Structure of Peanut Oil Triacylglycerols from Cultivars of Diverse Genetic Background

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ABSTRACT

Triacylglycerols (TG) were isolated from peanut cultivars chosen to embrace known extremes in oleic and linoleic acid content, and the TG structures were determined using pancreatic lipase hydrolysis. Molar concentrations of oleic and linoleic acid in *sn*-2-monoacylglycerols (MG) were highly correlated with molar concentrations in TG. Correlation coefficients were 0.99 in each case. Molar concentrations of oleic and linoleic in 2-MG are given by: $MG_{18:1} = 1.37TG_{18:1} - 20.8$; $MG_{18:2} = 1.71TG_{18:2} + 0.66$. Linoleic-oleic ratios ranged from 0.2-1.1 for TG, 0.2-2.6 for *sn*-2-MG, and 0.1-0.6 for acids in the 1(3)-positions of TG. Molar concentrations of linoleic acid in the 1(3)-positions of TG ranged from 7-26%. The linoleic-oleic ratios and molar concentrations of 1(3) linoleic acid in some cultivars were similar to values that have been reported for relatively nonatherogenic corn oil and randomized peanut oil.

INTRODUCTION

The triacylglycerols (TG) of many unsaturated seed oils are similar in several respects. The *sn*-2-position is occupied almost exclusively by unsaturated acids, usually of the 18 carbon series (1,2), and the data obtained thus far indicate asymmetric distribution of fatty acids between positions 1 and 3 of TG (3-6). Among cultivars within a given species a regular distribution pattern exists for the placement of fatty acids at all three positions (4-6). This pattern is perhaps best described by the 1-random-2-random-3-random hypothesis (7,8) which assumes a separate fatty acid pool for each position of the oil triacylglycerols. The concentration of an acid in any pool, and therefore in any position, appears to be governed primarily by the overall concentration of that acid in the oil, although some variation in this pattern has been observed and has been attributed to genetic factors (4,5).

Recent interest in the structure of peanut (*Arachis hypogaea*) oil TG has been stimulated by the observation that peanut oil is atherogenic to experimental animals. Peanut oil has been shown to be atherogenic to rats (9,10), rabbits 10-13), and Rhesus monkeys (14,15). This unexpected characteristic of peanut oil is not related to degree of saturation but has been attributed to TG structure (16,17). Autointeresterification of peanut oil, a process that redistributes fatty acids in a random manner among the three positions of TG, reduces the atherogenic potential of peanut oil to that of the relatively nonatherogenic corn oil (16).

Fat TG serves as an important source of metabolizable energy for animals and as precursor for other types of lipids. Triacylglycerols are not absorbed directly from the digestive tract but are first partially degraded by pancreatic lipase into *sn*-2-monoacylglycerols (MG) and "free" fatty acids derived from the 1 and 3 positions of TG. The fatty acids and MG are absorbed and together with endogenous fatty acids are reassembled into TG before release into the lymph as the primary constituent of chylomicrons (18,19).

Peanut oil triacylglycerols contain 8 fatty acids in concentrations equal to or greater than about 1%; however, oleic and linoleic account for ca. 75-80% of total acids. Among specific cultivars or genotypes the level of oleic acid may vary from 40-70% and that of linoleic acid from 10-40% (20). Among U.S. commercial varieties, the range in values is somewhat less and generally falls between 40 and 50% for oleic and 25 and 35% for linoleic acid. This paper reports on the structure of peanut oil TG obtained from cultivars chosen to embrace known extremes in oleic and linoleic acid content. The distribution of fatty acids in the 2-MG, and by difference in the 1(3)-positions of TG, was determined by hydrolysis with pancreatic lipase.

MATERIALS AND METHODS

The peanut cultivars were chosen from among a group of cultivars described in a previous study (20) and were grown at Tifton and Plains, GA. Choice of *A. hypogaea* cultivars was based strictly upon levels of oleic and linoleic acid. Seed of two selections were obtained from two crop years. These cultivars are not grown commercially, but two are similar to commercial varieties in fatty acid profiles. One selection of *Arachis villosulicarpa*, a species characterized by unusually high levels of behenic, lignoceric, and linoleic acid (20), was also included in the study. This species is used as food by Indians in the Mato Grosso region of Brazil and is the only other species within the genus *Arachis* used for food.

Oil was removed from mature seeds by Carver press and was stored at 0 C or below until time of analysis. Triacylglycerols were isolated by column chromatography on 60/80 mesh acid-washed Florisil by the method of Carroll (21). Lipase hydrolysis was carried out according to the procedure of Weber (4) but was scaled up to accommodate 100 mg samples (22). Products of hydrolysis were isolated by thin layer chromatography on 0.5 mm Silica Gel G plates developed in hexane/diethyl ether/acetic acid (65:35:1) and immediately converted to methyl esters with 3% sulfuric acid in methanol/benzene (3:2) (22). Fatty acid methyl esters were separated and quantitated on a MicroTek 220 gas chromatograph equipped with dual flame ionization detectors, an electronic integrator, and 1.86 m x 4 mm glass columns packed with 15% EGS on Chromosorb W (AW) (DMCS) (22). Linear regression equations were established from data plots. Data for *A. villosulicarpa* were not included in the statistical treatment.

RESULTS AND DISCUSSION

The molar concentration of fatty acids in the total TG, 2-MG, and 1(3)-positions of TG obtained from 10 peanut oils are listed in Table I. The fatty acids in the 1(3)-positions were calculated by difference (8). The genetic diversity within *A. hypogaea* is apparent from these data; however, the pattern of fatty acid distribution within the TG is similar to that reported previously for peanut oil (1,3,6,17) and for other seed oils (4,5). The saturated fatty acids and those with chain lengths greater than 18 carbons are restricted almost entirely to the 1(3)-positions, and the 2-position is filled almost entirely by oleic and linoleic acid. The molar concentrations of oleic and linoleic acid in the 2-MG are highly correlated with the concentrations of these acids in the total TG (Fig. 1). The high correlation coefficients (0.99) indicate that in this species the comple-

TABLE I

Structure of Peanut Oil Triacylglycerols

Variety number	Position	Fatty acid (mole percent)								18:2	Proportion at C-2	
		16:0	18:0	18:1	18:2	20:0	20:1	22:0	24:0	18:1	18:1	18:2
37	1,2,3	12.5	4.5	34.9	39.6	2.2	1.0	4.0	1.4	1.1		
	2	1.2	.3	27.2	70.3	.8	.2	---	---	2.6	27.0	59.3
	1(3)	18.1	6.6	38.7	24.2	2.9	1.4	6.0	2.1	.6		
50	1,2,3	11.4	2.3	43.3	37.2	1.1	1.2	2.5	1.0	.9		
	2	1.2	.4	38.2	60.2	---	---	---	---	1.6	29.4	53.9
	1(3)	16.5	3.2	45.8	25.7	1.6	1.8	3.7	1.5	.6		
48(1)[a]	1,2,3	10.2	1.5	52.3	27.7	1.0	2.0	3.4	1.9	.5		
	2	.8	---	47.4	51.8	---	---	---	---	1.1	30.2	62.3
	1(3)	14.9	2.2	54.7	15.6	1.5	3.0	5.1	2.8	.3		
48(2)[a]	1,2,3	12.2	1.3	44.2	33.8	1.0	1.9	3.7	2.0	.8		
	2	1.2	.3	42.9	54.7	.9	---	---	---	1.3	32.3	54.0
	1(3)	17.7	1.8	44.8	23.3	1.0	2.8	5.5	3.0	.5		
54	1,2,3	7.2	3.5	71.2	12.3	1.7	1.0	2.2	.8	.2		
	2	.5	---	80.7	18.8	---	---	---	---	.2	37.8	50.9
	1(3)	10.5	5.2	66.4	9.0	2.5	1.5	3.3	1.2	.1		
80	1,2,3	12.2	3.7	39.9	37.1	1.8	.9	3.3	1.2	.9		
	2	1.0	.2	33.0	65.7	.1	---	---	---	2.0	27.6	59.1
	1(3)	17.8	5.4	22.8	22.8	1.3	1.3	4.9	.5	.5		
58(1)[a]	1,2,3	6.5	3.4	73.3	12.0	1.5	.8	1.8	.8	.2		
	2	.5	---	77.1	22.4	---	---	---	---	.3	35.1	62.5
	1(3)	9.5	5.1	71.4	6.8	2.2	1.2	2.7	1.2	.1		
58(2)[a]	1,2,3	8.3	3.3	62.2	20.6	1.5	1.0	2.3	.9	.3		
	2	.6	---	62.4	37.0	---	---	---	---	.6	33.4	59.8
	1(3)	12.1	4.9	62.1	12.4	2.2	1.5	3.4	1.3	.2		
60	1,2,3	7.8	3.8	68.0	14.9	1.6	1.0	2.2	.9	.2		
	2	1.0	.4	72.7	25.9	---	---	---	---	.4	35.6	58.0
	1(3)	11.2	5.5	65.6	9.4	2.4	1.5	3.3	1.3	.1		
A. Villosulicarpa 120	1,2,3	9.9	1.7	16.2	51.6	1.5	2.2	12.5	4.4	3.2		
	2	.8	---	26.6	72.6	---	---	---	---	2.7	54.7	47.0
	1(3)	14.4	2.5	11.0	41.1	2.2	3.3	18.7	6.6	3.7		

[a]Seed from different crop years.

ment of enzymes governing the synthesis of TG is very similar in all genotypes and that the one factor controlling the concentration of oleic and linoleic acid in the 2-MG is the relative concentrations of these acids in the total fatty acid pool.

The linear regression equations generated by these data (Fig. 1) appear to be generally applicable to peanut oils. The values predicted by these equations agree quite closely with experimental values reported in the literature (1,3,6,17), and in most instances the differences between experimental and calculated values are within the expected experimental error of analytical techniques. It should be emphasized, however, that these equations cannot be applied to peanut oils that have been modified by chemical or physical techniques that alter the natural distribution of fatty acids in TG, or to TG fractions that have been isolated by such techniques (23). Similar sets of equations, applicable not only to the 2-position of TG but to positions 1 and 3 as well, have been published by Weber et al. (4) for corn, by Fatemi and Hammond (5) for soybean oils, and by Sanders for commercial varieties of peanuts (6).

Mattson and Volpenhein (1) reported that 33% (proportion) of oleic acid and 59% of linoleic acid in peanut oil was esterified at the 2-position. In the present study, these values ranged between 27 and 38% for oleic and 51 and 63% for linoleic acid (Table I). The linear regression equation for oleic acid (Fig. 1) predicts a positive correlation between TG oleic acid and the proportion esterified in the 2-position; this correlation was observed (r=.93) (22). In the regression equation for linoleic acid, the intercept (.66) is not significantly different from zero, and if we assume a zero intercept the equation predicts a proportion value of 57 for linoleic acid $[(1.71TG_{18:2}/3TG_{18:2})100]$. The observed value was 57.75 ± 3.99. The large variation in proportion values observed for linoleic acid does not appear to be due entirely to experimental error. Oleic and linoleic acid combined constitute ca. 97% of the fatty acids in 2-MG, and an error in estimating either acid would introduce an error of similar magnitude in the other. Levels of TG oleic acid accounted for 86% (r^2) of the variability in the proportion of oleic acid esterified in the 2-position.

The values obtained with *A. villosulicarpa* were distinctly different from those obtained with selections of *A. hypogaea*. These species are not closely related and are not cross fertile. The data from *A. villosulicarpa* were not included in the analysis of data but are included in Figure 1.

The low levels of other fatty acids found in the 2-position are in agreement with values reported in the literature. We examined one sample of commercially refined oil (data not given) and found slightly higher levels of stearic and long chain acids in the 2-position. During refining, peanut oil is usually subjected to a brief alkaline wash to remove nonesterified acids. This step may promote a low degree of acyl migration, and could account for the slightly higher levels of long chain acids sometimes found in the 2-position of refined oils. Acyl migration may also occur during lipase hydrolysis, particularly if the reaction and separation techniques are not carried out rapidly.

In peanut oil the unsaturation is due almost entirely to oleic and linoleic acid, and degree of unsaturation may be expressed as a ratio of these acids. The linoleic/oleic ratio among cultivars ranged from 0.2-1.1 for TG, 0.2-2.6 for 2-MG, and from 0.1-0.6 for the 1(3)-positions (Table I).

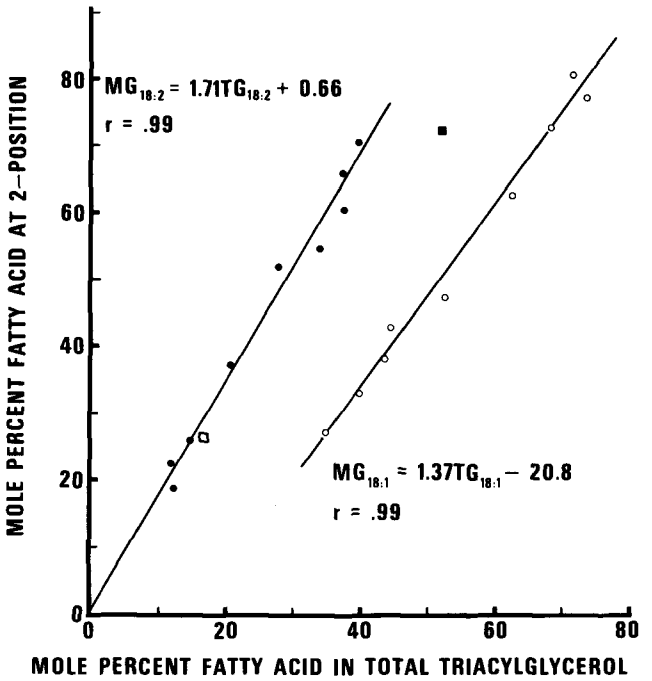

FIG. 1. Percentage of oleic (○,□) and linoleic (●,■) on the *sn*-2-position of triacylglycerols vs. the percentage in total triacylglycerols. (○,●) *A. hypogaea*, (□,■) *A. villosulicarpa*.

The molar concentrations of linoleic acid esterified in the 1(3)-positions likewise varied widely and ranged from ca. 7-26%.

If the unusual atherogenicity of peanut oil is related to TG structure, i.e., to low concentration of linoleic acid in the 1(3)-positions of TG and to linoleic/oleic ratio in the 2-position, then one would expect cultivars 37, 50, and 80 to show reduced levels of this characteristic as compared to oils that have been tested (10-17). The 2-MG linoleic-oleic ratio of variety 37 approaches that of corn oil (Table II), and this ratio in 37, 50, and 80 is much higher than that of randomized peanut oil reported to be no more atherogenic than corn oil (16). The molar concentration of linoleic acid in the 1(3)-positions of these cultivars is only marginally lower than that reported for randomized peanut oil (Table II). Varieties 54, 58 and 60 represent the other extremes in these parameters, and if TG structure is a factor, these varieties should be more atherogenic than peanut oils tested thus far (10-17).

In view of current understanding of the processes that occur during the digestion, absorption, and resynthesis of TG, it is not readily apparent how the relative proportions of linoleic acid in the 2 vs. 1(3)-positions of peanut oil TG or how the concentrations of certain TG molecular species could account for the unusual atherogenicity of this oil. During the digestion of TG, 2-MG and unesterified ("free") fatty acids derived from the 1(3)-positions are produced and are the molecular species absorbed from the intestinal tract. The integrity of the 2-MG is maintained during digestion and absorption, and during resynthesis of chylomicron TG (18,19), and it would seem, therefore, that the molecular species to be considered are the 2-MG and the molar concentrations of fatty acids in the nonesterified fatty acid fraction derived from the 1(3)-position of TG, rather than the distribution of specific TG molecular species within the native oil. While the random rearrangement of peanut oil TG fatty acids does increase the concentration of linoleic acid in positions 1(3) (Table II), the concentration of long chain acids in the 2-position is also increased. The levels of linoleic acid in positions 1(3) were increased to 27.7 mole percent by randomization (16), a value that is only marginally higher than the 22.6 mole percent estimated for an oil shown by Kritchevsky, et al. (16) to be atherogenic, and it seems unlikely that this difference alone could account for the observed difference in atherogenic potential. In this connection it should also be noted that rapeseed oil is relatively nonatherogenic (24), although it contains much lower concentrations (10-15%) (1) of polyenoic acids in the 1(3)-positions of TG than does the native peanut oils shown to be atherogenic.

Vesselinovitch et al. (15) suggested that aflatoxin might be responsible for the unusual atherogenic potential of peanut oil; howver, the addition of aflatoxin to peanut oil failed to produce quantitative or qualitative differences in atherosclerosis in Rhesus monkeys (25). Other active compounds may be present in the non-TG fraction of peanut oil, however, and it is possible that these compounds would be inactivated or removed under the conditions employed in the randomization of oil. The atherogenic potential of this fraction of peanut oil should be investigated.

TABLE II

Triacylglycerol Structure of Atherogenic and Nonatherogenic Oils

Sample	Position	Fatty acid (mole %) 18:1	18:2	18:2/18:1	Reference
Peanut oil[a]	1,2,3	50.8	27.8	.55	17
	2	50.8	46.2	.91	
	1(3)	50.8	18.7	.37	
Peanut oil[a]	1,2,3	46.1	35.6	.77	16
	2	42.4[c]	61.5[c]	1.45	
	1(3)	47.9[c]	22.6[c]	.47	
Corn oil[b]	1,2,3	25.7	56.7	2.21	17
	2	24.4	67.6	2.77	
	1(3)	25.9	51.0	1.97	
Randomized Peanut oil[b]	1,2,3	50.9	27.9	.55	17
	2	50.9	27.9	.55	
	1(3)	50.9	27.9	.55	

[a]Atherogenic.
[b]Nonatherogenic.
[c]Values estimated from equations, Figure 1.

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REFERENCES

1. Mattson, F.H., and R.A. Volpenhein, J. Biol. Chem. 236:1891 (1961).
2. Mattson, F.H., and R.A. Volpenhein, J. Lipid Res. 4:392 (1963).
3. Brockerhoff, H., and M. Yurkowski, Ibid. 7:62 (1966).
4. Weber, E.J., I.A. de la Roche, and D.E. Alexander, Lipids 6:525 (1971).
5. Fatemi, S.H., and E.G. Hammond,Ibid. 12:1032 (1977).
6. Sanders, T.H., Ibid. 14:630 (1979).
7. Tsuda, S., Yukagaku 11:408 (1962).
8. Litchfield, C., "Analysis of Triglycerides," Academic Press, New York, NY, 1972, pp. 196, 249.
9. Gresham, G.A., and A.N. Howard, Br. J. Exp., Pathol. 41:395 (1960).
10. Scott, R.F., E.W. Morrison, W.A. Thomas, R. Jones, and S.C. Nam, Exp. Mol. Pathol. 3:421 (1964).
11. Imai, H., K.T. Lee, S. Pastori, E. Ponlilio, R. Florantin, and W.A. Thomas, Ibid. 5:273 (1966).
12. Kritchevsky, D., S.A. Tepper, D. Vesselinovitch, and R.W. Wissler, Atherosclerosis 14:53 (1971).
13. Kritchevsky, D., S.A. Tepper, H.K. Kim, J.A. Story, D. Vesselinovitch, and R.W. Wissler, Exp. Mol. Pathol. 24:375

(1976).
14. Scott, R.F., E.S. Morrison, J. Jarmolych, S.C. Nam, M. Kroms, and F. Coulston, Ibid. 7:11 (1967).
15. Vesselinovitch, D., G.S. Getz, R.H. Hughes, and R.W. Wissler, Atherosclerosis 20:303 (1974).
16. Kritchevsky, D., S.A. Tepper, D. Vesselinovitch, and R.W. Wissler, Ibid. 17:225 (1973).
17. Myher, J.J., L. Marai, A. Kuksis, and D. Kritchevsky, Lipids 12:775 (1977).
18. Raghavan, S.S., and J. Ganguly, Biochem. J. 113:81 (1969).
19. Kayden, H.J., J.R. Senior, and F.H. Mattson, J. Clin. Invest. 46:1695 (1967).
20. Worthington, R.E., and R.O. Hammons, JAOCS 54:105A (1977).
21. Carrol, K.K., Ibid. 40:413 (1963).
22. Hokes, J.C., Factors Affecting the Oxidative Stability of Oils From Various Peanut Cultivars, M.S. Thesis, University of Georgia, Athens Georgia, 1977.
23. Gunstone, F.D., JAOCS 55:903 (1978).
24. Kritchevsky, D., H.K. Kim, and S.A. Tepper, Atherosclerosis 15:101 (1972).
25. Wissler, R.W., D. Vesselinovitch, J. Borensztajn, T. Schaffner, and R. Hughes, Fed. Prof. 35(3):294 (1976).

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