A New Approach to the Glyceride Structure of Natural Fats*

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ONE of the fundamental problems in fat chemistry is the glyceride structure of natural fats. Although it has long been known that fats are made up of glycerin combined with fatty acids, isolation of individual glycerides from natural fats has proved to be exceedingly difficult or impossible. For many years the only basic fact that was accepted was that the fatty acids tended to distribute themselves among the available glycerol molecules, i.e. homogeneous triglycerides, made up of a single fatty acid, were found to be rare except when one fatty acid greatly predominated. Theoretical considerations indicated that many isomeric glycerides could be present when a fat was made up of more than a few fatty acids, as is generally the case. It was also obvious that many of these glycerides would be so nearly identical in physical and chemical properties as to preclude their isolation by any available techniques.

Professor Hilditch and his co-workers (1) have developed analytical methods which can be considered reliable for at least certain types of glycerides. Thus, they have determined the trisaturated glyceride content of dozens of animal and vegetable fats and with this as a basis have theorized on the glyceride structure of the whole fat. Out of this has come the well known "rule of even distribution" as a general concept for all fats. This rule states that, in general, fatty acids tend to distribute themselves evenly over all the available glyceride molecules, or, more specifically, that no homogeneous triglycerides will be formed until the concentration of one acid exceeds about 60%. It was early realized that numerous exceptions to this rule existed; animal fats in particular consistently fell among these exceptions.

The rather qualitative nature of Hilditch's "rule of even distribution" has hampered a reliable evaluation of its validity. In a recent discussion of the theoretical and quantitative aspects of the hypothesis it has been shown that only an approximation of truly even distribution has been proposed and that only vegetable fats could be expected to conform to the proposed "even distribution" pattern (2). Animal fats, on the other hand, were shown to follow a pattern of random distribution such as is found in synthetic fats made by a direct esterification of glycerol and fatty acids. The glyceride distribution of such a fat may be calculated from its fatty acid composition, using equations based upon the law of probability (3, 4, 5). Thus, random distribution may be said to represent a condition of chemical equilibrium. Conversely, fats having an "even distribution" pattern are not in a state of chemical equilibrium, apparently having been formed by a directed rather than random synthesis.

With these facts in mind a new approach to glyceride structure determination appeared possible. It was already known that under certain conditions mixtures of two or more fats can be made to interesterify to a final product in which the glyceride structure is identical with what would be expected on the basis of a random collision or reaction of glycerol and the fatty acids present in the fat. This has been demonstrated experimentally with binary mixtures of synthetic glycerides as starting materials (6). It follows that a fat already randomly constituted should not be affected by such a treatment whereas a fat not so constituted would be expected to be altered to a measurable degree.

The technique employed has been to measure the melting characteristics and trisaturated glyceride content of a fat before and after it has been subjected to conditions conducive to interesterification. If no change is noted the fat may be said to have been constituted on a random basis; if significant changes occurred the original constitution of the fat must have been other than random. As will be seen in the experimental section, animal fats were found to be relatively unaffected by enforced interesterification. Vegetable fats, as was expected, were markedly altered, thus indicating that their glyceride structure was not based upon a random pattern. Obviously, this is by no means evidence that vegetable fats conform to the "even distribution" rule. It does, however, point up the significant difference between the glyceride distribution of animal fats and that of vegetable fats.

Experimental

1. Vegetable Fats:

(a) *Interesterification of soybean oil.* Refined soybean oil was heated to 140° C. and then wet stannous hydroxide catalyst (7) equivalent to 0.4% tin was added gradually. The temperature of the reaction mixture was raised to 225° C. and maintained there for 1.5 hours. As a result of this treatment the FAC melting point was increased from —7° to +5.5° C., and the softening point, from —13° to —0.5° C., as shown in Table I.

(b) *Interesterification of cottonseed oil.* Refined cottonseed oil was treated as described above for soybean oil. It was found that the FAC melting point increased from an initial value of 10.5° C. to a final value of 34° C., while the softening point increased from 8° to 17° C. Considerable precipitate separated from the treated cottonseed oil on standing. This was not the case with the untreated oil. Further, an approximate determination of the trisaturated glycerides (S_3) by an empirical acetone crystallization method (6) showed 1% trisaturated glycerides in the treated oil; none in the original oil. According to the random distribution theory, 2.1% should be present at equilibrium while on the even distribution basis none would be anticipated (2).

The results obtained on soybean oil and cottonseed oils would make it appear that these oils are definitely not randomly distributed.

2. Animal Fats:

(a) *Interesterification of oleo stock.* Oleo stock was treated as described above. In this case, however, the

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melting point changed from 49.5° to 49° C., and the softening point, from 47.5 to 46.5° C. The trisaturated glyceride content, originally 31.6%, decreased to a final value of 27.2%, 4.5% higher than that calculated for random distribution. This high value is caused by the precipitation of the di-saturated, mono-unsaturated glycerides which interfere with this determination. If oleo stock conformed to the random distribution hypothesis perfectly, no changes should have been observed. The fact that some change did occur indicates that oleo stock differs, at least slightly, from the random distribution pattern.

(b) *Interesterification of prime steam lard.* Interesterification of a sample of prime steam lard caused no change in the melting point and raised the softening point from 30.5 to only 31.5° C. The trisaturated glyceride content, however, increased from 1.4 to 4.0%. According to the random distribution hypothesis the trisaturated glyceride content should be 2.1%. This again is evidence for the hypothesis that animal fats fit random distribution closely but not perfectly. Additional evidence has been advanced by the glyceride composition data of Riemenschneider *et al.* (8).

3. FRACTIONATED FATS—OLEO OIL:

Oleo oil is not comparable with any of the classes of natural fats (1), since it does not occur as such in nature but results from the pressing of beef fat (oleo stock) to yield a hard "oleostearine" and a liquid oil (oleo oil). Consequently, it would not be expected to conform to either theory of natural fat structures inasmuch as the oleostearine which is removed contains most of the trisaturated and much of the disaturated glycerides of the oleo stock, leaving in the oleo oil a disproportionately large amount of the more unsaturated glycerides on the basis of its fatty acid composition. The relatively low proportion of trisaturated glycerides and high percentage (47.1%) of saturated fatty acids in oleo oil should make it susceptible to rearrangement under interesterifying conditions. This was found to be the case since on treatment similar to that outlined above the melting point increased from 26.5° to 44.5° C., and the softening point, from 23° to 40° C. The trisaturated glycerides, as determined by the crystallization method, increased from 0 to 16.2%. Obviously, a profound change has been produced in the glyceride structure of the fat.

Discussion

By subjecting samples of animal and vegetable fats to conditions causing interesterification and following any changes produced by determinations of melting and softening points, as well as trisaturated glycerides, it is possible to investigate the glyceride structure of fats by an independent method not previously used. On the basis of these interesterification experiments as well as a mathematical evaluation of the extensive data of Hilditch and co-workers (2), it appears that fats do not adhere strictly to either the "even" or the random theory of glyceride structure. Animal fats most nearly approach random distribution while seed fats most nearly approach "even." The only apparent explanation for the latter is that, in formation of fat in the seed, enzymes could exert a selective action in the synthesis. The basic reason for such a fundamental difference in the synthetic action of plant and animal enzymes would be well worth investigating.

The fat of the larger land animals (*viz.* lard), being derived from both animal and plant sources, would be expected to vary in glyceride structure, depending upon the diet of the animal and the extent to which the fat is absorbed as such or altered by interesterification, ester interchange, or hydrolysis and resynthesis during metabolism.

According to Frazer (9), during the digestion of fats lipolysis is only partial and hydrolysis of the triglyceride molecule is not regarded as an essential preliminary to its absorption. If this is correct the fat of the vegetarian land animals might be expected to have its normal random glyceride pattern altered somewhat by the ingested glycerides of "even" distribution. Consequently, we suggest that the natural glycerides of animal fats are constituted according to random distribution with the experimentally observed variations being the result of the glyceride structure of the dietary fats.

Conclusions

A new approach to the glyceride structure of natural fats has been achieved through the application of enforced interesterification to samples of animal and vegetable fats. The results obtained further substantiate the hypothesis that animal fats, in contradistinction to seed fats, are essentially randomly distributed.

REFERENCES

1. Hilditch, T. P. The Chemical Composition of Natural Fats. New York; Wiley; 1941.
2. Mattil, K. F. and Norris, F. A. Science *105*, 257 (1947).
3. Dean, H. K., The Utilization of Fats; London, A. Harvey (1938).
4. Longenecker, H. E. Chemical Reviews *29*, 201 (1941).
5. Bailey, A. E. Industrial Oil & Fat Products; New York. Interscience Publishers (1945).
6. Norris, F. A. and Mattil, K. F., Oil and Soap *23*, 289 (1946).
7. Van Loon, C. British Patent 249,916 (1924).
8. Riemenschneider, R. W., Luddy, Francis E., Swain, Margaret C., and Ault, Waldo C. Oil and Soap *23*, 276 (1946).
9. Frazer, H. C. Jour. Physiology *102*, 306 (1943).

TABLE I.

Effect of Interesterification on Selected Vegetable and Animal Fats.

		Minutes at 225° C.					% S_3 by Crystallization		Calculated % S_3	
		0	30	60	90	120	0 Min.	120 Min.	Even Distribution	Random Distribution
Soybean Oil	MP (°C.)	−7	+6	+5	+5.5	....	0	0	0	0.3
	SP (°C.)	−13	+0.5	−0.5	−0.5	....				
Cottonseed Oil	MP (°C.)	+10.5	33.5	34	....	+34	0	1%	0	2.1
	SP (°C.)	+ 8	16.5	16	....	+17				
Oleo Stock	MP (°C.)	49.5	48	48.5	....	49	31.6	27.2	0	22.7
	SP (°C.)	47.5	44.5	46	....	46.5				
Oleo Oil	MP (°C.)	26.5	42.5	44	44.5	....	0	16.2	0	10.5
	SP (°C.)	23	38	40	40	....				
Prime Steam Lard	MP (°C.)	43	40.5	41	....	43	1.4	4.0	0	2.1
	SP (°C.)	30.5	27	28	....	31.5				