J. Lipid Research, October 1963
Volume 4, Number 4

The specific distribution of unsaturated fatty acids in the triglycerides of plants

F. H. Mattson and R. A. Volpenhein

The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati 39, Ohio

[Manuscript received May 21, 1963; accepted July 10, 1963.]

SUMMARY

The distribution of fatty acids between the primary and the secondary positions of the triglyceride of 28 species of plants was determined. In confirmation of previous results, palmitic and stearic acids were found to be esterified predominantly at the primary positions. An earlier study demonstrated that fatty acids having a chain length of greater than 18 carbon atoms are also esterified predominantly at the primary positions. Examination of the data in the present and previous studies shows oleic, linoleic, and linolenic acids to have a common pattern of distribution. Each of these acids is approximately randomly distributed among the positions on the triglyceride molecule that are not occupied by palmitic or stearic acids or fatty acids having a chain length greater than 18 carbon atoms. The mechanisms by which these specific distributions could be brought about are discussed.

It has been demonstrated in plants that certain of the fatty acids occupy specific positions on the triglyceride molecule. Palmitic and stearic acids (1, 2) and fatty acids having a chain length of greater than 18 carbon atoms (3) are esterified predominately at the 1- and 3-positions of glycerol. The values obtained in these earlier studies clearly demonstrated that the 2-positioned fatty acids were almost exclusively oleic, linoleic, and linolenic acids. Because of the small number of species that had been examined, however, it was not possible to decide whether there were any differences among these acids in their distribution. Analytical values on an additional 28 species of plants have been obtained. Examination of these and the earlier values show oleic, linoleic, and linolenic acids each to be distributed among all three positions of the triglyceride molecule in a modified random pattern.

EXPERIMENTAL PROCEDURE

The fruits or seeds used in these studies were obtained from reliable commercial or private sources, so that the identity of the fats is certain. The classification of the 28 species of plants whose fatty acid distribution is reported in this paper is given in Table 1. Samples 20–25 are monocotyledons, the remainder are dicotyledons. In arriving at the pattern of oleic, linoleic, and linolenic acid distribution, the values reported here as well as those obtained in the earlier study (3) are included. For this reason, the sample numbers in Table 1 start at 20; the first 19 samples are in the earlier report.

The isolation and analytical procedures employed were identical with those previously described (3). The distribution of fatty acids in the triglyceride molecule was determined by the selective hydrolysis of the ester linkages at the primary positions with pancreatic lipase (4, 5). The details of the gas-liquid chromatographic techniques that were used in determining the fatty acid compositions of the original triglycerides and the monoglycerides resulting from hydrolysis by pancreatic lipase are given in the earlier paper (3).

RESULTS AND DISCUSSION

The fatty acid composition of the fats and the distribution of these acids on the triglyceride molecule are given in Table 2. The last line of numbers for each fat, the proportion value, is the percentage of that particular fatty acid of the triglyceride that is in the 2-position. If the fatty acids were distributed randomly, 33.3% of each fatty acid would be in the 2-position; obviously, such is not the case.

The results reported here confirm and extend to other species the earlier observations (1, 2) that palmitic and stearic acids are esterified predominantly at the 1- and

Downloaded from www.jlr.org by guest, on June 20, 2012

TABLE 1. ORDER, FAMILY, GENUS, SPECIES, AND COMMON NAME OF PLANTS STUDIED

Sample No.	Order	Family	Genus and Species	Common Name
20	Glumiflorae	Gramineae	*Oryza sativa*	Rice bran
21			*Triticum*	Wheat flour
22			*Triticum*	Wheat germ
23			*Zea mays*	Corn germ
24	Principes	Palmae	*Elaeis guineensis* jacq.	Palm fruit
25	Liliflorae	Liliaceae	*Allium cepa*	Onion seed
26	Myricales	Juglandaceae	*Carya illinoensis*	Pecan kernel
27			*Juglans regia* L.	Walnut seed
28	Fagales	Corylaceae	*Corylus avellana*	Filbert nut
29		Fagaceae	*Quercus palustris*	Acorn kernel
30	Centrospermae	Chenopodiaceae	*Spinacia oleracea*	Spinach seed
31	Ranales	Lauraceae	*Persea americana*	Avocado fruit
32	Rhoeadales	Moringaceae	*Papaver rhoeas*	Poppy seed
33	Rosales	Rosaceae	*Prunus communis*	Almond kernel
34		Leguminosae	*Glycine max*	Soybean seed
35	Geraniales	Linaceae	*Linum usitatissimum*	Linseed
36		Meliaceae	*Sapium sebiferum*	Stillingia tallow (fruit coat)
37	Sapindales	Anacardiaceae	*Anacardium occidentale*	Cashew nut
38	Malvales	Malvaceae	*Gossypium hirsutum*	Cottonseed (gland-free)
39			*Gossypium*	Cottonseed
40	Parietales	Guttiferae	*Dunkwa allanblackia*	
41			*Garcinia indica*	Kokum butter (seed)
42	Myrtiflorae	Lecythidaceae	*Bertholletia excelsa*	Brazil nut
43	Contortae	Oleaceae	*Olea europea*	Olive (fruit)
44	Tubiflorae	Solanaceae	*Lycopersicum esculentum*	Tomato seed
45		Pedaliaceae	*Sesamum indicum*	Sesame seed
46	Campanulales	Cucurbitaceae	*Cucumis sativus*	Cucumber seed
47			*Cucurbita pepo*	Squash seed
48		Compositae	*Carthamus tinctorius*	Safflower seed
49			*Helianthus annus*	Sunflower seed

3-positions. However, as will be discussed later, the distribution between these two positions is probably not random. The same pattern of distribution was observed for fatty acids having a chain length of greater than 18 carbon atoms (3). For ease of discussion, those fatty acids that are preferentially esterified at the 1- and 3-positions will be referred to as Category I acids.

An examination of the distribution of oleic, linoleic, and linolenic acids as reported in Table 2 and in the previous paper indicated that these acids, too, follow a pattern. Moreover, the pattern shown by all three acids was found to be similar. Consequently, oleic, linoleic, and linolenic acids are referred to as Category II acids.

Each of the Category II acids appeared to be randomly distributed among the positions on the triglyceride molecule that are not occupied by Category I acids. This hypothesis was tested by comparing, for each of the acids in Category II, its percentage in the total acids of this category in the triglyceride (Theory) with its percentage in the Category II acids at the 2-position (Found). For example, oleic acid constitutes 81% (Theory) of the Category II acids in palm fruit oil triglycerides (Sample 24), and if the hypothesis that is being tested is correct, it would also constitute 81% of the acids in the 2-position. It was actually shown to constitute 75% (Found) of the acids in the 2-position. Graphic presentations of the Theory vs Found values for oleic, linoleic, and linolenic acids are given in Fig. 1, 2, and 3, respectively. The points on these graphs were calculated from the values in Table 2 and from the earlier paper (3). The point for Sample 24, which was discussed above, is circled in Fig. 1. Since Samples 15, 36, 40, and 41 contain only a single Category II acid, and since Sample 31 contains a large amount of palmitoleic acid, they are not included in the figures. All of the remaining 41 species were used. The line best fitting the points in each figure was obtained by the method of Least Squares; it is labeled "Found." If the percentage of the acid in the Category II acids at the 2-position were the same as that in the Category II acids of the whole triglyceride, the Theory and Found lines would be superimposed.

The agreement between the theoretical and found values, as shown in these figures, is quite good. There is a deviation of about 10% in that the 2-position con-

Downloaded from www.jlr.org by guest, on June 20, 2012

TABLE 2. Fatty Acid Composition of Whole Triglyceride and of Fatty Acids at the 2-Position; and Proportion of Each Fatty Acid that is at the 2-Position.

	16:0	18:0	18:1	18:2	18:3*
			mol %		
20. Rice bran					
Triglyceride	18	1	41	38	2
2-Position	2	0	43	54	2
Proportion†	4	0	35	47	33
21. Wheat flour					
Triglyceride	4	4	27	59	7
2-Position	2	0	22	69	6
Proportion	17	0	27	39	29
22. Wheat germ					
Triglyceride	20		18	55	7
2-Position	1		18	75	6
Proportion	2		33	45	29
23. Corn					
Triglyceride	12	2	29	58	
2-Position	2	0	26	72	
Proportion	6	0	30	41	
24. Palm fruit					
Triglyceride	44	6	39	9	
2-Position	11	2	65	22	
Proportion	8	11	56	81	
25. Onion Seed					
Triglyceride	8	2	26	64	
2-Position	1	0	25	73	
Proportion	4	0	32	38	
26. Pecan					
Triglyceride	9	3	58	30	
2-Position	1	0	56	42	
Proportion	4	0	32	47	
27. Walnut					
Triglyceride	8	3	15	61	12
2-Position	1	0	17	71	11
Proportion	4	0	38	39	30
28. Filbert nut					
Triglyceride	7	2	82	8	
2-Position	1	0	87	12	
Proportion	5	0	35	50	
29. Acorn					
Triglyceride	9	1	69	20	
2-Position	1	0	68	31	
Proportion	4	0	33	52	
30. Spinach seed					
Triglyceride	12	1	24	60	2
2-Position	2	1	21	75	2
Proportion	6	33	29	42	33
31. Avocado‡					
Triglyceride	37		27	14	1
2-Position	7		41	37	2
Proportion	6		51	88	67
32. Poppy seed					
Triglyceride	10	2	11	76	
2-Position	1	0	9	89	
Proportion	3	0	27	39	
33. Almond					
Triglyceride	7	2	70	21	
2-Position	1	0	64	34	
Proportion	5	0	30	54	
34. Soybean					
Triglyceride	12	4	25	51	8
2-Position	1	0	22	69	8
Proportion	3	0	29	45	33
35. Linseed					
Triglyceride	6	4	22	15	52
2-Position	1	0	27	23	48
Proportion	6	0	41	51	31
36. Stillingia tallow					
Triglyceride	71	1	28		
2-Position	14	1	86		
Proportion	7	33	102		
37. Cashew nut					
Triglyceride	13	9	60	18	
2-Position	2	0	56	42	
Proportion	5	0	31	78	
38. Cottonseed (gland-free)					
Triglyceride	27	3	17	52	
2-Position	2	0	22	76	
Proportion	2	0	43	49	
39. Cottonseed					
Triglyceride	26	2	17	55	
2-Position	2	1	20	77	
Proportion	3	17	39	47	
40. Dunkwa allanblackia					
Triglyceride	2	54	45		
2-Position	2	5	93		
Proportion	33	3	69		
41. Kokum butter					
Triglyceride	2	61	37		
2-Position	2	2	96		
Proportion	33	1	86		
42. Brazil nut					
Triglyceride	17	12	33	39	
2-Position	1	1	40	58	
Proportion	2	3	40	50	
43. Olive					
Triglyceride	12	2	76	8	
2-Position	1	0	88	10	
Proportion	3	0	39	42	
44. Tomato seed					
Triglyceride	11	5	21	61	3
2-Position	1	0	22	74	3
Proportion	3	0	35	40	33
45. Sesame seed					
Triglyceride	10	6	40	44	
2-Position	1	1	43	55	
Proportion	3	6	36	42	
46. Cucumber seed					
Triglyceride	16	5	7	71	
2-Position	2	1	4	94	
Proportion	4	7	19	44	
47. Squash seed					
Triglyceride	17	7	16	60	
2-Position	1	1	17	81	
Proportion	2	5	35	35	

(continued on page 395)

Downloaded from www.jlr.org by guest, on June 20, 2012

TABLE 2 (contd.). FATTY ACID COMPOSITION OF WHOLE TRIGLYCERIDE AND OF FATTY ACIDS AT THE 2-POSITION; AND PROPORTION OF EACH FATTY ACID THAT IS AT THE 2-POSITION.

	16:0	18:0	18:1	18:2	18:3*
			mol %		
48. Safflower seed					
Triglyceride	8	3	14	75	
2-Position	1	0	13	86	
Proportion	4	0	31	38	
49. Sunflower seed					
Triglyceride	6	3	27	64	
2-Position	1	0	23	76	
Proportion	6	0	28	40	

* Fatty acid abbreviation system suggested by Dole et al. (15).

† $\frac{\text{"2-Position"}}{\text{"Triglyceride"} \times 3} \times 100$ = Proportion; i.e., percentage of fatty acid type that is esterified at the 2-position.

‡ Contains also 20% of 16:1, 22% of which is in the 2-position.

tains less oleic acid and more linoleic acid than would be expected if this pattern of distribution were followed strictly. Except for these differences, which in a few oils become quite marked, each of the acids of Category II is randomly distributed among the positions on the triglyceride molecule that are not occupied by Category I acids.

The mechanism by which this specific distribution of fatty acids in plant triglycerides is brought about remains to be established. In animal tissues, the main pathway of triglyceride synthesis appears to be α-glyceryl phosphate → phosphatidic acid → diglyceride → triglyceride (6). However, in the peanut cotyledon, phosphatidic acid is not formed by the acylation of α-glyceryl phosphate but rather by the phosphorylation of mono- or diglyceride (7). The

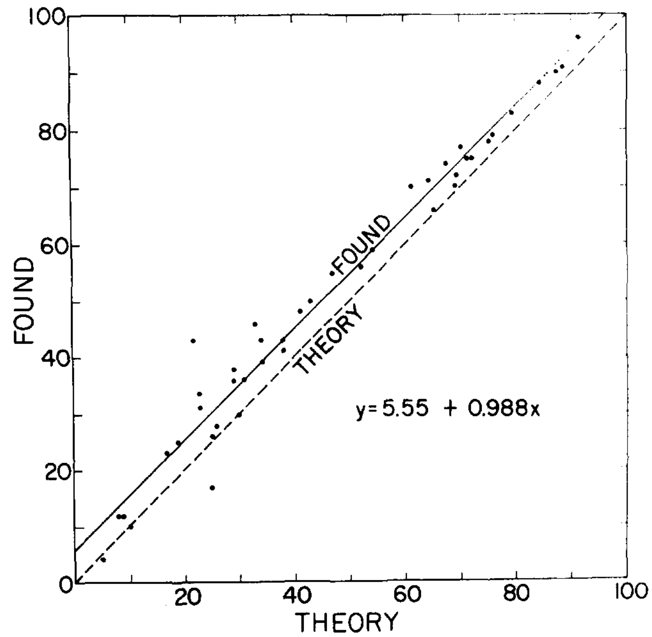

FIG. 2. Percentage of linoleic acid in Category II acids (oleic + linoleic + linolenic) present in the total triglyceride (Theory) vs its percentage in Category II acids at the 2-position.

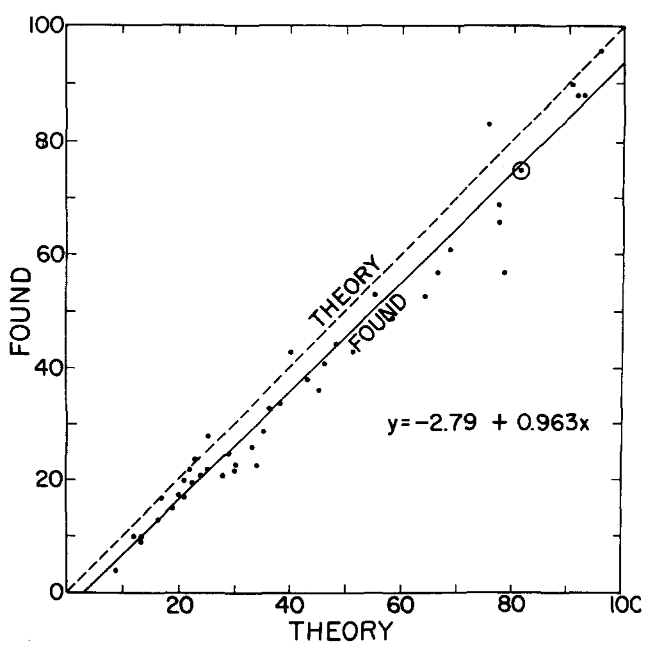

FIG. 1. Percentage of oleic acid in Category II acids (oleic + linoleic + linolenic) present in the total triglyceride (Theory) vs its percentage in Category II acids at the 2-position.

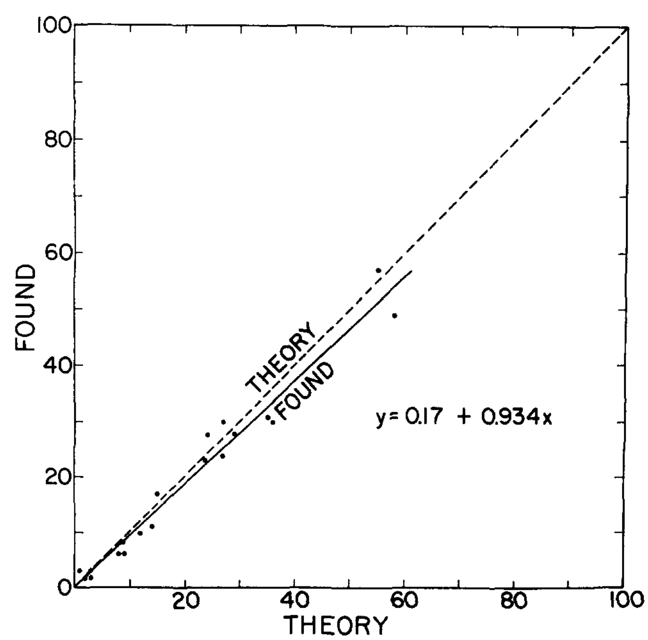

FIG. 3. Percentage of linolenic acid in Category II acids (oleic + linoleic + linolenic) present in the total triglyceride (Theory) vs its percentage in Category II acids at the 2-position.

Downloaded from www.jlr.org by guest, on June 20, 2012
JOURNAL OF LIPID RESEARCH
ASBMB

system resembles that reported in guinea pig brain tissue (8). On the other hand, the mesocarp of the avocado does synthesize phosphatidic acid from α-glyceryl phosphate (9). Thus, two completely different systems have been shown to exist in plants. It remains to be established which, if either, of these systems is the one generally found in the plant kingdom.

Savary and Desnuelle (10) have proposed the existence of two enzymes in plants to account for the distribution of fatty acids. They assume that the starting material is α-glyceryl phosphate. One enzyme is specific for oleic, linoleic, and linolenic acids and selectively esterifies these with the hydroxyl group next to the phosphate group, that is the β-position. The other enzyme has no fatty acid specificity and esterifies all fatty acids, regardless of structure, with the hydroxyl at the α'-position. The same enzyme, or one with a similar lack of fatty acid specificity, catalyzes the esterification of the α-position after removal of the phosphate group.

Gunstone (11) has calculated the proportion of glyceride types that would be found in a fat synthesized by various routes. His application of the formula suggested by A. S. Richardson and described by Vander Wal (12) covers the system proposed by Savary and Desnuelle. A part of the mechanism they have proposed is a random distribution of saturated and unsaturated fatty acids between the α- and α'-positions. Such random distribution would result in a monosaturated-diunsaturated glyceride level of no more than 50%. This maximum value would be attained when the saturated acids constitute one-third of the total acids of a fat. By the very nature of random distribution, any higher level of saturated fatty acids would result in an increase of disaturated-monounsaturated glycerides and a decrease, below 50%, of monosaturated-diunsaturated glycerides. In the data compiled by Hilditch (13), ten of the sixteen fats that contained from 20 to 50% saturated acids consisted of more than 50% of monosaturated-diunsaturated glycerides. The highest value, 68%, was observed in two fats. Levels of greater than 50% of this glyceride type are not compatible with the mechanism proposed by Savary and Desnuelle (10). To attain such levels, there must be a selective introduction of saturated fatty acids into one, and only one, of the primary positions of the triglyceride. If saturated fatty acids were preferentially introduced into the α'-position during the formation of phosphatidic acid, this would account for the structure of the resulting triglycerides. Such a mechanism affords a single phosphatidic acid pool with a fatty acid composition that is suitable for a precursor of either triglycerides or glycerophosphatides.

The data presently available indicate a high degree of fatty acid selectivity in the synthesis of plant triglycerides. It is likely that the mechanism of synthesis is specific with respect to the fatty acid and the position on the triglyceride molecule. There is the additional possibility of ester interchange which, if extensive, would mask any specificity, or lack thereof, that existed during the initial synthesis of the triglyceride.

The patterns of specific distribution of fatty acids in the triglycerides of vegetable fats as shown here and in the earlier reports (1, 3) are such that it is possible to calculate the distribution from the fatty acid composition of the triglyceride. The same is not true of animal fats. The limited number of species of animals that have been studied (1) has revealed no common pattern for any of the fatty acids, although there is a tendency for linoleic acid to be concentrated in the 2-position. There is the added complication that the depot fat of animals can be markedly altered by the dietary fat (14).

REFERENCES

1. Mattson, F. H., and E. S. Lutton. *J. Biol. Chem.* **233**: 868, 1958.
2. Savary, P., J. Flanzy, and P. Desnuelle. *Biochim. Biophys. Acta* **24**: 414, 1957.
3. Mattson, F. H., and R. A. Volpenhein. *J. Biol. Chem.* **236**: 1891, 1961.
4. Mattson, F. H., and L. W. Beck. *J. Biol. Chem.* **219**: 735, 1956.
5. Mattson, F. H., and R. A. Volpenhein. *J. Lipid Res.* **2**: 58, 1961.
6. Kennedy, E. P. *Federation Proc.* **20**: 934, 1961.
7. Bradbeer, C., and P. K. Stumpf. *J. Lipid Res.* **1**: 214, 1960.
8. Hokin, L. E., and M. R. Hokin. *Nature* **184**: 1068, 1959.
9. Barron, E. J., and P. K. Stumpf. *Biochim. Biophys. Acta* **60**: 329, 1962.
10. Savary, P., and P. Desnuelle. *Biochim. Biophys. Acta* **50**: 319, 1961.
11. Gunstone, F. D. *Chem. Ind.* (*London*) **No. vol**: 1214, 1962.
12. Vander Wal, R. J. *J. Am. Oil Chemists' Soc.* **37**: 18, 1960.
13. Hilditch, T. P. *The Chemical Constitution of Natural Fats*, 3rd Ed., New York, John Wiley and Sons, Inc., 1956, p. 298.
14. Tove, S. B., and F. H. Smith. *J. Nutr.* **71**: 264, 1960.
15. Dole, V. P., A. T. James, J. P. W. Webb, M. A. Rizack, and M. F. Sturman. *J. Clin. Invest.* **38**: 1544, 1959.

Downloaded from www.jlr.org by guest, on June 20, 2012