The Influence of Glyceride Structure on the Rate of Autoxidation[1]

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Abstract

The rate of autoxidation of mixtures of triunsaturated glycerides and tridecanoin was decreased by randomization with sodium methoxide. A theory, based on the hexagonal packing of glyceride acyl chains in the molten state, was proposed which accounted for the observed efforts on the rate of autoxidation. The theory suggests that the concentration of the unsaturated fatty acids on the 2-position of glycerol should stabilize a fat towards autoxidation. This was confirmed with experiments on natural fats.

Introduction

The composition and the structure of the fatty acids in a glyceride are known to have a profound influence on its rate of autoxidation, but the effect of glyceride distribution on the rate of autoxidation has not been investigated. The experiments reported in this paper show that the glyceride structure has considerable influence on the rate of autoxidation in some circumstances.

Methods

Decanoic acid was converted to methyl decanoate by refluxing for 6 hr with an excess of methanol and a sulfuric acid catalyst. The methyl esters were washed with aqueous sodium carbonate and distilled at 1 mm. The fraction boiling between 60 and 65C amounted to 84% of the charge and was 98% methyl decanoate and 2% methyl laurate by gas chromatography on butanediol succinate (5).

Tridecanoin was made by reacting a 5% excess of methyl decanoate with triacetin, using 0.5% of sodium methoxide as a catalyst. The reaction mixture was stirred with a magnetic stirrer; the pressure was reduced from 15 to 1 mm, and the temperature was increased to 60C over 6 hr. The methyl acetate which distilled amounted to 96% of theory. The catalyst was destroyed with 5% acetic acid solution, and the product was washed with aqueous sodium carbonate and water. The residual methyl esters were removed from the tricaprin by distillation in a Rota-film molecular still maintained at 100C and 0.5 mm.

Methyl linoleate was prepared from safflower oil by urea fractionation (7). This yielded a product containing 97% linoleic acid and 3% oleic acid, according to gas chromatographic analysis. Trilinolein was prepared like the tridecanoin. The polyenoates were protected from autoxidation whenever possible by a nitrogen atmosphere during sample preparation.

Methyl linolenate was prepared from linseed oil (14). This yielded a product containing 72.4% linolenic, 20.6% linoleic, and 7% oleic acid as determined by gas chromatography. This was converted to triglyceride by transesterification as before.

In the first experiment two glyceride mixtures were prepared from the tridecanoin, trilinolein, and trilinolenin. The first had a composition of 1.5% linoleate and 0.5% linolenate in tridecanoin, and the second had 1.5% linoleate in tridecanoin. Half of each glyceride mixture was randomized with 0.5% sodium methoxide under reduced pressure. Both the randomized and nonrandomized samples were washed with 5% acetic acid solution, sodium carbonate solution, and water, then were deodorized at 200C for 30 min at 1 mm.

In the second experiment a glyceride mixture containing 1.5% linoleate in tridecanoin was prepared from the tridecanoin and trilinolein. It was deodorized at 200C for 30 min at 1 mm. A sample was withdrawn at this stage. To the remainder of the mixture 0.5% sodium methoxide was added, and immediately another sample was withdrawn and the catalyst destroyed with 5% acetic acid solution. The remainder of the mixture was randomized for 6 hr at 60C and 1 mm. At the end of this period another sample was withdrawn and freed of catalyst. The remaining randomized sample was slowly cooled to −20C over 4 hr, where it crystallized. It was held at this temperature under reduced pressure overnight. The product was mixed rapidly with acetic solution to destroy the catalyst. All the samples were treated with an acetic acid wash, a sodium carbonate wash, and a final water wash so that the washing treatments were identical.

The glyceride mixture containing 10% linoleate in tridecanoin and the natural fats which were studied were randomized and deodorized as described in the first experiment.

The peroxide values were determined by the method of Hamm et al. (10). The thiobarbituric acid (TBA) test was carried out according to El-Negoumy and Hammond (6) except that 2 ml of water were added before the 1 ml of 1 N hydrochloric acid.

The oxidations of the synthetic glyceride mixtures were carried out in a water-jacketed air incubator maintained at 37C. The temperature of the incubator was uniform throughout, but as an additional precaution the samples to be compared were placed side by side on the same shelf. The fats were oxidized in open 250-ml Erlenmeyer flasks, and 200 g of the glyceride mixture were placed in each flask except in the second experiment, where 150 g of fat were used. Since the rates of oxidation were low in the early stages that were of experimental interest, no oxygen was bubbled through the fats. The more saturated natural fats were oxidized at 50C so that the randomized fats would be completely melted. The more unsaturated natural fats were oxidized at 21C to slow the rate of autoxidation for more convenient sampling. The natural fats were otherwise oxidized under the same conditions and with the same precautions as the synthetic mixtures except that the oxidations were carried out on 50-g samples in 125-ml Erlenmeyer flasks.

The peroxide values from experiment two were analyzed statistically by an analysis of variance. Changes in the values with time were fitted by using

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both linear and quadratic terms. It was assumed from the shape of the curves that terms of higher degree were unnecessary and could be pooled as an estimate of error for testing interaction and main effects. Since plots of the logarithm of the peroxide value versus time gave essentially a straight line, a similar analysis of variance was performed on the data after taking the logarithm of the peroxide values.

Results and Discussion

The peroxide values shown in Figure 1 indicate that the glyceride structure had a profound effect on the rate of autoxidation. The TBA results showed a similar pattern. It was possible that the results shown in Figure 1 were caused by some other effect than glyceride structure, such as the treatment with the alkaline catalyst or slight differences in deodorization conditions. To eliminate this possibility, the second experiment was carried out. The peroxide values obtained are shown in Figure 2. Again the TBA test showed parallel results. Statistical analysis of the peroxide values showed a significant interaction between interesterification treatments and the linear effect of days. The lower three curves are close together and show similar trends. Comparisons of the main effect of the short and directed interesterification with the long interesterification showed the differences to be highly significant. Plots of the logarithm of the peroxide values versus days gave quite straight lines and resulted in no interaction of treatment and days. Comparisons of the main effects of the short and directed interesterification with the long showed the differences to be highly significant. This indicated that the rate of autoxidation was faster if the unsaturated fatty acids were concentrated in simple triglycerides. A short exposure to the alkaline catalyst reduced the rate of autoxidation because of rapid interesterification; but the rate was still higher than that for a completely randomized sample. Directing the interesterification back toward glycerides containing more than one unsaturated acid per molecule significantly increased the rate of autoxidation.

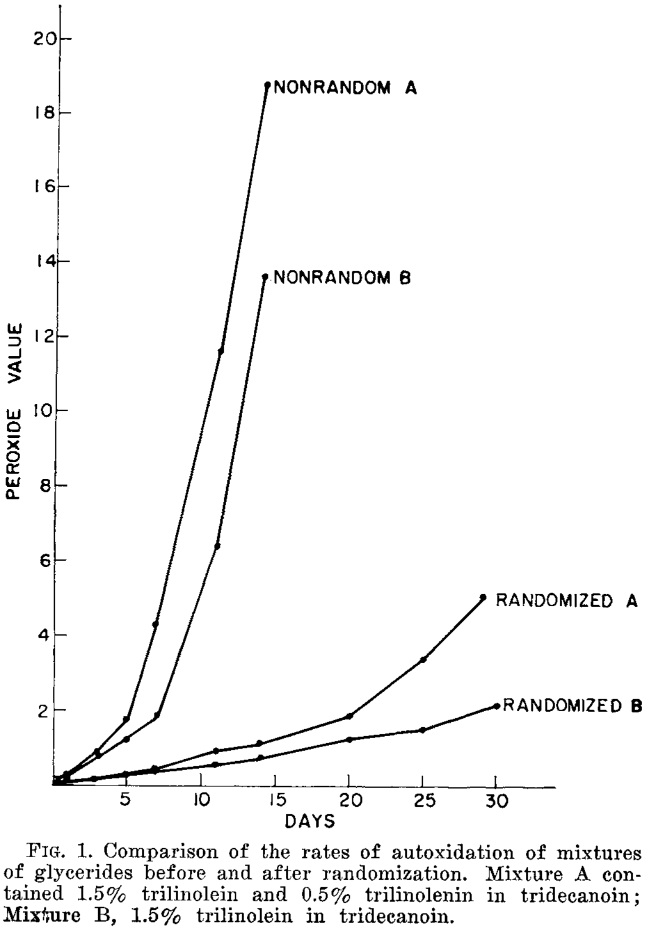


FIG. 1. Comparison of the rates of autoxidation of mixtures of glycerides before and after randomization. Mixture A contained 1.5% trilinolein and 0.5% trilinolenin in tridecanoin; Mixture B, 1.5% trilinolein in tridecanoin.

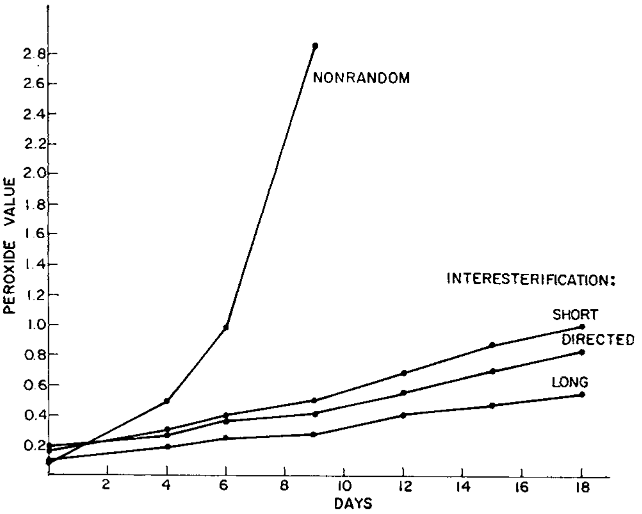
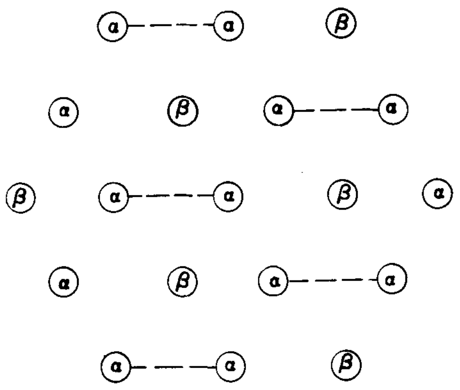
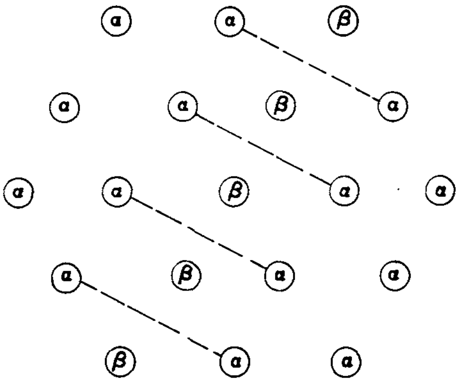


FIG. 2. Comparison of the rates of autoxidation of 1.5% trilinolein in tridecanoin before and after various interesterification treatments.

Evidently the close proximity of more than one unsaturated fatty acid on a glycerol molecule increases the rate of autoxidation. An explanation of these reaction-rate differences must rest not only in the glyceride structure of the fat but also in the physical structure of the liquid. Unfortunately the structure of the liquid state in fats is poorly defined; however, by making certain simplifying assumptions, one may account for the present data and suggest areas of further research. Some theories of liquid structure picture liquids as highly ordered and similar to crystal structures, but the degree of order is not certain (1,4). Glyceride crystals are usually pictured in the familiar tuning-fork arrangement, with alternate molecules turned 180° with respect to each other. Molten glycerides show but one short spacing on x-ray diffraction, and this is taken to indicate that the fatty acid chains are arranged in a hexagonal array. What is the relation of the two *alpha* chains of a particular triglyceride molecule in this hexagonal array? Figure 3 represents a cross-sectional view of this hexagonal packing and shows that there are two possible regular arrays: one, in which the two *alpha* chains from a given molecule (indicated by the dashed line) occupy the *ortho* positions of the hexagon, and one in which they are in *meta* positions. Besides these two, there are various irregular arrays that are possible where the fatty acid chains are arranged hexogonally, but the glycerol moieties lie at random angles. In any hexagonal array a particular fatty acid chain finds itself surrounded by six neighbors. Presumably, if a particular fatty acid contained a peroxy free radical, it could interact rapidly with any of its six hexagonal neighbors, but it would have to await some diffusion process to come in contact with other fatty acid chains. Figure 3 shows that, if the two *alpha* chains of a particular glyceride molecule are in the *ortho* array, they are hexagonal neighbors and should interact rapidly. If they are in the *meta* array, they are not hexagonal



ORTHO



META

FIG. 3. A cross-sectional representation of the *ortho* and *meta* hexagonal packing of molten glycerides in the tuning-fork configuration. The dashed lines indicate *alpha* chains attached to the same glycerol molecule.

neighbors and cannot become so as long as they remain in the *meta* array. If the assumption is true that a fatty acid can only interact rapidly with its hexagonal neighbors, then the glycerides must exist at least part of the time in the *ortho* array for the experiments show that the concentration of the unsaturated fatty acids of a glyceride mixture into triunsaturated glycerides increased the rate of autoxidation.

One can calculate the effect of glyceride structure on the rate of autoxidation if one assumes: a) the triglycerides are in the *ortho* array; b) differences in chain length and introduction of double bonds and peroxy groups cause no appreciable distortion of the array; c) oxygen is not limiting. In the *ortho* array a *beta* chain has only *alpha* chains for its hexagonal neighbors while an *alpha* chain has three *alpha* and three *beta* chains for hexagonal neighbors. If a triunsaturated glyceride were infinitely diluted with trisaturated glyceride, each *alpha* chain of the triunsaturated glyceride would find itself surrounded by one unsaturated and five saturated chains. Each *beta* chain would be surrounded by six saturated chains. The rate of autoxidation, when oxygen is not limiting, depends on the substrate concentration (2,11). Thus a peroxy free radical which finds itself surrounded entirely by unsaturated chains (substrate concentration = 1) should oxidize at a maximum rate, R. If the free radical finds itself surrounded by five saturated and one unsaturated chain, it should oxidize at 1/6th R. In the infinitely diluted triunsaturated glyceride two-thirds of the unsaturated chains find themselves surrounded by five saturated and one unsaturated fatty acid while one-third of the unsaturated chains are surrounded by saturated fatty acids, so

$$r = (2/3)\,(1/6)R + (1/3)0\,R$$

where r is the observed rate.

When the dilution is not infinite, a free radical may encounter unsaturated fatty acids belonging to glycerol molecules other than its own. The chance that any of its five neighbors, contributed from other molecules, will be unsaturated at any moment will be equal to the mol fraction of unsaturated fatty acid's, U, in the mixture. Then the relative rate,

$$\frac{r}{R} = \frac{2}{3}\left(\frac{5}{6}U + \frac{1}{6}\right) + \frac{1}{3}U = \frac{8}{9}U + \frac{1}{9}$$

If such a mixture is randomized, then the concentration of unsaturated fatty acids surrounding any free radical on either an *alpha* or *beta* chain will be U, and its relative rate of autoxidation will be U.

In these experiments the unsaturated fatty acid mol fraction was about 0.01. Thus the unrandomized mixture ought to have oxidized about 12 times as fast as the randomized in its initial step. However this value is too large because it is not the initial step alone which is measured by the peroxide values. In the unrandomized glyceride mixture the high rate is

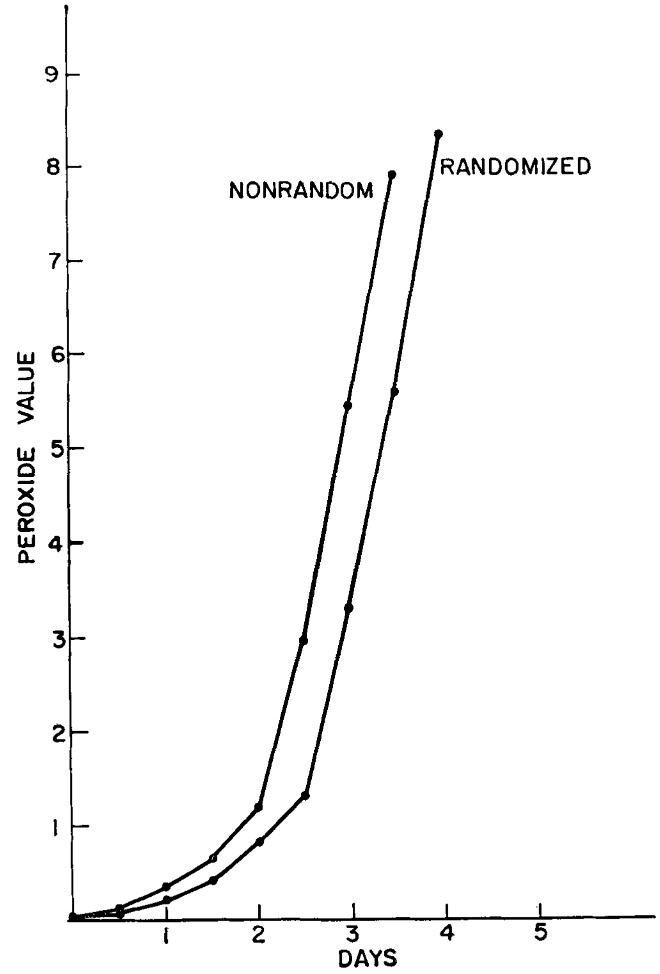


FIG. 4. Comparison of the rates of autoxidation of 10% trilinolein in tridecanoin before and after randomization.

mostly attributable to the autoxidation by an *alpha* chain of the other *alpha* chain on the same glycerol molecule. This step cannot be repeated so the rate at the next step will depend on the likelihood of another hexagonal neighbor being unsaturated at any given moment. This probability is difficult to calculate exactly but should be approximately the same as the random rate, that is, U, as long as U is small. If one then assumes that every other step in the chain occurs at the random rate, one concludes that the unrandomized fat ought to oxidize about 6.5 times as fast as the randomized. The observed value in Figures 1 and 2 is about 4 to 5 in the early stages of autoxidation when similar peroxide levels are compared. This is in good agreement with the theory.

The foregoing considerations also indicate that the rate of oxidation of unrandomized mixture compared with that of the randomized should decrease as the total concentration of unsaturated fatty acids increases. When the unsaturated fatty acid concentration reaches a mol fraction of 0.1, the rate of autoxidation of the unrandomized compound to the randomized mixture will drop to about 1.5. This was tested with a mixture of trilinolein in tridecanoin, and the results are shown in Figure 4. The observed ratio was actually about 1.4.

The synthetic glyceride mixtures considered so far have little relation to those of natural fats. In natural fats there seems to be a tendency for various fatty acids to be esterified at the 1-, 2-, and 3-position of the glycerol molecule (15), but the particular combinations that may occur together are otherwise random. Particularly in many fats there is a tendency for unsaturated fatty acids to be found mostly on the *beta* position. If the *ortho* array that seems to exist in melted glycerides is a regular one, with most of the glycerol groups aligned as they would be in a crystal, the configuration of natural glycerides should contribute to their stability. To consider an extreme case: if a fat contained less than one-third unsaturated fatty acids and these were entirely on the *beta* position, then they would always have only saturated fatty acids as hexagonal neighbors and the rate of autoxidation should be almost zero.

The rate of autoxidation can be predicted for a fat that follows the 1,3-random—2-random distribution of Vander Wal (15) if it is assumed that the various unsaturated fatty acids are distributed in the same ratio on the *beta* and *alpha* positions. The probability that a free radical will form on an *alpha* chain depends on the proportion of unsaturated alpha chains in the fat. The *alpha* chains are surrounded by three *alpha* and three *beta* chains. The probability of these hexagonal neighbors being unsaturated is proportional to the mol fraction of unsaturated fatty acid on the *alpha* and *beta* positions respectively. Similar reasoning holds for the *beta* position so:

$$\frac{r}{R} = \frac{2U_\alpha}{U_\beta + 2U_\alpha}\ (\tfrac{1}{2}U_\alpha + \tfrac{1}{2}U_\beta)\ + \frac{U_\beta}{U_\beta + 2U_\alpha}\ (U)_\alpha$$

where $U\alpha$ and $U\beta$ represent the mol fraction of unsaturated fatty acid on the *alpha* and *beta* positions, respectively. To represent this equation graphically, it is more convenient to introduce a new parameter, F, where

$$F = U_\alpha - U_\beta$$

Then substituting F in the previous equation and simplifying

$$\frac{r}{R} = \frac{(3U + F)\ (3U - F)}{9U}$$

This equation is plotted in Figure 5 and gives a family of curves for various values of unsaturated fatty acid in the fat. When F = O, the case is random and the relative rate of autoxidation is equal to U. This is a maximum value for all values of U, and the rate is smaller as the unsaturated fatty acid is concentrated either on the *alpha* or *beta* positions. The outer limits of the figure represent the boundaries inside which real glyceride compositions can exist. The figure shows that maximum stability is achieved by concentrating the unsaturated fatty acids on the *beta* position ($F < O$) although some stability is achieved by concentration of the unsaturation on the *alpha* position ($F > O$). Lard is one of the few fats that has a positive value of F. It is well known in the industry that the randomization of lard oil causes a decrease in its stability, but the reason for this has not been clear (16). Most other fats and oils have negative F values, and the vegetable oils, particularly, tend to fall along the left-hand limit of the figure. Highly unsaturated fats cannot concentrate enough of their unsaturated fatty acids exclusively on the *beta* position to have much influence on their stability. Certain vegetable oils, on the other hand, which have less than 40% unsaturated fatty acids should be stabilized considerably by their natural glyceride con-

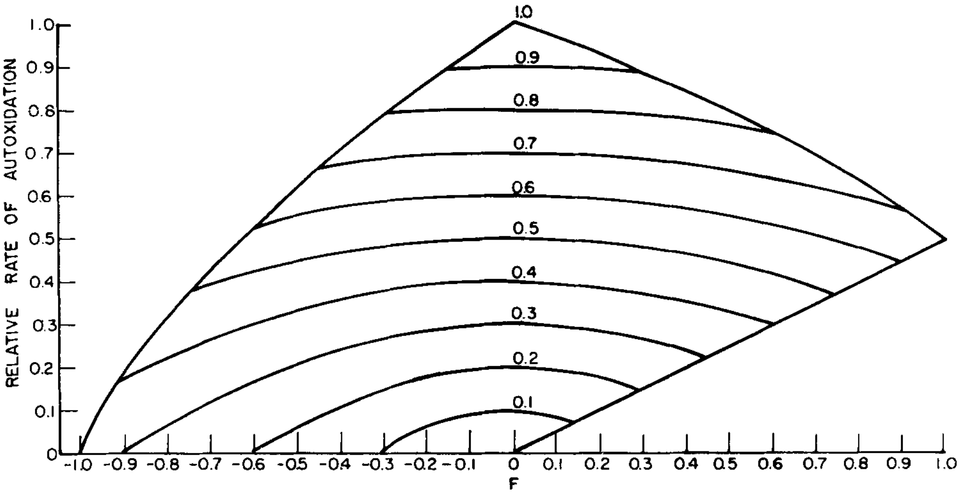


FIG. 5. Predicted relative rate of autoxidation of glycerides following a 2-random-1,3-random distribution. Each line represents a different value of U. $F = U_\alpha - U_\beta$.

TABLE I

Comparison of the Relative Rate of Oxidation of Randomized to Unrandomized Fats with Theoretical Predictions

Fat	U	F	Relative oxidation rate	Random / non-random	Composition reference
			observed	predicted	
Cocoa butter	0.40	−0.75	3.1	1.6	13
Borneo tallow	0.32	−0.80	2.5	3.2	13
Corn oil	0.86	−0.18	1.5	1.003	9
Soybean oil	0.84	−0.20	1.5	1.006	8

figuration compared with a random configuration.

The validity of these calculations was tested by comparing the rate of autoxidation of randomized and unrandomized samples of cocoa butter, Borneo tallow, corn oil, and soybean oil. The results are given in Table I. The results show that, in all four fats, randomization caused an increase in the rate of autoxidation. The two fats that were low in unsaturated fatty acids showed more change on randomization than those high in unsaturation. These facts support the theory, but the equation does not predict the observed magnitude of the change very well.

There are several reasons why the equation may be inaccurate. As before, the equation predicts only the rate of the first step in the chain reaction. Other steps in the chain will have different rates if the unsaturated acids are not randomly distributed. This will make the observed ratios somewhat lower than those predicted by the equation. In the natural fats under investigation the unsaturation is high enough so that the assumption that every other step occurs at the random rate is not reliable. The assumption that the different unsaturated fatty acids are distributed in the same way on the *alpha* and *beta* positions of the glyceride is not valid for natural fats. For example, the linoleic acid in the fats may oxidize so much more rapidly than the oleic acid that calculations based on the distribution of the total unsaturation, U, may not be reliable. Recent evidence has indicated that the two *alpha* positions of a natural glyceride may have different fatty acid compositions (3). The importance of this effect will depend on how regularly the glycerol moieties are aligned in a molten fat. If the 1- and 3-positions of the glycerol are always oriented in the same way in a hexagonal array, a study of the *ortho* array in Figure 3 shows that a fatty acid chain on the 1-position is surrounded by only chains from the 2- and 3-positions. Thus a fatty acid on the 1-position might be insulated from other fatty acids on the 1-position by its hexagonal neighbors just as the 2-position was in the case already discussed. There are antioxidants and perhaps pro-oxidants in the natural fats. The effects of the randomization reaction and the alkaline catalyst on these compounds are unknown. Finally the basic assumptions of the calculation are approximations. For example, the degree of regularity of the hexagonal array in liquid fats is unknown. Also many triglycerides are believed to exist in a "double tuning-fork" or "chair" form in the crystalline state, and it is not known how such glycerides behave in a liquid state.

It remains to be proved that organisms derive any benefit from having their glycerides conform to a particular distribution pattern. It has been suggested that it is advantageous for an organism to limit the amount of long-chain trisaturated glyceride in its depot fat because of the crystallization of such glycerides at physiological temperatures (12). The possible benefit, in terms of increased stability to oxidation, that a particular structure may confer on triglycerides as well as other lipid species should also be considered.

The relation of glyceride structure to oxidative stability may have technological implications. For example, it should be possible to synthesize fats of greater stability by concentrating the unsaturated fatty acids on the *beta* position.

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REFERENCES

1. Bailey, A. E., "Melting and Solidification of Fats," Interscience Publishers Inc., New York, 1950, 9–11.
2. Bolland, J. L., Trans. Faraday Soc. *44,* 669 (1948).
3. Brockerhoff, H., J. Lipid Res. *6,* 10 (1965).
4. Chapman, D., "The Structure of Lipids," John Wiley and Sons Inc., New York, 1965, p. 241.
5. Craig, B. M., and W. L. Murthy, JAOCS *36*, 549 (1959).
6. El-Negoumy, A. M., and E. G. Hammond, J. Dairy Sci. *45,* 848 (1962).
7. Eshelman, L. R., and E. G. Hammond, JAOCS *35*, 231 (1958).
8. Gunstone, F. D., and F. B. Padley, Ibid. *42,* 957 (1965).
9. Gunstone, F. D., and M. I. Qureshi, Ibid. *42*, 961 (1965).
10. Hamm, D. L., E. G. Hammond, V. Parvanah and H. E. Snyder, JAOCS *42,* 920 (1965).
11. Henderson, J. F., and H. A. Young, J. Phys. Chem. *46,* 670 (1942).
12. Kartha, A. R. S., "Studies on the Natural Fats," Vol. I, published by the author, Ernakulam, India, 1951, pp. 113–117.
13. Mattson, F. H., and E. S. Lutton, J. Biol. Chem. *233,* 868 (1958).
14. Parker, W. E., R. E. Koos and D. Swern, "Biochemical Preparation," Vol. 4, John Wiley and Sons, New York, 1955, p. 86.
15. Vander Wal, R. J., JAOCS *37,* 18 (1960).
16. Vander Wal, R. J., personal communication.

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