The Component Triacylglycerols of Avocado Fruit-Coat[1]

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ABSTRACT

The component triacylglycerols of avocado fruit-coat fat have been determined by thin layer argentation chromatography and by pancreatic lipolysis. The fat was found to contain a total of 33 triacylglycerols (10 major and 23 minor) belonging to triunsaturated (43.0%), diunsaturated-monosaturated (44.1%), disaturated-monounsaturated (12.4%) and trisaturated (0.5%) types of acylglycerols. The results of the distribution of individual fatty acids show a 1,3-random-2-random distribution pattern. Among the unsaturated fatty acids, linoleic acid was esterified at the 2 position with greater preference than either oleic or palmitoleic acid.

INTRODUCTION

Persea americana is a subtropical fruit of the Lauraceae family. The fat content is usually about 20% although variation from 5-30% fat has been reported (1). The main fatty acids of avocado fat are palmitic ($C_{16:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids (2). The fat also contains a significantly high proportion of palmitoleic acid ($C_{16:1}$), 7-10%, which is uncommon among the fats of higher flora (2).

Although avocado mesocarp has been used to study the biosynthesis of long chain fatty acids by Squires and coworkers (3) and Stumpf and Barber (4), the triacylglycerol (TAG) composition of this fat has not been previously reported. A study of the structure of avocado TAGs was therefore considered important for the better understanding of the mechanism of the biosynthesis of avocado lipids. The present communication reports the component TAGs and positional distribution of fatty acids in TAGs as determined from the data on pancreatic lipase hydrolysis and fatty acid composition. Since fatty acids higher than C_{18} or lower than C_{16} are not present, avocado fat provides a good model for study of the distribution of C_{16} and C_{18} fatty acids in TAGs.

MATERIALS AND METHODS

Reagents

All chemicals used during extraction, isolation and purification were reagent grade. Reference TAGs, diacylglycerols, monoacylglycerols, methylesters of fatty acids for gas liquid chromatography (TLC) and thin layer chromatography (TLC) were purchased from Mann Research Laboratory, New York. Bio-Sil-H silicic acid (100 mesh) was obtained from Bio-Rad LaboLaboratories, Richmond, Calif. Oxgall and steapsin were obtained from Sigma Chemical Co., St. Louis, Mo. Miami (Florida) grown Taylor variety of avocado was purchased from the local food store.

Preparation of Triacylglycerols

Exposure of lipids to oxygen was minimized by the use of a nitrogen atomsphere during extraction and purification.

The fresh and ripe avocado was peeled, the seed removed and about 200 g of the fleshy mesocarp was extracted by

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TABLE I

Fatty Acid Composition Triacylglycerol (TAG) Fractions, Monoacylglycerols From Lipase Hydrolysis, and Proportion of Each Fatty Acid in 2 Position

	Double bonds per molecule		Amount of fraction,[a] %		Fatty acid, mole %				
	Found[b]	Calc.[c]	Found	Calc.[d]	16:0	18:0	16:1	18:1	18:2
Whole TAG					23.0	0.5	8.6	55.6	12.3
2-Monoacylglycerol					5.0		4.0	70.0	21.0
Proportion[e]					7.0		16.0	42.0	57.0
TAG fraction	0	0	2.0	0.5	100.0		---	---	---
2-Monoacylglycerol					100.0		---	---	---
Proportion					33.0		---	---	---
TAG fraction	1	1	9.6	9.2	65.5		3.4	30.4	---
2-Monoacylglycerol					11.9		5.2	83.0	---
Proportion					6.0		51.0	88.0	---
TAG fraction	2	2	33.0	31.9	37.9		3.1	57.4	1.7
2-Monoacylglycerol					3.2		4.1	74.7	17.9
Proportion					3.0		44.0	43.0	---
TAG fraction	3	3	35.8	37.8	12.9		7.4	70.0	9.7
2-Monoacylglycerol					10.1		5.3	55.3	29.3
Proportion					26.0		24.0	26.0	101.0
TAG fraction	4+	3.4	17.8	17.0	5.8		10.4	66.4	17.7
2-Monoacylglycerol					3.0		3.4	36.9	56.7
Proportion					17.0		11.0	19.0	107.0

[a]TAG fractions were separated according to the number of double bonds per TAG molecule by Ag^+ TLC.

[b]Found by comparison of the R_f values obtained by thin layer chromatography with the R_f values of known TAGs.

[c]Calculated on the basis of fatty acid composition.

[d]See Table II.

[e]Proportion represents the percentage of fatty acids esterified in the 2 position of the TAG molecule. It was calculated by the equation: Proportion = 2 position x 100/whole TAG x 3. (11).

TABLE II

Component Triacylglycerols (TAGs) of Avocado Fruit-Coat Fat

	Minor TAGs				Major TAGs		
TAG[a]	Type	Mole %		TAG	Type	Mole %	
PPP[b]	SSS	0.5	0.5	POP	SUS	7.2	9.3
PPO	SSU	1.6		PLP	SUS	2.1	
PPP_O	SSU	0.4	2.3	POP_O	SUU	4.8	
PPL	SSU	0.3		POO	SUU	21.8	36.7
PP_OP	SUS	0.4	0.4	POL	SUU	3.6	
PP_OP_O	SUU	0.3		PLO	SUU	6.5	
PP_OO	SUU	1.2		P_OOO	UUU	7.4	
PP_OL	SUU	0.2	4.1	OOO	UUU	16.4	34.1
PLP_O	SUU	1.4		OOL	UUU	5.4	
PLL	SUU	1.0		OLO	UUU	4.9	
P_OPO	USU	0.5					
OPO	USU	1.2	2.1	Total,			80.1
OPL	USU	0.4					
P_OOP_O	UUU	0.8					
P_OP_OO	UUU	0.4					
OP_OO	UUU	0.9					
OP_OL	UUU	0.3					
P_OOL	UUU	1.2	7.6				
LOL	UUU	0.4					
P_OLP_O	UUU	0.5					
OLL	UUU	1.6					
P_OLO	UUU	1.1					
P_OLL	UUU	0.4					
Total,			17.0				

[a]Calculated by the method of Coleman (18).

[b]Abbreviations: P = palmitic acid; P_O = palmitoleic acid; O = oleic acid; L = linoleic acid; SUS = 1-saturated, 2-unsaturated, 3-saturated; SUU = 1-saturated, 2-unsaturated, 3-unsaturated; plus 1-unsaturated, 2-unsaturated, 3-saturated; UUU = 1-unsaturated, 2-unsaturated, 3-unsaturated; SSS = 1-saturated, 2-saturated, 3-saturated; SSU = 1-saturated, 2-saturated, 3-unsaturated plus 1-unsaturated, 2-saturated, 3-saturated; and USU = 1-unsaturated, 2-saturated, 3-unsaturated.

homogenization in a Waring Blendor with four volumes of chloroform-methanol 2:1 v/v. The homogenate was allowed to stand for 60 min at room temperature and then filtered under suction. The extract was washed with 0.7% aqueous solution of NaCl to remove the water soluble nonlipid components (5). The chloroform layer was evaporated in a rotary evaporator at 37 C and immediately taken up in a small portion of chloroform-methanol 2:1 v/v. Separation of lipid classes was carried out on a silicic acid column (18 x 1 in) using stepwise elution with petroleum ether-diethylether solvent mixtures (6). The TAG fraction obtained by this method was further purified by preparative TLC on 20 x 20 cm glass plates coated with a 1.0 mm layer of Silica Gel G (E. Merck, Darmstadt, Germany) and using a solvent system of petroleum ether(b.p. 30-60 C)-diethylether 4:1 v/v (7).

Argentation TLC of Triacylglycerols

Preparative silver ion (Ag^+) TLC of TAGs was carried out essentially according to the procedure described by Barrett et al. (8). Glass plates (20 x 20 cm) coated with Silica Gel G (0.5 mm) containing 12.5% aqueous $AgNO_3$ w/v were used for separations with a solvent system of 1% ethanol in alcohol-free chloroform v/v. After development the plate was dried under N_2 and sprayed lightly with a 0.2% solution of 2', 7'-dichlorofluorescein in 95% ethanol w/v. Bands were located in the long wavelength region with the aid of an UV lamp and scraped off into small chromatography columns containing ca. 1 g of silicic acid which removed any fluorescein present in the lipid fractions. TAGs were eluted from the columns with 100 ml of dry diethyl ether. The solvent was evaporated to dryness with a jet of N_2 and residual TAG fraction was stored at -10 C. The amount of TAG in each fraction was determined by the method of Van Handel (9) using purified mahua *Madhuca butyracea*, Sapotaceae) oil as standard.

Gas Liquid Chromatography

The fatty acid composition of TAGs was determined by GLC analysis of the methyl esters of the constituent fatty acids prepared by transmethylation of 10-50 mg of the TAGs with sodium methoxide in anhydrous methanol. GLC analyses were carried out on an Aerograph gas chromatograph equipped with a thermal conductivity detector and a 76 x 0.4 cm (ID) copper column containing 18% Hi-EFF-IBP (diethylene glycol succinate polyester) on 60-80 mesh Chromosorb W (Applied Science Laboratories, State College, Pa.). The column was operated isothermally at 170-180 C with a helium carrier gas flow of 70 ml/min. Peaks were identified by using known fatty acid methyl esters as reference standards. The quantitative response factors of various methyl esters of fatty acids were determined by chromatography of reference mixtures of known composition under similar operating conditions. Peak areas were measured by triangulation and the fatty acid composition was expressed in mole per cent.

Pancreatic Lipase Hydrolysis

The lipase hydrolysis of triacylglycerols was carried out by the procedure of Mattson and Volpenhein (10). The hydrolyzed lipids were extracted with diethylether, washed thrice with water and dried over anhydrous sodium sulfate. The extract was reduced to dryness with a jet of N_2. The residual lipids were dissolved in a suitable volume of petroleum ether and separated by TLC using 0.5 mm layer Silica Gel G preparative plates (20 x 20 cm) and diethylether-petroleum ether-acetic acid 67:31:2 v/v/v as the developing solvent. The monoacylglycerol band was located by fluorescence under short wave UV light, scraped off the TLC plate and eluted with ether and methanol. The solvent was removed in a stream of N_2. The fatty acid composition of the monoacylglycerols was determined by GLC. Procedure and conditions of analysis were the same as those used for the analysis of the fatty acid composition of TAGs.

RESULTS AND DISCUSSION

Composition and Positional Distribution of Fatty Acids

Fatty acid composition of the whole TAG, TLC-TAG

TABLE III

Component Triacylglycerol (TAG) Types in Avocado Fruit-Coat

Calculation method	TAG type, mole %				Isomers, mole %			
	S_3[a]	S_2U	SU_2	U_3	SUS[b]	SSU	USU	UUS
Coleman (18)	0.5	11.9	43.7	41.7	9.7	2.2	2.1	40.8
Vander Wal (23)	0.5	12.4	44.1	43.0	10.2	2.2	2.3	41.8
Gunstone (22) (Theo. 1)	---	11.9	44.5	42.0				

[a]Abbreviations: S_3, S_2U, SU_2 and U_3 represent trisaturated, disaturated-monounsaturated, diunsaturated-monosaturated and triunsaturated respectively.

[b]For abbreviations see fn.[b] Table II.

fractions and 2-monoacylglycerols in avocado is given in Table I. The original fat shows a high proportion of unsaturated fatty acids (76.5%) which is characteristic of vegetable fats. Among the unsaturated acids, oleic acid (55.6%) is found in the highest proportion followed by linoleic acid (12.3%) and palmitoleic acid (8.6%). Palmitic acid (23%) is the only major saturated fatty acid present in avocado fat. Stearic acid is present only in trace amounts. These data are within the range of the literature values (2).

The proportion of each fatty acid esterified at the 2 position of the alcohol component in TAGs was calculated by the method of Mattson and Volpenhein (11) from the fatty acid composition of 2-monoacylglycerols obtained by pancreatic lipase hydrolysis (12). Results (Table I) clearly show that both $C_{16:0}$ and $C_{18:0}$ acids are preferentially esterified at the 1 and 3 positions in the whole TAG. This is in agreement with the general distribution pattern of the saturated fatty acids reported for other vegetable fats (11,13,14). About 5.0% of the total saturated fatty acids are also found in the 2 position.

The proportion of $C_{18:1}$ and $C_{18:2}$ acids in the 2 position in the whole TAG are 42.0% and 56.9% respectively. These values are significantly higher than 33.3% indicating that the distribution is nonrandom. The proportionally higher concentration of $C_{18:1}$ and $C_{18:2}$ in the 2 position has been ascribed to the fact that the specific distribution of saturated fatty acids ($C_{16:0}$ in this case) in 1 and 3 position forces a higher proportion of $C_{18:1}$ and $C_{18:2}$ acids in the 2 position of the TAG molecule (11,15). Thus fatty acids in avocado TAGs appear to follow a 1,3-random-2-random distribution pattern (10) which is a characteristic of the vegetable oils (11,14,16-19).

Avocado fat was separated according to the degree of unsaturation by argentation TLC. Five TAG fractions were isolated and based on the comparison of R_f values obtained by TLC with the R_f values of known TAGs, the TAG fractions were characterized as containing 0, 1, 2, 3 and 4 double bonds per molecule. Results are listed in Table I. Excellent agreement was obtained between the amounts of individual fractions of TAGs and those predicted by calculation in the whole TAG assuming a 1,3-random-2-random distribution. The number of double bonds per TAG molecule in the individual TAG fractions as determined by TLC is in good agreement with those calculated on the basis of fatty acid composition. The only exception appears to be in the 4 double bond TAG fraction which is different from the value of 3.4 double bonds per TAG molecule calculated on the basis of fatty acid composition; this could be accounted for by a partial overlap of the bands containing 4 double bond and 3 double bond TAG fractions in the TLC.

The positional distribution of fatty acids in individual fractions was also determined and compared with that of the whole TAG. Results are given in Table I. As expected, $C_{16:0}$ acid distribution in all the fractions was in good agreement with the pattern found in the case of whole TAG. Monoenoic acids ($C_{16:1}$ and $C_{18:1}$) appear to follow an identical distribution pattern in all fractions and, like whole TAG, in fractions containing one or two double bonds per molecule the proportions of these acids in the 2 position are significantly greater than 33.3% which should be expected if the distribution were random. In contrast to these results, fractions containing 3 or more double bonds per molecule showed less than 33.3% of monoenoic acids in the 2 position.

In this connection it is interesting to note that almost 100% of the $C_{18:2}$ acid is found in the 2 position. Furthermore as the molar concentration of $C_{18:2}$ acid increases in TAG fractions, the proportion of $C_{16:1}$ and $C_{18:1}$ acids decreases (Table I). These results are interpreted to mean that $C_{18:2}$ acid takes precedence over $C_{16:1}$ and $C_{18:1}$ acids and forces their distribution in favor of the 1 and 3 positions. That $C_{18:2}$ acid is acylated at the 2 position more effectively than other C_{18} unsaturated acids ($C_{18:1}$ and $C_{18:3}$) has also been reported for *Jatropha* (20) and rubber seed oils (21) but to our knowledge the influence of $C_{18:2}$ on the distribution of $C_{16:1}$ acid has not been reported before. The mechanism for this preferential acylation of $C_{18:2}$ acid at the 2 position is still unknown.

Component Triacylglycerols

The component TAGs of the avocado fruit-coat have been calculated from the results of the fatty acid composition of the whole TAG, lipase hydrolysis and Ag^+ TLC by the methods employed by Coleman (18), Gunstone (22) and Vander Wal (23). Results obtained by these methods appear in Tables II and III. It was found that 10 major TAGs are present in avocado fat (Table II). About 80% of the total TAG is composed of the following major glycerides: POP (7.2%), PLP (2.1%), POP_O (4.8%), POO (21.8%), POL (3.6%), PLO (6.5%), P_OOO (7.4%), OOO (16.4%), OOL (5.4%) and OLO (4.9%). (For abbreviations see fn.[b] Table II.) Seventeen per cent of total TAGs is formed by 23 minor TAGs (Table II) with unsaturation varying from 0 to 4 (or more) double bonds per TAG molecule. About 3% of the oil is unaccounted for. Thus, out of possible 64% TAGs avocado fat contains 33. Unsaturated TAG with saturated fatty acids in the 2 position constitutes only a small fraction (less than 5%) of the avocado fat. This is in agreement with the general pattern that vegetable oils contain a very small fraction, if any, of trisaturated TAGs (22).

The types of TAGs found in avocado fruit-coat are SSS, SUS, SSU, USU, SUU and UUU (Table II). The composition of each type of TAG calculated by various methods is given in Table III. The results are in excellent agreement. From these data it is apparent that disaturated-monounsaturated acylglycerols (S_2U) should be mainly of the symmetrical type (SUS), and the monosaturated-diunsaturated acylglycerols (SU_2) should be of the unsymmetrical type (SUU).

Although results reported here suggest 1,3-random-2-random distribution pattern, the 1-random-2-random-3-

random distribution cannot be ruled out because 1, 3 positions in TAGs appear to contain unequal proportions of fatty acids (24). However, further investigations of the stereospecific distribution of fatty acids in 1,3 and 2 positions in vegetable TAGs are needed in order to understand fatty acid distribution in vegetable fats.

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