General Properties and the Fatty Acid Composition of the Oil from the Mophane Caterpillar, *Imbrasia belina*

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ABSTRACT: A preliminary investigation of the bulk properties of the oil from the edible mophane caterpillar (phane), *Imbrasia belina*, showed a significant difference in the iodine values of the oils from mature and young phane. Detailed analysis of the fatty acid composition of the two oil samples was thus carried out by capillary gas chromatography (GC) and complemented with ^{1}H and ^{13}C nuclear magnetic resonance (NMR) studies to investigate the degree of unstauration in the two oil samples. While these studies showed that the oil samples from the mature and young mophane caterpillar were much the same in fatty acid composition, the data revealed a significant divergence from a literature report on phane oil. This earlier report puts the ratio of total saturated to total unsaturated fatty acids at approximately 1:1 (48.2:48.8, in percentages) and estimates the fatty acid composition for the major fatty acids as 16:0 (31.9%), 18:0 (15.2%), 18:1 (20.4%), 18:2 (9.9%), and 18:3 (19%). The data collected from the present work, however, showed the fatty acid composition for total saturated and total unsaturated fatty acids to be 40.5 and 57.0%, respectively. This work estimated the fatty acid composition for the major fatty acids as 16:0 (27.2%), 18:0 (12.3%), 18:1 (16.1%), 18.2 (10.7%), and 18:3 (29.0%). Thus, linolenic acid was the most abundant fatty acid in the phane oil. The GC results of the present analysis were largely corroborated by studies of the composition of fatty acid classes in the phane oil estimated from integrals of ^{1}H and ^{13}C NMR signals. Oils from other edible Lepidoptera larvae are also known to be much richer in unsaturated than saturated fatty acids.

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The mophane caterpillar, locally called phane, is the larval stage of the mophane moth, *Imbrasia belina* (Westwood) (Lepidoptera: Saturniidae). This larval stage goes through five developmental stages, or instars, before pupating. The mophane caterpillar derives its name from the host plant, *Colophospermum mophane*, on whose leaves it feeds. Phane traditionally used to be an important food source mainly for the people of the northeastern part of Botswana. However, phane is now eaten all over Botswana and has indeed become a much sought-after delicacy in southern Africa. The mophane caterpillar has thus become an important trading commodity in the southern Africa.

Being such an important food source for the people of Botswana and for other ethnic groups in southern Africa, phane has attracted some attention, particularly with regard to its nutritional value. The boiled and then dried mophane caterpillar (one of two common methods of preparation) has been reported to contain about 47.5% crude protein in the dried mass (1). In a comparative study, the mophane caterpillar has been reported to contain over twice as much protein as cooked beef or raw chicken (2). The reported fatty acid profile of the oil from phane puts the ratio of total saturated fatty acids to total unsaturated fatty acids at approximately 1:1 (48.2:48.8, in percentages), with palmitic acid being the dominant fatty acid (31.9%) (3).

As part of general studies to determine the physical and chemical properties of seed oils to evaluate the general quality of seed oils in Botswana, it was decided to determine similar data for phane oil because such data have not been reported. As shown in Table 1, physical and chemical parameters were determined for mature phane oil (i.e., late fifth instar) and young phane oil (i.e., late fourth to early fifth instars). Table 1 shows significant differences in the iodine values (IV) and the acid values (AV) of the two oil samples. The different IV suggested the possibility of different degrees of

TABLE 1
Physical and Chemical Properties of the Oil from *Imbrasia belina* Caterpillar

Physical + chemical properties	Mature phane oil	Young phane oil
Refractive index	1.473	1.469
Density	0.901	0.917
Acid value, mg KOH/g	15.7	9.90
Saponification value, mg KOH/g	184 ± 2	180 ± 2
Iodine value (Wijs)	94 ± 3	71 ± 3
Peroxide value, meq/kg	0.27	0.18
Unsaponifiable matter, % (w/w)	2.05	2.02
Total phosphorus, % (w/w)	0.26	0.25
% Yield of oil (w/w)	29.6	22.9

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unsaturation in the samples. It was therefore decided to carry out detailed analysis of the fatty acid composition of the two oil samples to investigate any possible differences in their fatty acid profile which might be attributable to age differences.

EXPERIMENTAL PROCEDURES

Materials. The *I. belina* caterpillars, phane, were collected in the surrounding villages of Francistown in the north-east of Botswana and were divided into two groups: (i) mature phane, i.e., late fifth instar larvae about to pupate, and (ii) young phane, i.e., late fourth instar to early fifth instar larvae that were still actively feeding. The two phane groups were treated in the same way by roasting in hot ash for about 20 min before storing in a deep freezer.

Extraction. The dried phane were macerated in a Waring blender. The mature sample (174.8 g) was extracted with chloroform/methanol (2:1) by the method described by Christie and was given a "Folch" wash (4). The final chloroform extract yielded a greenish-brown oil (51.7 g = 29.6%, w/w). The young phane powder (207 g) was extracted with chloroform in a Soxhlet apparatus to yield another greenish-brown oil (46.0 g = 22.9%, w/w).

Bulk properties. The physical and chemical properties of the oil samples (Table 1) were determined by standard IUPAC methods for the analysis of oils and fats (5). All experiments were performed in triplicate

Fatty acid composition. Sample preparation. The oil samples were transesterified by refluxing in dry methanol that contained ethanoyl chloride to produce fatty acid methyl esters (FAME) (4).

Instrumentation and separation conditions. The fatty acid composition was determined with a Varian 3500 GC (Palo Alto, CA) with an on-column injector, a flame-ionization detector, and a DB-Wax [30 m × 0.25 mm i.d. (J&W, Folsom, CA)] capillary column. The carrier gas was helium at a flow rate of 2.3 mL/min. The oven temperature was programmed, starting at 130°C (2 min), increasing to 150°C (30°/min), and holding for 10 min. Injector and detector temperatures were held at 130°C and 250°C, respectively. Reference compounds were either standard mixtures GLC-68A (Nu-Chek-Prep, Elysian, MN) or Memixes (Larodan Fine Chemicals, Malmo, Sweden).

Nuclear magnetic resonance (NMR) analysis. Proton NMR spectra were acquired at 500 MHz, and ^{13}C spectra were acquired at 125.8 MHz with a Bruker DMX-500 (Karlsruhe, Germany) NMR spectrometer. The ^{13}C spectra were run by utilizing an inverse gate decoupled pulse sequence with a sufficiently long relaxation delay, thus suppressing nuclear Overhauser enhancement and minimizing effects from differing spin-lattice relaxation times. This technique facilitates quantitative comparisons of the different signals in the olefinic region.

RESULTS AND DISCUSSION

The physical and chemical properties listed in Table 1 show oil profiles that are rather similar to those of seed oils, especially with regard to the iodine values. The AV are rather high, but they are quite comparable with AV for unprocessed palm oil and virgin olive oils.

The difference between the two IV of *ca.* 20 units was thought to be sufficiently significant to warrant further investigation to ascertain whether any differences in the degree of unsaturation existed in the two oil samples that could either be due to the age difference in the two groups of larvae or to the different extraction methods employed in this work.

The detailed fatty acid profile of the two oil samples, as revealed by the GC analysis (Table 2), dispels any suspicions about different degrees of unsaturation in the two oil samples. Table 2 clearly shows the young and mature phane oils to be virtually identical. Thus, the chloroform/methanol (2:1) extraction, followed by "Folch wash," isolated the same lipid classes as the Soxhlet extraction with chloroform. The difference in yield (Table 1) between the two extraction methods could be attributed to the age difference in the two larvae groups: the mature phane had attained their maximum weight and had slowed down in feeding prior to pupating, while the young phane were actively feeding for a few more days before reaching the pupating stage.

The GC analysis of the two oil samples estimated the ratio (average of the two samples) of saturated, mono-, di-, and triunsaturated fatty acids as: 40.47:17.32:10.71:28.99 (in percentage; Table 2). Thus, the ratio of total unsaturated to saturated fatty acids present was 57.02:40.47. Furthermore, the GC analysis showed that the order of abundance of the major fatty acids in the phane oil was: 18:3n-3 (29.0%) > 16:0 (27.2%) > 18:1n-9 (16.1%) > 18:0 (12.3%) > 18:2n-6 10.7%).

The results of the GC analysis were largely in agreement with ^{1}H and ^{13}C NMR analyses of the fatty acid classes in the oil samples, as shown in Tables 3 and 4. ^{1}H NMR analysis somewhat underestimated diunsaturated fatty acids (mainly linoleic acid) and overestimated total saturated acids. How-

TABLE 2
Fatty Acid Composition (in percent) of the Oil from *Imbrasia belina* Caterpillar as Determined by Capillary Gas Chromatography

Fatty acid	Mature phane	Young phane
14:0	0.31	0.31
16:0	27.24	27.08
17:0	0.39	0.41
18:0	12.03	12.51
20:0	0.30	0.35
16:1n-7	0.86	0.90
17:1	0.12	0.12
18:1n-9	15.99	16.26
18:1n-7	0.18	0.20
18:2n-6	10.55	10.86
18:3n-3	29.44	28.54
Unknowns	2.58	2.47
Total	100	100
Saturated	40.28	40.66
Monounsaturated	17.16	17.48
Diunsaturated	10.55	10.86
Triunsaturated	29.44	28.54

TABLE 3
Composition of Classes of Fatty Acids in the Oil of Young *Imbrasia belina* Caterpillar as Estimated from Integrals of ^{1}H Nuclear Magnetic Resonance (NMR) Signals

Proton signal	Chemical shift (ppm)	Integral
Olefinic	5.26	2.14
Methoxy	3.56	3.00
Double allylic	2.72	1.29
C-3	2.20	2.03
Allylic	1.97	2.30
C-2	1.54	2.03
Methylene	1.17–1.22	19.02
Methyl n-3	0.88	0.87
Methyl, others	0.79	2.29
Methyl, total		3.16
Estimated amount of fatty acids		
α-Linolenic		27%
Diunsaturated		6%
Monounsaturated		21%
Saturated		45%
Estimated average carbon number		17.05

ever, the results generally agreed with the results of the GC analysis (Tables 2 and 3).

The ^{13}C studies were carried out by reference to standard works in this area to help identify the various olefinic signals (6,7). Because the experimental conditions minimize nuclear Overhauser effects and differences in spin-lattice relaxation, quantitative comparisons could be made. Thus, the composition of the fatty acid classes was determined by estimating the integrals for the signals in the olefinic regions. The results obtained (Table 4) were in good agreement with the results of the GC analysis.

TABLE 4
Composition of Fatty Acid Classes in the Oil from Young *Imbrasia belina* Caterpillar as Estimated from Integrals of ^{13}C NMR Signals

Carbon signal[a]	Chemical shift (ppm)	Integrals for young phane	
Ln-16	131.65	1.0000	
Ln-9	129.96	1.0030	
L-13	129.90	0.3777	
O-10	129.75	0.5634	
L-9	129.74	0.4059	
O-9	129.51	0.5732	
Ln-13	128.06	0.0170	
Ln-12	128.04	0.9953	
L-10	127.90	0.3557	
L-12	127.76	0.3619	
Ln-10	127.59	0.962	
Ln-15	126.97	0.9866	
Fatty acid classes		Average integrals	Estimated content (%)
Linolenic		0.9940	28
Linoleic		0.3753	11
Oleic		0.5683	16
Saturated[b]			45

[a]Ln = linolenic acid; L = linoleic acid, O = oleic acid.
[b]Estimated from proton integrals. For other abbreviation see Table 3.

The fatty acid composition of the lipid content of *I. belina* caterpillar as determined by capillary GC in this work is in disagreement with an earlier report by Zinzombe and George (3). These authors reported the abundance ratio of total saturated to total unsaturated fatty acids to be approximately 1:1 (48.2:48.8, in percent) and further reported the order of abundance of the major fatty acids as 16:0 (31.9%) > 18:1 (20.4%) > 18:3 (19%) > 18:0 (15.2%) > 18:2 (9.9%). This earlier report and our current work agree that, among the major fatty acid constituents, linoleic acid (18:2) is the least abundant in the phane oil. However, we found linolenic acid to be the most abundant fatty acid in the phane oil and, indeed, the abundance of total unsaturated fatty acids (57%) was higher than total saturated fatty acids (40.7%).

Variations in the fatty acid composition of the oil from the *I. belina* caterpillar could arise from the methods of preparing the sample as well as from the method of extraction. Hexane extraction, the method used in the earlier report, will isolate mainly the neutral lipids and leave much of the polar lipids, which are often rich in unsaturated fatty acids. The method of boiling phane in salted water and allowing it to dry in the sun is reported by the rural phane traders to give a tastier product than the method of roasting in hot ash. However, prolonged boiling could bring about the breakdown of tissue cell walls and release oil into the aqueous medium. The method of preparing phane by roasting in ash, which was adopted in this work, has the advantage of being a faster process and hence less likely to cause loss of oil.

The different methods of sample preparation might result in differences in the fatty acid composition of the phane oil. However, as quoted by Zimzombe and George, oils from other edible Lepidoptera larvae, such as *Imbrasi ertili* and *Heliothis zea,* are composed of up to about 70% unsaturated fatty acids. Indeed, linolenic acid is the most abundant fatty acid (32%) in the oil from *H. zea* larvae (3,8). Thus, the fatty acid composition determined in this work is not unique for *I. belina* but appears to be quite normal among edible Lepidoptera larvae.

Our findings in this work have demonstrated that the oil from the caterpillar of *I. belina* is much more like an unsaturated seed oil than a typical animal fat. Phane is certainly a better source of the essential fatty acids linolenic and linoleic acids than many other animal food sources.

REFERENCES

1. van Voorthuizen, E. G., The Mophane Tree, *Botswana Notes and Records 8*:223–230 (1976).
2. Sekhwela, M.B.M., The Nutritive Value of Mophane Bread-Mophane Insect Secretion (Mophote or Maboti), *Botswana Notes and Records 20*:151–154 (1989).
3. Zinzombe, I.M., and S. George, Larval Lipid Quality of Lepidoptera: *Gonimbrasia belina*, *Botswana Notes and Records 26*: 167–173 (1994).
4. Christie, W.W., *Lipid Analysis*, Pergamon Press, Oxford, 1982, pp. 52-53.

5. *IUPAC Standard Methods for the Analysis of Oils, Fats and Derivatives*, 6th edn., compiled by C. Paquot (Part 1), Pergamon Press, Oxford, 1979
6. Renou, P.J., M. Bonnet, and C. Denoyer, High Resolution ^{13}C NMR Spectroscopy of Rendered Animal Fats: Degree of Saturation of Fatty Acid Chains and Position on Glycerol, *Int. J. Food Sci. Technol. 25*:399–408 (1990).
7. Gunstone, F.D., High Resolution ^{13}C NMR. A Technique for the Study of Lipid Structure and Composition, *Prog. Lipid Res. 33*:19–28 (1994).
8. DeFoliart, G.R., Edible Caterpillars: A Potential Agroforestry Resource?, *Food Insects Newletter 4*(2): 3–4 (1991).

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