11). In 1935, Hilditch (12) was able to show that most of the palmitic acid in lard is attached at the beta position of the glyceride molecule, instead of the more numerous alpha positions. This work has been confirmed with more refined techniques (13). Disaturated beta palmito stearo-glycerides are now recognized as the cause of the coarse crystal structure of lard. Although randomized lard still contains approximately 25% disaturated glycerides, less than 5% are of the beta palmito type.

The late A. E. Bailey (14) associated the crystallization habits of lard with its poor creaming power and apparently was the first to use interesterification for the purpose of breaking up the responsible glyceride configuration. The resultant product was comparable to good quality vegetable fat shortenings in appearance and creaming quality. Small amounts of interesterified lard were produced in the late 1930's (15). The reaction was carried out in the absence of air at about 200°C. with sodium hydroxide plus glycerol as catalyst. The product was handicapped by the absence of natural antioxidants like the tocopherols which occur in cottonseed and soybean oil but not in lard.

Low-Temperature Interesterification

Two technological advances finally triggered commercial lard interesterification. One of these was the development and approval of effective antioxidants. The American Meat Institute played a major role in this. The other was the discovery of low-temperature interesterification, first disclosed by Eckey (16).

Since reaction rates increase with temperature, fat interesterification was originally carried out at temperatures as high as practicable. Even so it took hours to arrive at a random equilibrium at 200°C. Eckey however was trying to direct the reaction away from its usual random endpoint. In the course of his work he found that under the proper conditions some catalysts were still active below the melting point of fat, and at 50°C. they were so active that equilibrium was reached within 30 minutes (17).

Sodium and potassium and their lower alcoholates, amides, and hydrides are all very effective low-tem-

perature catalysts. It seems likely that these materials are actually precursors of the real catalyst, which probably is a compound of the alkali metal and a glyceride. According to Eckey, compounds of sodium with materials much more acidic than alcohol will not bring about interesterification at low temperatures.

The alcoholates are the most convenient to use, and sodium methoxide is the most popular of these. Special precautions are necessary when the alkali metals and hydrides are used, because hydrogen is liberated. When sodium amide ages, it may form nitrogen oxides which can detonate spontaneously (18). Sodium methoxide is easier to handle, but its use also requires care because the reaction between water and sodium methoxide is very exothermic. There have been instances where open containers have ignited in moist atmospheres.

The use of sodium methoxide for low-temperature lard interesterification has been patented (19,20). Patents have also been granted for its use in preparing other shortenings (21,22), as well as confectionery fats which contain coconut oil (23,24). It has a number of advantages over the old high-temperature methods. The reaction can be carried out in open kettles, equilibrium is reached more quickly, and the danger of thermal damage to the fat is greatly reduced. However high- and low-temperature interesterifications have the same effect on glyceride composition, crystal structure, and creaming properties if they are continued until a random equilibrium exists.

Fats which are to be interesterified should be low in moisture, free fatty acids, peroxides, and other extraneous materials which will react with sodium methoxide. The catalyst concentration should be kept as low as practicable to prevent excessive losses resulting from saponification by the sodium radical and methyl ester formation by the methyl radical. Lard which has been filtered and vacuum dried but not refined requires about 0.20% sodium methoxide.

The fat should take on a reddish brown hue a few minutes after the catalyst has been added. This color probably is due to the sodium-glyceride compound believed to be the true catalyst. If it does not appear, more catalyst is required. In the temperature range of 60 to 85°C. the reaction reaches equilibrium within thirty minutes after the color change occurs. With many fats either the melting point or cloud point can be used to follow the reaction. With lard these determinations are of no value since interesterification changes the melting point of lard only slightly. Therefore cooling curves or single point dilatometric measurements are generally used. The cooling curve of lard has a characteristic hump which disappears after interesterification.

After the fat has been interesterified, the catalyst may be inactivated either with water or acid. When water is used the saponified acids can be removed with conventional refining equipment. The alkaline water also removes most of the color bodies formed during the reaction. If the fat is to be used as a food, the methyl esters must be removed. These come out readily during steam deodorization.

Directed Interesterification

A random arrangement of fatty acids is not always the most desirable one. Eckey (17) found that interesterification can be directed away from its usually random endpoint if the fat is allowed to crystallize during the reaction. The trisaturated glycerides crystallize first. This removes them from the reaction and upsets the equilibrium of the liquid phase, which in turn forms more trisaturates in an effort to regain equilibrium. This continues until most of the saturated acids have been precipitated. Eckey was able to get 19% trisaturated glycerides from cottonseed oil, which contains 25% saturated acids. If this fat had been randomly interesterified, it would have contained only about 1.5% trisaturated glycerides and more than 40% disaturated mono-unsaturated glycerides.

Directed interesterification has also been used to separate disaturated mono-unsaturated glycerides. To accomplish this, catalyst was added to chilled oils in which most of the crystals were disaturated mono-unsaturated glycerides. These served as nuclei for the formation of more of the same crystals (25,26).
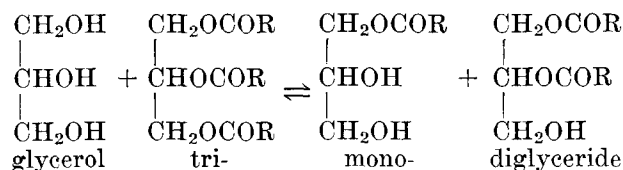
Nonrandom interesterification has many possibilities. Among them are the preparation of shortenings, confectionery fats, and margarines (16) as well as the removal of saturated fatty acids from oils. However as with random interesterification, the primary application at the present time is concerned with the preparation of lard shortening. Randomly rearranged lard is softer and has a lower melting point than conventional shortening. Consequently it has to be firmed up with about 10% of trisaturated glycerides. With directed interesterification the trisaturated glycerides are one of the reaction products. The resultant fat also has a better plastic range because it is lower in disaturated mono-unsaturated glycerides (27).

Directed interesterification is relatively slow because of the lower temperatures used and the time required for crystallization. Solid catalysts like sodium metal and sodium methoxide tend to become coated, which slows down the reaction still more. This coating effect has been overcome by the use of a dispersion of liquid sodium-potassium alloy. These liquid spherical particles slough off the coating as it is formed, and as a result the fat is always exposed to active catalyst (27).

After the catalyst has been added, the lard is sent through a series of heat exchangers to lower the temperature gradually. The course of the reaction can be followed by cloud-point determinations. When the desired amount of trisaturates has been formed, the catalyst is destroyed. This is done while the fat is still in a semisolid state because any rise in temperature while the catalyst is active will reverse the reaction. Water plus carbon dioxide has been recommended to reduce saponification (27). After the catalyst has been destroyed, the fat is melted so that the soaps can be removed.

Monoglycerides

Monoglycerides may be prepared either by the esterification of fatty acids with glycerol or the glycerolysis of triglycerides. Glycerolysis is generally used for the preparation of food grade emulsifiers.

$$
\begin{array}{ccccc}
CH_2OH & CH_2OCOR & & CH_2OCOR & CH_2OCOR \\
| & | & & | & | \\
CHOH & + \quad CHOCOR & \rightleftarrows & CHOH & + \quad CHOCOR \\
| & | & & | & | \\
CH_2OH & CH_2OCOR & & CH_2OH & CH_2OH \\
glycerol & tri- & & mono- & diglyceride
\end{array}
$$

Both alcoholysis and interesterification occur. At

equilibrium there is an essentially random mixture of glycerol, mono-, di-, and triglycerides. The reaction cannot be driven to completion by adding an excess of glycerol, because of its limited solubility. It ranges from 22.5% at 200°C. to 40% at 250°C. (28). Most commercial monoglyceride mixtures are prepared at about 200°C. with sodium hydroxide as catalyst and contain 40 to 45% alpha monoglycerides plus about 4% beta monoglycerides. Mutual solvents have been proposed to increase the monoglyceride content (29), but most concentrates are made by molecularly distilling mono- and diglyceride mixtures. The distilled products have a monoglyceride content of 90% or more.

Phosphoric acid has been recommended for destroying the alkaline catalyst (30). The reaction should be halted before any cooling occurs, otherwise glycerol will drop out of solution and reduce the yield of monoglycerides.

Acidolysis plus Interesterification

Fats like coconut oil, which contain low molecular weight acids, may be modified by displacing the short chain acids with longer chain acids (31). The reaction can be carried out under reduced pressure in a vessel equipped with a fractionating column through which the lower molecular weight acids are continuously removed as they are freed (32). The product has a random distribution of the remaining acids. The usual high-temperature interesterification catalysts are effective. A similar result can be obtained by using monoesters instead of the free acids (32,33).

Interesterification and allied ester reactions are also used quite extensively in the manufacture of paints and plasticizers. These applications were reviewed by Formo in the 1954 A.O.C.S. Short Course (34).

REFERENCES

1. Friedel, C., and Crafts, J. R., Annalen 133, 207 (1865).
2. Norris, F. A., and Mattil, K. F., Oil and Soap 23, 289 (1946).
3. Desnuelle, P., and Naudet, M., Bull. soc. chim. 13, 90 (1946).
4. Bailey, A. E., "Industrial Oil and Fat Products," 2nd ed., Interscience Publishers Inc., New York (1951), p. 834.
5. Normann, W., Chem. Umschau 30, 250 (1923).
6. Normann, W., German Patent 417,215 (1925).
7. Van Loon, Chr., British Patent 249,916 (1926), Dutch Patent 16,703 (1927), U. S. Pat. 1,873,513 (1932).
8. Grün, Ad., Ztschr. angew. chem. 38, 827 (1925).
9. Grün, Ad., "Chemie und Technologie der Fette," Julius Springer, Vienna (1936), Vol. 1, p. 285.
10. Gooding, C. M., U. S. Patent 2,309,949 (1943).
11. Bömer, A., Limprich, R., Kronig, R., and Kuhlmann, J., Z. Untersuch. Nahr. Genussm. 26, 559 (1913).
12. Hilditch, T. P., and Stainsby, W. J., Biochem. J. 29, 99 (1935).
13. Quimby, O. T., Wille, R. L., and Lutton, E. S., J. Am. Oil Chem. Soc. 30, 186 (1953).
14. Bailey, A. E., "Industrial Oil and Fat Products," Interscience Publishers Inc., New York (1945). pp. 301, 681.
15. Bailey, A. E., Proceedings of the Fifth Research Conference, American Meat Institute (1953), p. 11.
16. Eckey, E. W., British Patent 574,807 (1946), U. S. Patent 2,442,531-2, 2,442,536-7 (1948).
17. Eckey, E. W., Ind. and Eng. Chem. 40, 1183 (1948).
18. Encyclopedia of Chemical Technology, Interscience Publishers, Inc., New York (1954), Vol. 12, p. 600.
19. Vander Wal, R. J., and Van Akkeren, L. A., U. S. Patent 2,571,-315 (1951). Reissue 23,499 (1952).
20. Mattil, K. F., and Nelson, D., U. S. Patent 2,625,483 (1953).
21. Nelson, D. W., U. S. Patent 2,855,311 (1958).
22. Steffen, A. H., U. S. Patent 2,855,310 (1958).
23. Cochran, W. M., and McGee, R. F., U. S. Patent 2,726,158 (1955).
24. Cochran, W. M., and Ott, M. L., U. S. Patent 2,783,151 (1957).
25. Abbott, A. D., U. S. Patent 2,442,538 (1948).
26. Eckey, E. W., U. S. Patent 2,442,535 (1948).
27. Hawley, H. K., and Holman, G. W., J. Am. Oil Chem. Soc. 33, 29 (1956).
28. Feuge, R. O., and Bailey, A. E., Oil and Soap 23, 259 (1946).
29. Eckey, E. W., and Richardson, A. S., U. S. Patent 2,251,693 (1941).
30. Eckey, E. W., and Clark, C. C., U. S. Patent 2,065,520 (1936).
31. Barsky, G., U. S. Patent 2,182,332 (1939).
32. Eckey, E. W., U. S. Patent 2,378,006, 2,378,007 (1945).
33. Paterson, W. J., U. S. Patent 2,521,742 (1950).
34. Formo, M. W., J. Am. Oil Chem. Soc. 31, 548 (1954).

Dietary Fat and Heart Disease

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WITHIN THE PAST FEW YEARS all of us in the fats and oils industry have become acutely aware of the research indicating a relationship between heart disease and dietary fats. Besides the potential effects on our markets, these programs are of vital concern in terms of the health of the nation, our families, and ourselves.

This is a controversial topic and an active area of research. There are important differences between experimental animals and human beings in the ways in which blood vessels react to the dietary factors presumed to lead to atherosclerosis and heart disease. Therefore available data are inconclusive to the extent that they provide at least some degree of support for each of several points of view.

My purposes in the present review are to outline the nature of the evidence now available, to indicate the limitations in the data, and to discuss the interpretations and hypotheses on which current clinical research is based.

First of all, there is no doubt that fats are excellent foods. They are highly concentrated sources of energy and contribute much to the enjoyment we get out of our meals. As a matter of fact a certain amount of fat is necessary to provide the essential fatty acids which our bodies are unable to synthesize and, in addition, to carry into our tissues the fat-soluble vitamins which are so important to health. Lipid materials, of which fats are one class, are important constituents of all living cells and life as we know it would be impossible without these fatty materials. Thus, there is no doubt that fats are excellent foods.

However, recent clinical studies have clearly shown that there are important differences in the way in which our bodies metabolize fats from different sources and that these differences may be associated with the development of coronary heart disease.

One of the bits of evidence linking heart disease with dietary fats has come from population and epidemiological studies. Statistics are being amassed in all parts of the world on local incidences of various diseases, the foods people eat, and the conditions under which they live. There are very serious limitations to the accuracy of such data and consequently they must be interpreted with caution. For example, medical records from the United States are not directly comparable to those from India where few people ever see a doctor. In addition, reliable dietary histories are difficult to obtain so the information available generally relates to the quantities of food available in the mar-