Triglyceride Composition of Chrysalis Oil, an Insect Lipid

U.K. MAJUMDER and **A. SENGUPTA**, Department of Pharmacy, Jadavpur University, Calcutta – 700 032, India

ABSTRACT

Chrysalis oil, an insect fat obtained from the spent silk worm pupae, *Bombyx mori*, is a by-product of sericultural industry and represents a potential source of 1750 tons of linolenic-rich oil per annum for India. Fatty acid and triglyceride compositions of chrysalis oil have been determined by the combination of the techniques of lower temperature segregation, lipolysis, thin layer and gas liquid chromatography. Percentage contents of the component acids are: $C_{14:0}$, 0.6; $C_{16:0}$, 19.3; $C_{18:0}$, 3.9; $C_{18:1}$, 17.7; $C_{18:2}$, 9.8, and $C_{18:3}$, 48.7. Major component triglycerides are, LLnLn, 5.2%; PLnO, 6.4%; OLnLn, 9.6%; LnLnLn, 10.5% and PLnLn, 14.0% (P, palmitic; O, oleic; L, linoleic and Ln, linolenic acids. On low temperature crystallization, Chrysalis oil yielded two fractions amounting together to 40% of the total with composition quite similar to that of linseed oil.

INTRODUCTION

Chrysalis oil, an insect fat obtained from the cocoon of the silk worm, has recently gained industrial significance in this country. About seven million kilograms (dry weight) of spent silkworm pupae belonging to the mulberry (*Bombyx mori*), Tassar (*Antheraea mylitta*), Eri (*Philosami ricini*) and Muga varieties are produced annually in India. Over 70% of this total comes from mulberry silkworm pupae. Mulberry and Tassar pupae are reported to contain 28.4% and 26.9% oil, rich in linolenic acid. As such, spent silkworm pupae, the by-product of sericultural industry, represent a potential source for 1750 tons of linolenic-rich oil per annum. Spent pupae of these varieties are also rich in protein (50%), and nutritional studies have indicated that spent pupae make good poultry and cattle feed (1). Long ago, Kimura (2) and Bergmann (3) determined the fatty acid composition of Chrysalis oil by methods now considered inadequate for the purpose. The changes in fatty acid composition of lipid at various stages of ontogeny of the insect were determined by Niemierako (4) and Herodek and Farkas (5). The latter group used the technique of paper chromatography. According to Sridhara and Bhat (6), unsaturated neutral triglycerides (42-43%) along with high concentration of phospholipids (18-24.3%) constitute the silkworm fat. They also observed an increase in the content of oleic acid and free fatty acids with concomitant decrease in the content of saturated acid in the lipid during the process of metamorphosis of the insects. Ito and Nakasone (7,8) studied the nutritional requirements of the silkworm and found that fatty acids, especially the polyunsaturated ones, are essential in developmental stages. But Sridhara and Bhat (9,10) showed that certain fatty acids are biosynthesized from the acetate in *Bombyx mori*. Nakasone and Ito (11) later used GLC to determine the fatty acid composition of the lipids of *Bombyx mori* in its different developmental stages from egg to adult, of larval organs and of mulberry leaves, the main diet of the insects. These studies indicated that linoleic acid (33% of the total) predominates throughout its developmental process. Fatty acid composition varies greatly during the larval period, and young larvae contain more stearic and linoleic acids and less palmitic and oleic acids than the older ones. All these earlier studies were primarily oriented towards metabolism of lipids in *Bombyx mori*, but there is not much data in the literature on the triglyceride composition of insect lipids. Triglyceride composition of the lipids of four scale insects were deduced by Hashimota et al. (12) by determining the fatty acid composition at the 2-position of the glycerine moiety.

In view of the potential importance of Chrysalis oil in industry, it was thought desirable to study the fatty acid and triglyceride compositions of the lipid of spent silkworm pupae by modern techniques. This communication reports the findings on the triglyceride compostion of this insect lipid.

EXPERIMENTAL

The techniques used were low temperature segregation, selective enzymatic hydrolysis, thin layer chromatography and gas liquid chromatography. Repeated and systematic crystallization of the oil from acetone, first at -60 C and thereafter successively at higher temperatures in 10 C increments were carried out by the method suggested by Foreman and Brown (13). Supernatent liquids at each step were removed as Fraction F (-60 C), E (-50 C), D (-40 C), C (-30 C), and B (-20 C). The final precipitate left at -20 C was designated Fraction A.

Lipolysis was carried out as suggested by Coleman (14) at pH 8.5 and 37.5 C using a purified pancreatic lipase preparation with the addition of Ca^{2+} ions and bile salts.

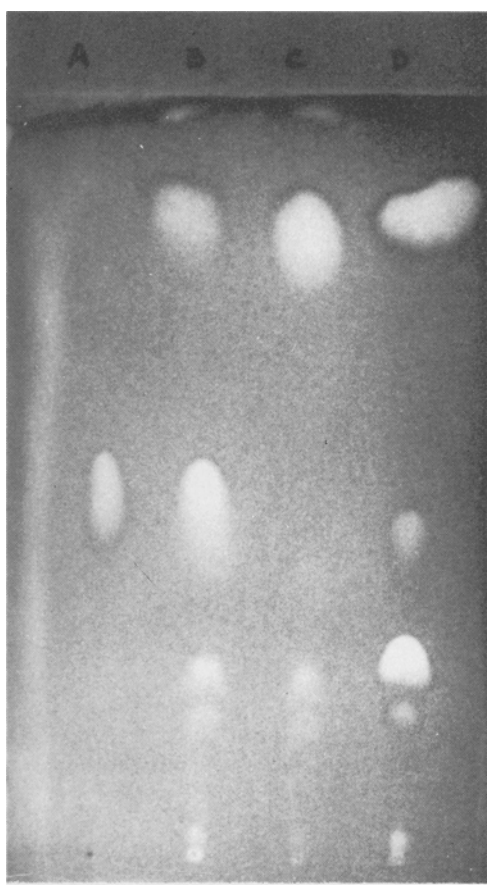

FIG. 1. Thin layer chromatography of Chrysalis oil on silica gel. Spots: A – Stearic acid; B – Chrysalis oil. C – Refined chrysalis oil; D – Tripalmitin. Solvent system: n-Hexane/diethyl ether/acetic acid (80:20:0.25). Spray Reagent - 2,7, dichlorofluorescein.

TABLE I

Characteristics of Refined Chrysalis Oil and Its Mixed Fatty Acids

Characteristics	Oil	MFA
Saponification equivalent	294.9	276.2
Iodine value (Wijs 60 min.)	161.8	167.5
Nonsaponifiable matter (% Wt.)	5.1	---

The partial glycerides were separated on a thin layer (0.3-0.4 mm) of silica by developing with the solvent system of *n*-hexane/diethylether/acetic acid (75:25:0.12). The monoglyceride fraction was detected with 2',7'-dichlorofluorescein, and the original triglycerides were converted to methyl esters by the semimicro method of Ludy et al. (15).

Gas liquid chromatography of the methyl esters was carried out with the Hewlett Packard analytical gas chromatograph (model 700-R12) equipped with flame ionization detector. The column (6 ft x 1/4 in.) packed with 10% diethylene glycol succinate on 60/80 mesh Gas Chrom Z was operated at 160 C with a carrier gas flow of 40 ml/min. Peak areas were determined as the product of peak height and the width of half height. The weight percentages were converted into mole percentages. The sample of the oil was analyzed by the method of Fiske and Subba Rao (16) to determine the content of total phosphorus. About 8 mg of the sample was digested with 2.5 ml. of 10 N sulfuric acid in a 10 ml micro Kjeldahl flask until all the fumes were liberated. One drop of concentrated nitric acid was added, and boiling was continued until the evolution of fumes ceased. After cooling, the content of the flask was transformed to a 50 ml flask. Five milliliters 2.5% solution of ammonium molybdate and 2. ml 0.25% 1,2,4 aminonaphthol sulfonic acid were added. The volume was made up and optical density of the solution was measured against a blank at 660 mμ.

RESULTS

The sample of Chrysalis oil was extracted with petroleum ether (bp 40-60 C) from the cocoon of the silkworm pupae *Bombyx mori* supplied by a reputed chemical and pharmaceutical concern of Calcutta. Upon chromatography on a thin layer of silica gel G with the solvent system of *n*-hexane/diethyl ether/acetic acid (80:20:0.25) triglycerides and free fatty acids only, were detected but no phospholipids (Fig. 1). Analysis by the method of Fiske and Subba Rao (16) also did not indicate the presence of phospholipids in the sample.

A portion of the oil (free fatty acids 22.4% as oleic) was refined with alkali as 50% (w/w) solution in hexane (17). The refined oil and the mixed fatty acids isolated therefrom on analysis showed the characteristics given in Table I. Refined Chrysalis oil (42.1) g) was segragated by low temperature crystallization technique of Foreman and Brown (13) into six fractions with varying degrees of unsaturation. First crystallization was effected at -60 C and the temperature was raised by 10 C at each of the subsequent crystallizations. Fatty acid compositions of all the six fractions (A-F) and of the oil itself were determined by gas liquid chromatography of their methyl esters. Chrysalis oil and its fractions (A-F) were then subjected separately to hydrolysis by pancreatic lipase (14). The 2-monoglycerides were isolated by thin layer chromatography and were converted to methyl esters by the method of Luddy et al. (15). Those esters were analyzed by GLC. Fatty acid compositions of the triglycerides and the corresponding 2-monoglycerides were all converted into mole percentages, and the results are shown in Table II. Triglyceride compositions of

TABLE II

Fatty Acid Composition (mole %) of Triglyceride and 2-Monoglyceride of Refined Chrysalis Oil and Its Different Fractions

Component acids	Oil		A(4.0%)		B(30.2%)		C(15.7%)		D(26.3%)		E(13.8%)		F(10.0%)		Computed values[a]	
	I.V., TG	161.8 MG	I.V., TG	90.1 MG	I.V., TG	122.6 MG	I.V., TG	162.1 MG	I.V., TG	185.5 MG	I.V., TG	196.5 MG	I.V., TG	203.8 MG	I.V., TG	162.3 MG
C14:0	0.6	---	0.2	---	0.2	---	---	---	---	---	---	---	---	---	0.1	---
C14:1	---	---	0.5	---	---	---	---	---	---	---	---	---	---	---	tr	---
C16:0	19.3	5.3	32.0	11.6	29.9	6.6	19.3	6.4	9.7	3.6	6.7	3.8	8.8	3.0	17.7	5.2
C18:0	3.9	0.6	16.7	1.6	6.9	2.4	4.9	1.0	2.2	---	1.3	---	0.8	---	4.4	0.9
C18:1	17.7	16.8	21.1	37.8	21.6	25.7	17.7	16.9	20.2	13.0	17.3	11.0	12.8	11.9	19.2	18.0
C18:2	9.8	9.7	5.6	6.3	6.4	8.1	10.3	9.1	12.6	11.3	17.4	11.9	13.2	10.3	10.8	9.8
C18:3	48.7	67.6	23.9	42.7	35.0	57.2	47.8	66.6	55.3	72.1	57.3	73.3	64.4	74.8	47.8	66.1

[a]Computed values from fractions (A-F).

TABLE III

Triglyceride Composition[a] (M%) of Refined Chrysalis Oil and Its Fractions (A-F)

Triglycerides[b]	Oil	Fractions A (4.0%)	B (30.2%)	C (15.7%)	D (26.3%)	E (13.8%)	F (10.0%)	Computed values (A-F)
SSS	0.7	6.0	2.4	0.7	0.1	---	---	0.6
SSU	2.4	5.9	4.5	3.2	1.0	0.7	0.6	2.6
SUS	10.2	38.6	23.7	9.9	2.6	0.9	1.5	8.7
USU	2.8	1.3	2.1	3.5	2.5	3.1	2.4	2.9
SUU	41.1	38.6	45.6	40.8	25.9	17.5	21.8	39.5
UUU	42.8	9.6	21.7	41.9	67.9	77.8	73.7	45.7

[a]Glycerides present more than 5% are shown below:
Oil: PLnO, 6.4, PLnLn, 14.0; OLnLn, 9.6; LLnLn, 5.2 and LnLnLn, 10.5
Fraction A: OPO, 6.7; POST, 7.8; PLnP, 7.6; PLnSt, 8.8; PLnLn, 5.2.
Fraction B: PLnP, 9.9; POLn, 5.2; PLnO, 9.2; PLnLn, 11.4; OLnLn, 5.4.
Fraction C: PLnO, 6.2; PLnLn, 13.2; OLnLn, 9.2; LLnLn, 5.6; LnLnLn, 9.8.
Fraction D: PLnLn, 8.6; OLnLn, 16.0; LLnLn, 9.0; LnLnLn, 15.9.
Fraction E: PLnLn, 6.0; OLnL, 6.0; OLnLn, 14.8; LLnLn, 14.5; LnLnLn, 17.8.
Fraction F: PLnLn, 10.4; OLnLn, 11.8; LLnLn, 13.0; LnLnLn, 26.2.
[b]S = Saturated acyl groups and U = Unsaturated acyl groups, and the order indicated is the order of linkage with glycerine moiety.

TABLE IV

Fatty Acid Composition (Wt. %) of *Bombyx mori* Lipid

Sample		Fatty acids $C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{16:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Bombyx mori	(2)	---	25.0		---	22.0	38.0	15.0
Bombyx mori[a]	(3)	---	20.0	4.0	2.0	35.0	12.0	25.0
Bombyx mori[b]	(19)	---	---	---	---	29.8	48.9	21.3
Bombys mori	(5)							
	L[b]	---	10.0	10.8	---	18.1	25.6	34.0
	PP	---	26.9	4.1	---	20.8	14.7	33.5
Bombyx mori	(6)							
	L	---	20.0	18.0	---	26.0	12.0	26.0
	P	---	16.0	11.0	---	35.0	12.0	26.0
Bombyx mori	(11)							
	L (5th instar)	0.4	16.4	7.2	0.7	14.7	16.0	44.7
	PP	0.4	27.2	4.4	2.2	21.6	7.7	36.4
Bombyx mori (Present work)	P	0.5	18.0	4.0	---	18.2	10.0	49.3

[a]-$C_{20:0}$, 1.0 %, and higher unsaturated acids 1.0%.
[b]C > 18 acids 1.5%. L = Larva; P = Pupa; PP = Prepupal Larva after Cocoon spinning.

the Chrysalis oil and of its different fractions were calculated from the fatty acid compositions of the original triglycerides and of the corresponding 2-monoglycerides using the assumptions of Vander Wal (18) and Coleman (14). The fatty acids have been grouped as: P - $C_{16:0}$; St - $C_{14:0}$ and $C_{18:0}$; 0 - $C_{18:1}$ and $C_{14:1}$; L - $C_{18:2}$ and Ln - $C_{18:3}$. Results are shown in Table III.

DISCUSSION

Of the previous reports on the fatty acid composition of *Bombyx mori* lipid obtained from the insect at its various stages of ontogeny (5,6,11,19) only that of Nakasone and (11) was based on gas chromatography. Earlier works revealed $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ acids as the major constituents of this insect lipid, linolenic acid being present in very high proportions. Findings of the present investigation along with the previous ones are shown in Table IV. These results indicate a close parallelism between the fatty acid composition of the Chrysalis oil and that of the lipids of the 5th instar stage of the larva after cocoon spinning as reported by Nakasone and Ito (11). Minor discrepencies (not more than 5%) observed in the content of C_{18} acids may be due to difference in the developmental stages of the insects or due to difference in the composition of their dietory lipids in the two instances. In agreement with the observations of Sridhara and Bhat (6), the present sample of *Bombyx mori* lipid was also found rich in the content of free fatty acids, but the presence of phospholipid as reported by them (6) could not be detected in the present sample. This may be due to difference in the methods of extraction. Hashimoto et al. (12) reported the triglyceride compositions of the four scale insects by determining the fatty acid composition at the 2-position of the glycerine molecule. We also observed that upon pancreatic lipase hydrolysis the 2-position of the glycerol moiety is predominantly occupied by C_{18} unsaturated acids (94%). Following the assumptions of Coleman (14) and Vander Wal (18) originally proposed for seed lipids, the major triglycerides comprising the Chrysalis oil are palmitodilinolenin, 14.0%; trilinolenin, 10.5%; oleodilinolenin, 9.6%; palmito linoleno olein, 6.4%; linoleodilinolenin, 5.2%, and palmitolinoleo palmitin, 4.7%.

A good drying oil should contain at least 50% inolenic and 20% linoleic acids (20). Chrysalis oil with 49% linolenic and 10% linoleic acids does not satisfy this minimum and as such can not be used as a drying oil. But, upon low temperature crystallization, Chrysalis oil yielded two fractions (D, 26.3% and E, 13.8%) with compositions much similar to that of linseed oil as shown in Table V.

So it is evident that on low temperature crystallization Chrysalis oil yields a fraction amounting to 40% of the total, which shows a potential of utilization in paint and

TABLE V

Fatty Acid Composition (Mole %)

Acids	Linseed oil (21)		Chrysalis oil Fraction (present work)	
	Argentina	India	D (26.3%)	E (13.8%)
Saturated	17.7	15.7	11.9	8.0
Oleic	15.2	13.7	20.2	17.3
Linoleic	14.9	14.4	12.6	17.4
Linolenic	52.2	56.2	55.3	57.3

varnish industry. Low temperature crystallization also yielded another fraction, B, (30% of the total) whose composition (35% saturated and 22% oleic acids) indicates the possibility of its being utilized for other industrial purposes.

Gunstone et al. (22) introduced the concept of selectivity factor for seed lipids to compare the affinity of different acyl groups for the 2-position of the glycerine molecule. According to them, linoleic acid shows a selectivity factor of 1-1.2, while the corresponding value for oleic and linolenic acid is 0.8-1. Selectivity factors were also calculated by the method of Gunstone et al. (22) for all the C_{18} unsaturated acids present in the chrysalis oil and its different fractions (A-F) obtained by low temperature crystallization. The selectivity factor for linolenic acid was found to vary between 1-1.2, while the corresponding values for oleic and linoleic acids varied between 0.6-1 and 0.7-0.9, respectively. Whether selectivity factor is a speciality of insect lipid or whether it is dependent on the relative abundance of individual C_{18} acids in the mixture needs confirmation by further studies.

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