Structure of Unsaturated Vegetable Oil Glycerides: Direct Calculation From Fatty Acid Composition[1]

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Abstract

Composition and structure of unsaturated glycerides of vegetable oils can be calculated directly from the fatty acid composition of the oil. Fatty acid distribution on the 2 position as normally determined by lipase hydrolysis is calculated from the composition of the whole oil by applying the following three rules in their respective order: Saturated fatty acids and those with chain length greater than 18 carbons are first distributed equally and randomly on the 1 and 3 position of the glycerol moiety; oleic and linolenic acids are treated equally, or as a unit, and distributed equally and randomly on all three glyceride positions with any excess from the 1 and 3 position being added to the 2 position; and all remaining positions are filled by linoleic acid. Remarkably good agreement between the calculated and experimentally determined fatty acid distributions is shown for soybean, linseed, safflower and many other vegetable oils whose compositions are reported in the literature. An association between oleic and linolenic acid within the glyceride structure of some vegetable oils is evident.

Introduction

Random distributions along with various modifications have been proposed for the fatty acid positional structure of vegetable oil triglycerides (8,10,18,22,28, 29,33,35). Statistical random distribution based solely on the concentration of the fatty acids in the whole oil was demonstrated to be in error by changes in physical properties of a catalytically randomized fat (23). A modified random distribution that restricted the saturated fatty acids in vegetable oils to the primary hydroxyl positions on the glycerol moiety was first proposed by Richardson as cited by Mattson and Volpenhein (22) and by Vander Wal (33). This restricted randomization theory has been formalized as the 1,3-random-2-random theory by the work of Vander Wal (34,35), Coleman (4,5) and others (6,17,32,36).

Proof of the 1,3-random-2-random theory is based on the lipase hydrolysis technique, which allows an accurate fatty acid analysis to be made of those acids attached at the secondary hydroxyl position. This specific analysis allows the calculation of the total composition of both primary positions but does not distinguish between the 1 and 3 position. Lipase hydrolysis investigations indicate a distinct specificity of the C_{16} and C_{18} saturated fatty acids in vegetable oils for the primary positions of the glycerol molecule (20,26). The specificity of the saturated acids forces a proportionally higher concentration of the unsaturated acids into the 2 position. Specific distribution patterns with respect to all three glyceride positions for individual unsaturated fatty acids in vegetable oils have not been reported, except for corn oil (2).

Brockerhoff (2) reported that the distribution of the major fatty acids in corn oil between position 1 and 3 is almost random and that linolenic acid is present in all three positions. Savory and Desnuelle (25) proposed that two enzymes are operative in plants, one enzyme under specific conditions will esterify glycerol with oleic, linoleic and linolenic acids at the 2 position, and the other is a nonspecific enzyme which will indiscriminately esterify with all acids on the 1 and 3 positions. Gunstone (13) in his review attributes to Hilditch the theory that oleic and other unsaturated acids are preferentially esterified at the 2 position and that the remaining acids become associated in some manner with the 1 and 3 positions.

Mattson and Volpenhein (22) suggest that oleic, linoleic and linolenic acids have a common pattern of distribution, which is random in all positions on the glycerol molecule that are not occupied by Category I Acids. Category I Acids are defined as those acids that preferentially occupy the 1 and 3 position. Except for a few discrepancies which are marked, a deviation of about 10% from theory was reported for oleic and linoleic acid in the 2 position. Gunstone et al. (14) recently reported that in *Jatropha oils* unsaturated C_{18} acids occupy the 2 position with linoleic acid taking a slight precedence over oleic and linolenic acid. Earlier Gunstone (13) had concluded that in vegetable oils the secondary hydroxyl is preferentially acylated with unsaturated C_{18} acids and that the two primary hydroxyl groups are acylated subsequently with saturated acids and with unsaturated C_{18} acids not required at the 2 position. In three theories on glyceride structure which Gunstone (13) examined, the first requirement was that the 2 position hydroxyls are preferentially acylated by unsaturated C_{18} acids.

Glyceride structure calculations are complicated especially when restrictions are placed on specific acids or on glyceride positions. In vegetable oils, it is possible for the first time to calculate the fatty acid distribution directly from the fatty acid analysis of the whole oil. Calculated distributions are based on three simple rules and the results agree exceptionally well with lipase hydrolysis data. Basic evidence fundamental to the reliability and validity of the calculations is presented and a theory of glyceride structure discussed.

Experimental Procedures

Calculations

Soybean, linseed and safflower fatty acid composition and the lipase hydrolysis data previously presented (12) were used for development of the method. The extensive data of Mattson and Volpenhein (21,22) on vegetable oils as well as the published data of Coleman (4), Gunstone et al. (14), Jurriens et al. (16,17) and others (3,35, Evans, unpublished data) were used to illustrate the reliability of the method. All data are used as reported and not re-

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TABLE I
Calculated Glyceride Structure

Fat	Fatty acids	Glyceride, Total	Position 1	Position 2 (Mol %)	Position 3
Soybean	16:0	10.9	5.45	0.00	5.45
	18:0	4.1	2.05	0.00	2.05
	18:1	22.5	7.50	7.50	7.50
	18:2	54.3	15.60	23.10	15.60
	18:3	8.2	2.73	2.73	2.73
Linseed	16:0	6.3	3.15	0.00	3.15
	18:0	3.6	1.80	0.00	1.80
	18:1	17.9	5.97	5.97	5.97
	18:2	16.2	3.75	8.70	3.75
	18:3	56.0	18.67	18.67	18.67

calculated to a mole per cent basis but have been normalized to total 100% for computer analysis. Calculation of glyceride structure in vegetable oils embodies three rules: (a) palmitic and stearic fatty acids and acids whose chain length is greater than 18 carbons are exclusively assigned to the 1 and 3 position and assumed to be randomly distributed; (b) oleic and linolenic acids are treated alike (or as a unit) and distributed randomly and equally in all three positions; any excess of these acids from the 1 and 3 position are added to the 2 position; (c) all remaining glyceride positions are filled by linoleic acid.

The reliability of the calculated glyceride structure method depends upon the accuracy of the fatty acid composition and upon the accuracy of the lipase determined data with which it is compared. The t-test of the mean differences between calculated and reported values for oleic, linoleic and linolenic acids was not significant, which indicated that the two methods are in agreement. Excluded from the calculations were data on linoleic acid for rape seed and palm oils, as was the single oleic acid value for the cottonseed oil. The oleic acid value for the one sample of cottonseed oil is most likely in error, while the consistently large differences observed for linoleic acid in the Cruciferae and fats with high saturate contents is in need of further investigation. Small errors either in individual fatty acid analysis, or when the total fatty acid composition does not add up to 100%, can result in large differences between the calculated and determined fatty acid distribution values. When the percentage composition of any fatty acid becomes relatively low (>5%) the accuracy of its determination is much poorer and differences between the calculated and determined structure are greatly exaggerated. Another source of difference is the authenticity and reliability of the original oil sample. The sample must be representative of the whole natural oil; compositional data of winterized, fractionated or selectively modified oil cannot be used. Nonagreement of calculated structure data in some instances indicated that the reported fatty analyses were for a modified oil.

TABLE II
Unsaturated Glyceride Structure of Vegetable Oils

Oil	Lo,[a] %	Ol + Ln, %	Ol + Ln 2 position, %	Lo to fill 2 position, %	Proportion[b] of Lo Calculated	Proportion[b] of Lo Found
Soybean	54.3	30.7	10.2	23.1	42.5	43.7
Soybean	50.3	33.8	11.3	22.0	43.7	45.2
Soybean[c]	51.0	33.0	11.0	22.3	43.8	45.0
Soy hypocotyl	57.0	28.6	9.5	23.8	41.7	43.5
Linseed	16.2	73.9	24.6	8.7	53.7	45.1
Linseed	14.5	75.6	25.2	8.1	55.8	47.4
Linseed[c]	15.0	74.0	24.7	8.6	55.6	51.0
Safflower	78.3	12.0	4.0	29.3	37.4	37.6
Safflower	80.5	10.7	3.6	29.7	36.9	37.6
Safflower[c]	75.0	14.0	4.7	28.6	38.2	38.0

[a] Ol, oleic; Lo, linoleic; Ln, linolenic.
[b] Proportion, per cent of total Lo in the 2 position (21).
[c] Data from reference 22.

TABLE III
Composition of Soybean Hypocotyl Oil

Sample	Fatty acids, % Pal[a]	St	Ol	Lo	Ln	Ol + Ln
I	12.1	3.8	13.8	55.3	14.9	28.7
II	12.8	3.6	13.6	56.4	13.5	27.1
III	11.9	3.5	12.3	55.1	17.1	29.4

[a] Pal, palmitic; St, stearic.

An example of calculations made in this manner for the fatty acid distribution in soybean and linseed oils are shown in Table I. A comparison is given in Table II between the calculated results and those obtained by lipase hydrolysis techniques for soybean, linseed and several other vegetable oils.

Discussion

Background information and justification for the rules of calculation are based on evidence obtained from studies on soybean, linseed and wheat lipids. Data from the literature show that the calculated compositions are in remarkably good agreement with experimental results for the unsaturated fatty acid distribution in vegetable oils.

Rule 1 states that the saturated fatty acids are distributed exclusively on the 1 and 3 position, and it is widely used by most investigators in glyceride structure studies (22,26). For the highly unsaturated vegetable oils, which contain only low levels of saturated fatty acids, the distribution of stearic acid can be stated as being almost exclusively in the 1 and 3 position (21,22). Our data on soybeans, safflower and linseed oils would indicate that at palmitate levels of 12% or less, palmitic acid can also be regarded as being found exclusively in the 1 and 3 position. The data of Mattson and Volpenhein (21,22) indicate that in vegetable oils with high palmitate levels the amounts in the 2 position are relatively small. Kartha (19) does not accept the specificity of pancreatic lipase for the 2 position, regarding the method as more qualitative than quantitative. However, he reports the absence of trisaturated glycerides in corn and cottonseed oils, which would be most logically explained by the absence of palmitic and stearic acids from the 2 position.

Rule 2 distributes oleic and linolenic acid equally and randomly to all positions. Perception of rule 2 developed from our compositional studies on soybean oils and soybean hypocotyl oils (Evans, unpublished data). Table III shows that soybean hypocotyl oil

TABLE IV
Preference of Linoleic Acid for the 2 Position in Vegetable Oil Triglycerides

Oil	Linoleic acid, % Total acids	Total Lo in 2 position	Total unsaturated fatty acids
Soybean	54.3	43.7	63.9
Soybean	50.3	45.2	59.8
Soybean[a]	51.0	45.0	60.7
Soy hypocotyl	57.0	43.5	66.6
Linseed	16.2	45.1	18.0
Linseed	14.5	47.4	16.1
Linseed[a]	15.0	51.0	16.8
Safflower	78.3	37.6	86.7
Safflower	80.5	37.6	88.3
Safflower[a]	75.0	38.0	84.3

[a] Data from reference 22.

has about three times more linolenate than is present in soybean oil. This increase in linolenic acid was exactly balanced by a reduction in oleic acid. This inverse relation of oleic to linolenic in soybean oil was shown by Scholfield and Bull (27) and in linseed oil by Eckey (11) who plotted the data of Painter (24) for 128 samples. Although the inverse relation between oleic and linolenic acids in soybean and linseed oils is not perfect as in soybean hypocotyl oil, the degree of association in all three oils is sufficient to indicate a biological relationship between these two fatty acids.

A preference of oleic, linoleic and linolenic acid for the 2 position has been observed (3,13,22). However, in most vegetable oils 40% to 50% of the linoleate present is in the 2 position (Table IV). Linoleate preference holds whether this acid makes up only 20% of the unsaturated acids, as in linseed oil, or 80% as in safflower oil. A preference mechanism for linoleic acid may exist, or the exact opposite may be true, i.e., that linoleic acid has the least or no preference in whatever mechanism exists in the synthesis and deposition of vegetable seed storage fats. Work on fat synthesis in vivo with isotopic tracers indicates that formation of the polyunsaturated acids in higher plants follows the formation of oleic acid (15,30). Palmitic and stearic acids are not precursors of the unsaturated C_{18} fatty acids (15). Apparently only myristic acid and saturated acids of shorter chain lengths can act as precursors of oleic acid and then only in the presence of molecular oxygen (1,15,31). Two separate pathways are postulated for synthesis of fatty acids in plants, one for saturated and the other for unsaturated acids (15,31). A satisfactory and acceptable theory on the specificity of glyceride structure in plants will have to await a clear understanding of the modes of fatty acid biosynthesis.

Since linoleate makes up such a large percentage of the fatty acids in vegetable oils, calculations based on random distributions will most likely require severe restrictions in order to obtain the rather constant 2 positional arrangement of linoleic acid. No logical explanation for the preference of linoleate for the 2 position has been found. However, when linoleic acid distribution is considered last in the calculation of glyceride structure, the results agree with the lipase hydrolysis data reported for most vegetable oils (Table V).

Table V, arranged in alphabetical order, does not show the association sometimes desired for making botanical or analytical comparisons within sets of analyses. The excellent agreement between determined values and calculated results for the *Jatropha* oils (14) gives strong support for this unique three-rule method of calculating glyceride structure in vegetable oils directly from the fatty acid analysis. Although all the published data by Mattson and Volpenhein (21,22) are not included, the agreement is very good for soybean, cottonseed, corn, linseed and other common vegetable oils. The lipase distribution data of Jurriens and Knoesen (17) do not agree as well to calculated distributions as other published data, but neither do their lipase data agree well with literature values. Our data on linolenate distribution (12) within the various unsaturated triglycerides of soybean and linseed oils agree, within experimental error, to the theoretical random distribution of 33.3%.

Fats high in saturated acids, such as palm oil, or

TABLE V

Percentage of Unsaturated Fatty Acids in the Position of Vegetable Oils

Oil	Fatty acid	Proportion[a]		Reference
		Calculated	Reported	
Acorn	Ol	33.3	33	22
	Lo	50.0	52	
Almond	Ol	33.3	30	22
	Lo	47.6	54	
Brazil nut	Ol	33.3	40	22
	Lo	58.1	50	
Cashew nut	Ol	33.3	31	22
	Lo	74.1	78	
Cocoa butter	Ol	86.7	81	21
	Lo	100.0	122	
Cocoa butter	Ol	87.0	84.1	4
	Lo	100.0	100.0[b]	
Cocoa butter	Ol	85.1	84.3	17
	Lo	100.0	66.7	
Cocoa butter	Ol	86.5	81.5	3
	Lo	100.0	76.2	
Cocoa butter	Ol	82.1	77.7	
	Lo	100.0	122.0	
Corn	Ol	33.3	30	22
	Lo	41.4	41	
Corn	Ol	33.3	34.3	34
	Lo	41.4	41.7	
Cottonseed	Ol	33.3	39	22
	Lo	50.3	47	
Cottonseed	Ol	33.3	56.9	17
	Lo	53.4	42.4	
Jatropha curcas	Ol	33.3	34.6	14
	Lo	51.4	49.5	
J. multifda	Ol	33.3	32.9	14
	Lo	51.1	48.9	
J. gossypifalia	Ol	33.3	35.9	14
	Lo	39.8	38.6	
Kokum butter	Ol	90.1	86	22
Linseed	Ol	33.3	41	22
	Lo	55.6	51	
	Ln	33.3	31	
Linseed	Ol	33.3	39.7	12
	Lo	53.7	45.1	
	Ln	33.3	33.8	
Mustard	Ol	76.7	88	21
	Lo	100.0	87	
	Ln	76.7	74	
Palm fruit	Ol	60.7	56	22
	Lo	100.0	81	
Palm oil (Sumatra)	Ol	62.0	52.7	17
	Lo	100.0	65.0	
Palm oil (Congo)	Ol	58.0	56.6	16
	Lo	100.0	66.4	
Peanut	Ol	33.3	33	21
	Lo	55.6	58	
	Ln	33.3	0	
Peanut	Ol	33.3	27.8	17
	Lo	50.3	53.0	
	Ln	33.3	44.4	
Rapeseed	Ol	50.9	56	21
	Lo	100.0	82	
	Ln	50.9	55	
Rapeseed	Ol	58.3	63	21
	Lo	100.0	80	
	Ln	58.3	67	
Rice bran	Ol	33.3	35	22
	Lo	50.0	47	
	Ln	33.0	33	
Safflower	Ol	33.3	31.7	12
	Lo	37.5	37.6	
Safflower	Ol	33.3	31	22
	Lo	38.2	38	
Soybean	Ol	33.3	29	22
	Lo	43.8	45	
	Ln	33.3	33	
Soybean	Ol	33.3	30.4	4
	Lo	46.2	45.0	
	Ln	33.3	30.4	
Soybean	Ol	33.3	37.1	17
	Lo	42.4	41.2	
	Ln	33.3	23.3	
Soybean	Ol	33.3	30.0	12
	Lo	42.5	43.7	
	Ln	33.3	31.7	
Soybean hypocotyl	Ol	33.3	31.6	12
	Lo	41.7	43.4	
	Ln	33.3	26.7	
Stillingia tallow	Ol	100.0	102	22
Sunflower	Ol	33.3	28	22
	Lo	38.0	40	
Tomato seed	Ol	33.3	35	22
	Lo	42.0	40	
	Ln	33.3	33	
Walnut	Ol	33.3	38	22
	Lo	39.3	39	
	Ln	33.3	30	
Wheat flour	Ol	33.3	27	22
	Lo	37.8	39	
	Ln	33.3	29	
Wheat germ	Ol	33.3	33	22
	Lo	45.4	45	
	Ln	33.3	29	

[a] Proportion, per cent of total Lo in the 2 position (20).

[b] Assumed Lo at 2%.

those high in category I acids (22), like the Cruciferae, have almost all the linoleate in the 2 position. Calculated values for linoleate may show its distribution in the 2 position up to 100%. The difference in linoleate distribution between calculated and found is not so great as might be expected since the actual distribution in the Cruciferae differs markedly from that in other vegetable oils. Lipase data show that in Cruciferae oils about 80–90% of the linoleate is in the 2 position compared to 40–50% for other vegetable oils. In spite of the differences for linoleate in the 2 position, distribution of the other unsaturated acids in the Cruciferae oils agrees remarkably well with the lipase hydrolysis data. This agreement would indicate that in Cruciferae either all the category I acids, which includes erucic acid, are not distributed exclusively on the 1 and 3 position or that the fatty acids are distributed in a manner different from most of the other vegetable oils. Craig (7) has shown a linear relationship between oleic and erucic acids in a varietal study of rapeseed. Downey and Craig (9) reported the same relationship in a genetic study where the erucic acid content was at different levels. These latter authors observed no regular variation for either the saturated acids or the linolenic acid throughout the genetic series. Such relationships might indicate that a different biosynthetic fatty acid mechanism exists for the Cruciferae.

Vegetable oils are usually characterized by high percentages of linoleic acid. Poorer agreement shown between calculated and determined results for the low level linoleic acid oils would indicate the need of further investigations with considerable emphasis being placed on an accurate determination of the distribution of linoleic acid.

Plant butters and tallows also offer special examples where saturated acids are more than sufficient to fill the 1 and 3 position. Calculated fatty acid distributions show excellent agreement with the lipase distribution data for cocoa butter, kokum butter and stillingia tallow.

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