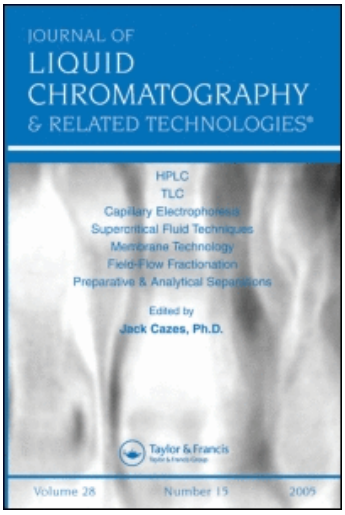

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Kathryn Horutz[a]; Lawrence R. Layman[b]; Bernard Fried[a]; Joseph Sherma[b]
[a] Department of Biology, Lafayette College, Easton, Pennsylvania [b] Department of Chemistry, Lafayette College, Easton, Pennsylvania

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HPLC DETERMINATION OF TRIACYLGLYCEROLS IN THE DIGESTIVE GLAND-GONAD COMPLEX OF *BIOMPHALARIA GLABRATA* SNAILS FED HEN'S EGG YOLK VERSUS LEAF LETTUCE

KATHRYN HORUTZ[1], LAWRENCE R. LAYMAN[2], BERNARD FRIED[1], AND JOSEPH SHERMA[2]
[1]*Department of Biology*
[2]*Department of Chemistry*
Lafayette College
Easton, Pennsylvania 18042

ABSTRACT

HPLC was used to analyze triacylglycerols in the digestive gland-gonad (DGG) complex of the planorbid snail Biomphalaria glabrata fed either hen's egg yolk or leaf lettuce. Both lettuce and yolk samples were also analyzed for their triacylglycerol composition. Identification of specific triacylglycerols was made according to retention times that corresponded to those of triacylglycerol standards. Triacylglycerols identified in the hen's egg yolk were rac-glyceryl-1,2-oleate-3-stearate (OOS), trilinolein, and rac-glyceryl-1,3-palmitate-2-oleate (POP). Triacylglycerols from snails fed leaf lettuce were identified as rac-glyceryl-1-oleate-2,3-stearate (OSS), OOS, trilinolein, and rac-glyceryl-1-oleate-2,3-palmitate (OPP), whereas those from snails fed hen's egg yolk were identified as OSS, trilinolein, PSO, and POP.

INTRODUCTION

Determination of the lipid components in the digestive gland gonad complex (DGG) and hemolymph of the planorbid snail Biomphalaria glabrata has been the focus of recent research in our laboratory. Thin layer chromatography (TLC) has been the major

technique used to analyze lipids in the DGG and hemolymph of this snail (1). Studies have focused on the separation of phospholipids in the DGG (2), cholesterol esters in the DGG (3), and sterols in the hemolymph (4) by TLC. A recent study involved separation of triacylglycerols in snail DGG by silica gel, reversed phase, and argentation TLC (5). Separation and determination of triacylglycerols in the DGG by high performance liquid chromatography (HPLC) and comparison to TLC are the subjects of this study.

Triacylglycerol standards with known partition numbers (PN) were used to identify triacylglycerols based on their retention times. Partition numbers are inversely related to retention times and can be calculated using the following formula: PN = CN-2m, where CN denotes the carbon number and m the number of double bonds in the triacylglycerol molecule (6,7).

EXPERIMENTAL

Standards

The following triacylglycerol standards were obtained from Sigma (St. Louis, MO USA): tristearin, tripalmitin, triolein, trilinolein, and trimyristin. Standards obtained from Matreya Inc. (Pleasant Gap, PA USA) were as follow: rac-glyceryl-1,3-palmitate-2-oleate (POP), rac-glyceryl-1,2-oleate-3-stearate (OOS), rac-glyceryl-1-palmitate-2-stearate-3-oleate (PSO), rac-glyceryl-1-oleate-2,3-stearate (OSS), rac-glyceryl-1-palmitate-2-oleate-3-stearate (POS), rac-glyceryl-1-oleate-2,3-palmitate (OPP), rac-glyceryl-1.3-stearate-2-oleate (SOS), and rac-glyceryl-1-stearate-2,3-palmitate (SPP). Table 1 lists the standards, their saturation classes, double bond positions, and partition numbers.

TABLE 1

Triacylglycerol Standards, Saturation Classes (SAT), Double Bond positions (POS), and Partition Numbers (PN)

STANDARDS	PN	SAT	POS*
Triolein	48	M_3	MMM
Trilinolein	42	D_3	DDD
Tristeatin	54	S_3	SSS
Tripalmitin	48	S_3	SSS
Trimyristin	50	S_3	SSS
POS[1]	50	S_2M	SMS
OOS[2]	50	SM_2	MMS
OSS[3]	52	S_2M	MSS
OPP[4]	48	MS_2	MSS
POP[5]	48	S_2M	SMS
PSO[6]	50	S_2M	SSM
SOS[7]	52	S_2M	SMS
SPP[8]	48	S_3	SSS

* S- saturated M- monoenoic D- dienoic

1 rac-glyceryl-1-palmitate-2-oleate-3-stearate
2 rac-glyceryl-1,2-oleate-3-stearate
3 rac-glyceryl-1-oleate-2,3-stearate
4 rac-glyceryl-1-oleate-2,3-palmitate
5 rac-glyceryl-1,3-palmitate-2-oleate
6 rac-glyceryl-1-palmitate-2-stearate-3-oleate
7 rac-glyceryl-1,3-stearate-2-oleate
8 rac-glyceryl-1-stearate-2,3-palmitate

Sample Preparation

Stock cultures of Biomphalaria glabrata were maintained as previously described (8). Each culture consisted of 10 snails (shell diameter 12-15 mm) fed either leaf lettuce or hen's egg yolk ad libitum for one week (8). A total of 30 cultures were used, 15 fed leaf lettuce and 15 fed hen's egg yolk. A pool of approximately 400 mg (wet weight) of DGG was collected from each culture.

Lipids were extracted from the DGG with 5 ml of chloroform-methanol (2:1), and the non-lipid material was removed from the

extract by a Folch wash (0.88% KCl). The lipid-containing phase was dried under a stream of nitrogen at room temperature. The lipid residue was reconstituted in 2 ml of chloroform.

Preparative TLC was used to isolate the triacylglycerol fraction (9). Baker-flex IB2 silica-gel plates were developed in petroleum ether-diethyl ether-acetic acid (80:20:1). The triacylglycerol band was identified through co-migration of triolein standards on guide strips (9). The triacylglycerol band was scraped off the plate and eluted with 500 ul of chloroform through a disposable glass pipet plugged with glass wool.

Ten percent samples [500 mg/ 5 ml (2:1) chloroform:methanol] of hen's egg yolk and leaf lettuce were prepared in the same manner as described above for snails.

HPLC

Twenty ul of the isolated triacylglycerol fraction were injected into a Waters u-Bondapack C18 column attached to a Waters M-45 solvent delivery system through a Rheodyne 7125 injector with a 20 ul loop. Triacylglycerols were monitored at 208 nm using a Waters 481 LC UV detector. The mobile phase used was acetonitrile-tetrahydrofuran (7:3). The tetrahydrofuran contained no preservatives, so it was necessary to prepare fresh mobile phase each day. The solvent was eluted through the column for 10 min prior to use in order to equilibrate the system. A triolein standard was injected into the column and its retention time compared to a control value to insure that the system was working properly. Flow rate was set to 1.00 ml/minute, absorbance range 0.20, and attenuation 2.00. Chromatograms were recorded for the triacylglycerols isolated from the 10% yolk solution, the 10% leaf lettuce solution, yolk-fed snails, and lettuce-fed snails. Chromatograms were also obtained for each triacylglycerol standard.

TABLE 2

Average Retention Times (RT) in Min of Triacylglycerol peaks from Liquid Chromatograms of 10% Yolk Solution, Biomphalaria glabrata (B.g.) Fed Lettuce, B. glabrata Fed Hen's Egg Yolk, and Triacylglycerol Standards*

RT	10% yolk	B.g. fed lettuce	B.g. fed yolk
1.0			
1.5			
2.0			2.19
2.5		2.53	
3.0	3.04	3.24	3.03
3.5			
4.0			
4.5			
5.0			
5.5			
6.0			6.34
6.5		6.73	6.80
7.0			
7.5			7.57
8.0	8.29	8.01	
8.5			8.95
9.0		9.22	
9.5	9.63		
10.0			10.30
10.5		10.55	
11.0	11.26		11.41
11.5			
12.0			12.40
12.5			
13.0			
13.5	13.50		13.87
14.0		14.29	
14.5			
15.0			
15.5			15.74
16.0	16.20		
16.5			
17.0			
17.5			
18.0			
18.5			
19.0			
19.5	19.88		19.68
20.0			

*Standards (retention times in min)

1. triolein 17.70
2. trilinolein 8.19
3. tristearin 3.80
4. tripalmitin 21.80
5. trimyristin 19.11
6. PSO 8.75
7. OOS 9.42
8. OSS 6.75
9. OPP 14.13
10. POP 19.50
11. SOS 17.44
12. SPP 16.50

RESULTS

Table 2 lists the average retention times of triacylglycerols from the 10% yolk solution, the snails fed hen's egg yolk, and the snails fed lettuce. Preparative TLC of the 10% leaf lettuce sample did not show a triacylglycerol band that migrated with the same mobility as the triolein standard. HPLC confirmed the absence of triacylglycerols in the lettuce sample. Partition numbers were assigned to each peak based on the inverse relationship between RT and PN, but were not used for identification since they paralleled the retention times.

As seen in Table 2, the 8.29 min peak from the 10% yolk sample correlated with the trilinolein standard, the 9.63 min peak from the 10% yolk solution corresponded to the triacylglycerol standard OOS, and the 19.88 min peak from the 10% yolk sample corresponded to POP. The 6.73, 8.01, 9.22, and 14.29 min triacylglycerol peaks from lettuce-fed snails corresponded to OSS, trilinolein, OOS, and OPP, respectively. The 6.88, 8.98, and 19.68 min peaks in chromatograms of triacylglycerols isolated from snails fed hen's egg yolk corresponded to standard peaks of OSS, PSO and POP, respectively. Calculated standard deviations for the retention times of triacylglycerol peaks from yolk and lettuce-fed snails were approximately 0.26 min, which showed that there was little discrepancy between the retention times of peaks of individual compounds from different snail or standard chromatograms.

DISCUSSION

Results from this study indicate that the triacylglycerol composition of the DGG of the planorbid snail Biomphalaria glabrata is different depending upon its diet. This was determined by analyzing the correspondence between retention times of standard

triacylglycerols, triacylglycerols from each diet, and triacylglycerols from the DGG of snails on each diet.

Through preparative TLC and HPLC, it was determined that leaf lettuce did not contain triacylglycerols. Thus, the triacylglycerols in the DGG of snails maintained on the lettuce diet probably represent those synthesized by the snail.

As seen in Table 2, snails maintained on egg yolk showed more triacylglycerol peaks than either the egg yolk or snails maintained on lettuce. Several peaks in the 10% yolk and yolk-fed snail chromatograms had similar retention times. Likewise, several peaks from the triacylglycerols isolated from snails maintained on leaf lettuce had similar retention times to those from snails maintained on the hen's egg yolk. The only similarity between the 10% yolk solution and the lettuce-fed snails was found between the 8.29 min peak on the 10% yolk chromatogram and the 8.01 min peak on the lettuce-fed chromatogram.

The 8.29 min peak from the 10% yolk solution had a retention time similar to the trilinolein standard (Table 2). The 9.63 min peak from the 10% yolk solution had a retention time that was similar to OOS (Table 2). Previous work using argentation thin layer chromatography found PSO as the major component of triacylglycerols isolated from 10% yolk solution, lettuce-fed, and yolk-fed snails (5).

The 6.73, 8.01, 9.22, and 14.29 min peaks representing triacylglycerols isolated from snails fed lettuce were identified as OSS, trilinolein, OOS, and OPP, respectively. Previous work, using argentation thin layer chromatography, found that triacylglycerols isolated from lettuce-fed snails could be classified as either PSO or OSS (5). Triacylglycerol peaks at 6.88, 8.98, and 19.68 min from snails fed hen's egg yolk were

identified as OSS, PSO, and POP, respectively (Table 2). Masterson et al. (5), using argentation TLC, indicated that the main triacylglycerol fraction of yolk-fed snails was either PSO or OOS.

The results from this study confirm those of Masterson et al. (5) in identifying the major triacylglycerol components in the DGG complex of B. glabrata snails fed either hen's egg yolk or leaf lettuce. In that study, argentation thin layer chromatography found the major triacylglycerol components of the DGG of B. glabrata fed lettuce to be PSO or OSS, and PSO or OOS for snails fed hen's egg yolk (5). Additionally, we found trilinolein, OOS, and OPP in the lettuce-fed snails, but PSO was not identified. Also, POP was found in the yolk-fed snails, but not OOS.

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