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Further Lipid Profiling of the Oil from the Mophane Caterpillar, *Imbrasia belina*

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Abstract A comprehensive lipid profiling of the oil from the edible mophane caterpillar, *Imbrasia belina*, has been carried out as part of the study of the nutritional value of the caterpillar. GC-MS analysis revealed the composition of the major FA classes as 18:3 (29.98%), 16:0 (25.64%), 18:1 (17.97%), 18:0 (12.49%) and 18:2 (11.81%), which was in agreement with reported GC-FID analysis of the phane oil. ESI-FTICR mass spectrometric analysis showed phane oil to contain 20 TAG classes, with C54:4 (14.59%), C52:3 (14.71%) and C52:2 (10.49%) being the dominant classes, whilst ^{13}C-NMR studies of the TAGs regiochemistry showed that occupancy of the *sn*-2 position was dominated by linolenyl and linoleoyl groups whereas the *sn*-1/3 positions were dominated by saturated groups. Normal-phase HPLC analysis of the unsaponifiable matter showed the presence of α-tocopherol (71.39 μg/g) and γ-tocopherol (1.66 μg/g) as the only tocol content in phane oil. GC-MS analysis of the total acetylated unsaponifiable matter gave the relative composition of the major sterols as cholesterol (53.77%), β-sitosterol (24.16%), 22-dehydrocholesterol (14.58%) and campesterol (6.26%), whilst GC-MS analysis of an SPE pre-fractionated unsaponifiable matter gave the absolute 4-desmethylsterol content (μg/g) as cholesterol (4482.44), β-sitosterol (1861.95), 22-dehydrocholesterol (1274.53), campesterol (503.83) and stigmasterol (21.78). Perhaps the adverse effect of such high dietary cholesterol content on humans could be mitigated by the presence of the substantial amounts of β-sitosterol and campesterol which are known to be blood plasma cholesterol lowering phytosterols.

Keywords ESI-FTICR · Fatty acid classes · GC-MS · Mophane caterpillar · *Imbrasia belina* · Normal-phase HPLC · Triacylglycerol classes · Sterols · Tocopherols · Tocotrienols

Introduction

The mophane caterpillar, more popularly known as phane, is the larval stage of the emperor moth, *Imbrasia belina* (Westwood) (Lepidoptera: Saturniidae). The larval stage of *Imbrasia belina* derives its local name from its host plant, *Colophospermum mophane*, whose leaves constitute the principal food source for the larvae. Phane traditionally used to be an important food source mainly for the people in the Northeastern district of Botswana but has now become a much cherished delicacy throughout Botswana and the surrounding countries like Namibia, South Africa and Zimbabwe, thus cutting for itself a niche market in the southern Africa region.

Being an edible worm, the nutritional value of the mophane caterpillar has received considerable attention in the southern Africa region. In comparison with beef (cooked), biltong and chicken (raw), Sekhwela [1] reported that phane had the highest content of protein (56.8%), fat (16.4%), carbohydrate (13.8%) and calcium (0.458%) in g/100 g sample. Thus in addition to its unique taste, the mophane caterpillar serves as a source of good nutrition to its consumers. In pursuance of the studies on its nutritional value, the physicochemical properties and the FA profile of the oil from phane were previously investigated by one of

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us. Yeboah et al. [2] reported an oil yield of 29.6% (w/w) from phane and estimated the fatty acid composition from GC-FID analysis as 18:3n-3 (29.4%), 16:0 (27.2%), 18:1n-9 (16.1%), 18:0 (12.3%), 18:2n-6 (10.7%), making the ratio of total unsaturated to total saturated FAs as 57.02:40.47%. Thus the mophane caterpillar provides a good dietary source of the ω-3 essential FA, α-linolenic acid. The study indeed showed that the oil from the mophane caterpillar was more similar to an unsaturated vegetable oil than a typical animal fat.

The present study seeks to further characterize the lipid content of the mophane caterpillar by carrying out structural and compositional studies on the triacylglycerols and the minor components, i.e., sterols, tocopherols and tocotrienols in the oil of the mophane caterpillar. The regiochemistry, i.e., the distribution of the acyl groups on the glycerol backbone in oils and fats has been shown to have important physiological and nutritional effects on humans [3], whilst the composition of the triacylglycerols can serve as fingerprint in confirming the authenticity of oils and fats [4]. Sterols, tocopherols and tocotrienols constitute the major components in the unsaponifiable matter in oils and fats. The composition of sterols in oils and fats has been shown to be characteristic of each plant or animal source and hence can be used to determine the identity of a particular oil or fat [5]. Moreover certain sterols have been shown to have such important bioactive properties as cancer prevention [6] and lowering of cholesterol in blood plasma [7]. Tocopherols and tocotrienols, on the other hand, are natural antioxidants for protection against oxidative attack on lipids and other biomolecules. Therefore detailed information about all the above constituents will greatly enhance our knowledge of the quality of the mophane caterpillar as an important food source in the southern African region. This study has attempted to provide some of this information.

Experimental Procedures

Materials

Dried mophane caterpillar (*I. belina*) 200 g was obtained from an open air market in Gaborone, Botswana.

Solvents and Extraction

Solvents and reagents used in this work, unless otherwise stated, were all of analytical grade. Solvents used for high-resolution MS were of HPLC grade. Solvents were obtained from Rochelle Chemicals, South Africa, BDH (Merck Chemicals, Pty Ltd. UK), Riedel-de Haën (Sigma Aldrich, GmbH) or JT Baker Chemicals Co. (Phillipsburg).

After thorough cleaning, the dried mophane worms were macerated in a Waring commercial blender (Gateshead, UK). The powder was extracted with a mixture of *n*-hexane/2-propanol (3:1, v/v) in a Soxhlet apparatus for 6 h.

Separation of Acylglycerols

Triacylglycerols (TAG), diacylglycerols (DAG) with free fatty acids (DAG + FFA) and monoacylglycerols (MAG) in the oil sample were separated by gradient elution on silica gel (5% H_2O, Saarchem Pty Ltd.) using benzene (100%), benzene: diethyl ether (9:1) and diethyl ether (100%), sequentially.

Fatty Acid Composition: Sample Preparation

FAME

The oil sample (2 g) was transesterified by refluxing in dry methanol that contained ethanoyl chloride to yield a mixture of fatty acid methyl esters (FAME), which was stored under nitrogen and later used for GC-MS and ^{1}H-NMR analyses.

Instrumentation and Separation Conditions

The mixture of FAMEs in dichloromethane was analysed in a ThermoQuest Voyager GC-MS coupled to ThermoQuest Trace GC 2000 SERIES (San Jose, California, USA). Xcalibur version 1.3 software from Thermo Fischer Scientific (San Jose, California, USA) was used to process the data. Separation of the FAMEs was carried out using a CP-WAX 52 CB capillary column (0.25 μm × 0.25 mm × 30 m) from Chrompak International BV (Middelburg, The Netherlands) consisting of a 100% polyethylene glycol phase. An injection temperature of 200 °C and an interface temperature of 240 °C were used. UHP helium gas was used as the carrier gas at a flow rate of 1 mL/min. An initial temperature of 60 °C was held for 1 min and then ramped to 240 °C at the rate of 5 °C per minute, after which it was held isothermally for 10 min.

Nuclear Magnetic Resonance Analysis

A proton-NMR spectrum of the mixture of FAMEs, dissolved in $CDCl_3$, was acquired at 300 MHz using a Bruker Avance DPX 300 spectrometer. The relative composition of the saturated, monounsaturated, diunsaturated and α-linolenic fatty acids, together with their average chain lengths, shown in Table 2, were determined from the relative sizes of the integrals of the signals for the allylic, diallylic and methyl protons using Holmback's equations [8].

Analysis of Triacylglycerols (TAG)

High Resolution Mass Spectrometric Analysis

TAG extract (0.15 mg) was dissolved in methanol and the methanolic solution was introduced continuously via a syringe pump into an electrospray ionization source (APPOLO) on a high resolution FTICR mass spectrometer (Bruker Daltonics Apex III) at a flow rate of 2 μL min^{-1}. All data were acquired with 512 K data points and zero-filled to 2048 K by averaging 32 scans and applied to Fourier transform and magnitude calculation. After acquisition, mass spectral raw data were post-processed using Bruker XMASS acquisition and processing software (Version 6.12) according to full width half maximum (FWHM) criteria and taken as input data for calculations. Molecular formulas were assigned by use of the software above. Molecular formulas were limited to 200 ^{12}C, 300 ^{1}H, 30 ^{16}O and 1 ^{23}Na atoms.

^{13}C-NMR Analysis of Triacylglycerols

Standard compounds (tri-palmitin, tri-olein, and tri-linolein) were obtained from Fluka and used without further purification. About 200 mg of the oil sample was dissolved in deuterated chloroform ($CDCl_3$) and the solution (700 μL) was placed in a 5-mm (diameter) NMR tube. The ^{13}C-NMR spectrum was recorded on a Varian Mercury 400 spectrometer operating at 100.6 MHz. The ^{13}C-NMR spectrum for quantitative analysis was recorded with a spectral width of 1,300 Hz, 64 K data points, a pulse repetition time of 60 s, a 90° flip angle and full proton decoupling. Two hundred scans were accumulated per spectrum. The FIDs were zero-filled to 128 K prior to Fourier transform resulting in a digital resolution of 0.02 Hz/point. A line broadening of 0.05 Hz was used for the exponential weighting. Peaks were assigned by spiking the standard compounds into the real test samples.

Analysis of Tocopherols and Tocotrienols

Saponification

The phane oil sample for the analysis of tocopherols and tocotrienols was saponified according to the method reported by Panfili et al. [9]. The oil sample (2.0 g) in an amber screw capped bottle was flushed with nitrogen and 10 M potassium hydroxide (2.0 mL) was added. Absolute ethanol (2.0 mL) and 0.2 M sodium chloride solution (2.0 mL) were then added and the mixture flushed again with nitrogen. Ethanolic pyrogallol (0.5 M, 5.0 mL) was finally added as an antioxidant and then flushed with nitrogen. The bottle was placed in a 70 °C water bath and mixed after every 5 min for 45 min, after which the bottle was cooled in ice and 0.2 M sodium chloride solution (15.0 mL) added. The suspension was extracted twice with 9:1 (v/v) *n*-hexane/ethyl acetate (15.0 mL). The combined organic layer was evaporated to dryness. The dry residue was dissolved in *n*-hexane/isopropanol (99:1) and passed through silica Chromabond™ SPE cartridge. The filtrate was dried and re-dissolved in *n*-hexane (10.0 mL). This was then appropriately diluted prior to HPLC-FLD analysis.

HPLC-FLD Analysis

A Merck HPLC system (Darmstadt, Germany) that consisted of a Merck-Hitachi HPLC L-7100 Intelligent pump, a Rheodyne injector fitted with a 5-μL loop and a Merck L-7480 fluorescence detector was used for the tocopherol and tocotrienol analysis. The analysis was achieved using normal-phase HPLC on Nucleosil 100-5 column (5 μm × 4 mm × 25 cm) from Macherey–Nagel (Düren, Germany). The mobile phase was *n*-hexane/isopropanol (99.7:0.3 v/v) at a flow rate of 1.0 mL/min. Fluorometric detection of all peaks was performed at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. Tocopherol peaks were identified and quantified against authentic tocopherols used as external standards. For each extract quantitative analysis was performed in triplicate.

Analysis of Phytosterols

Saponification of Oil Sample

To the oil sample (2.5 g) in a 250-mL round-bottomed flask was added 0.5 M ethanolic potassium hydroxide solution (25.0 mL) and the mixture refluxed for 1 h. Water (100.0 mL) was then added down the condenser and the mixture was extracted with diethyl ether (100 mL × 3) in a 500-mL separatory funnel. The combined ether solution was washed with water (40.0 mL × 3), and then washed successively with 0.5 M aqueous potassium hydroxide (40.0 mL), distilled water (40.0 mL) and repeatedly with aqueous 0.5 M KOH (40.0 mL) before finally washing with more 40.0 mL portions of distilled water until the washings were neutral to phenolphthalein indicator. The ether solution was then dried under anhydrous magnesium sulphate, and the solvent evaporated by distillation on a water bath to release the unsaponifiable matter.

Acetylation

The whole unsaponifiable matter (UM) was acetylated according to the method of Wilson et al. [10]. The UM

(2.0 mg) was dissolved in 2:1 (v/v) pyridine-acetic anhydride solution (600 μL) and the reaction was allowed to proceed at room temperature overnight. The excess reagents were then removed with slight warming under a stream of nitrogen gas. The mixture was re-dissolved twice in dichloromethane and the solvent removed in a stream of nitrogen. The acetylated products were transferred into a sample vial and dissolved again with dichloromethane, flushed with nitrogen and stored at 4 °C for GC-MS analysis.

Analysis by GC-MS

The acetylated lipids in dichloromethane were analysed in a ThermoQuest Voyager GC-MS coupled to ThermoQuest Trace GC 2000 SERIES (San Jose, California, USA). Xcalibur version 1.3 software from Thermo Fischer Scientific (San Jose, California, USA) was used to process the data. A DB-5MS capillary GC column (0.25 μm × 0.25 mm × 30 m) from J & W Scientific (California, USA) was used for separation and UHP helium was used as the carrier gas at a flow rate of 1 mL/min. The injection temperature was 220 °C, while the interface temperature was 300 °C. The initial temperature was 60 °C held for 1 min and then ramped to 200 °C at a rate of 15° per minute. It was then held for 1 min before the second ramp at a rate of 5 °C per minute to 300 °C. This was then held isothermally for 25 min.

SPE Fractionation of Sterols

The SPE method of Damirchi and Dutta [11] was modified for this determination. A 500-mg, 3-mL silica solid phase extraction (SPE) cartridge from Macherey–Nagel (Düren, Germany), was used per sample. The cartridge was attached to a Macherey–Nagel vacuum manifold (Düren, Germany). Waste tubes were positioned to collect the conditioning solvent. The cartridges were conditioned by passing *n*-hexane (5.0 mL) through them. After conditioning, the *n*-hexane was discarded and a clean test tube was positioned to collect the phytosterol fraction. The UM (2.0 mg) sample aliquots in 2 mL *n*-hexane were then loaded onto the cartridges. The UM was then washed with *n*-hexane/ethyl acetate (10.0 mL), 99:1 (v/v), to remove any non-sterol compounds. Vacuum was applied at 5 mmHg for each elution. This fraction was discarded after checking with analytical TLC that it did not contain compounds of interest. 4-Methylsterols were then eluted with *n*-hexane/ethyl acetate (10.0 mL), 99:1 (v/v), followed by 14.0 mL of 97:3 (v/v) *n*-hexane/ethyl acetate. Prior to eluting pure 4-desmethylsterols, additional *n*-hexane/ethyl acetate (2.0 mL), 97:3 (v/v), was used to wash the cartridges. 4-Desmethyl sterols were then eluted with *n*-hexane/ethyl acetate (25.0 mL), 97:3 (v/v). The fractions were dried, spiked with 10 μg of 5α-cholestane as an internal standard and acetylated prior to GC-MS analysis. The components were quantified against 5α-cholestane.

Data Analysis

Unless otherwise stated experiments for the determination of tocopherols, tocotrienols and sterols by HPLC and GC-MS were carried out in triplicate and results are expressed as mean values ± SD. NMR and FTICR-MS results are expressed as values for single runs

Results and Discussion

The attainment of refractive index (RI) of 1.473 and iodine value (IV) of 94 (Wij's) in the previous study, by Yeboah et al. [2], gave an early indication that the oil from the mophane caterpillar was more polyunsaturated than would be expected from a typical animal fat. This observation was soon confirmed by the results of the GC-FID analysis of the FAMEs, which gave the degree of polyunsaturation at about 40% in the phane oil [2]. The results of the GC-MS analysis of the FAMEs from the phane oil obtained in the present study are given in Table 1 together with the results of the earlier GC-FID analysis. The two results can be seen to be in close agreement with each other. Both analyses agree that the most dominant FA in the oil of the mophane caterpillar was α-linolenic acid (ca. 29%), followed by palmitic acid (ca. 25%). Again both analyses give similar ratios of total unsaturated to total saturated FAs, which are in good agreement with the ratio of total unsaturated to total saturated FAs obtained in this study from proton NMR estimation of the FA classes in the phane oil given in Table 2. Two minor areas of diversion between the GC-FID and GC-MS analyses were (a) the detection of trace amounts of myristic acid, 14:0, (0.31%) by the GC-FID analysis and (b) the GC-MS detection of 8,11–20:2 fatty acid (0.39%). These minor differences could be attributed to the fact that the two experiments analysed two different oil samples extracted from different phane harvests.

In the light of current awareness of the importance of detailed information about the TAGs that constitute the bulk of oils and fats [3, 4], this study attempted to determine the composition of intact TAGs and their regiochemistry by a combination of high resolution mass spectrometry-Fourier transformed ion cyclotron resonance interfaced with an electrospray ionization source, ESI-FTICR, and ^{13}C-NMR spectrometry, respectively. We recently described the essential details of the two techniques in an earlier publication [12]. The results of the ESI-FTICR mass spectrometric results are given in Table 3, which shows the

Table 1 Fatty acid composition in percent of phane oil by GC-MS and by GC-FID

FA	GC-MS[a]	GC-FID[b]
12:0	ND	ND
14:0	ND	0.31
9–16:1	1.29 ± 0.01	0.86
16:0	25.64 ± 0.03	27.24
17:0	0.43 ± 0.0	0.39
9,12,15–18:3	29.98 ± 0.03	29.44
9,12–18:2	11.81 ± 0.02	10.55
9–18:1	17.97 ± 0.02	15.99
11–18:1	ND	ND
18:0	12.49 ± 0.03	12.03
9a–18:1	ND	ND
9a,11t–18:2	ND	ND
8,11–20:2	0.39 ± 0.01	ND
11,13–20:2	ND	ND
9–20:1	ND	ND
20:0	ND	0.30
Unknown	ND	2.57
Total unsaturated	61.44	57.59
Total saturated	38.56	40.28

[a] Data from this investigation; values represent the average of three replicate analyses ± SD

[b] Data from previous work [2]

Table 2 Composition of fatty acid classes in phane oil as estimated from integrals of ^{1}H-NMR signals

Proton signals	Chemical shift (ppm)	Integrals
$CH_{2\ envelope}$	1.27	19.68
Diallylic	2.80	1.18
Allylic 1	2.06	2.20
Me	0.88	2.17
Me ω-3	0.98	0.90
Percentages of the estimated amount of fatty acids		
α-Linolenic acid	27.0	
Diunsaturated acid	6.0	
Monounsaturated acid	21.0	
Total saturated acids	45.0	
Total unsaturated acid	54.0	
Average carbon number	17.8	

percentage composition of TAG classes, C*x*:*n*, where *x* denotes the total number of carbon atoms and *n* denotes the total number of double bonds in the three fatty acids in each TAG class. In the ^{13}C-NMR determination of the regio-distribution of the FAs on the glycerol backbone, the results of which are shown in Table 4, the integrated carbonyl resonances of saturated, oleoyl and linoleoyl/linolenyl groups were used to estimate the acyl distribution between the *sn*-1/3 and *sn*-2 glycerol positions [13].

The distribution of saturated acyl groups, as shown in Table 4, is over 70% at the *sn*-1/3 primary positions whilst the *sn*-2 position is 100% occupied by unsaturated acyl groups, with linolenyl followed by linoleoyl being the dominant acyl groups at this position. This regiochemistry of the TAGs in the phane oil appears to be more consistent with lipids of plant origin than of animal origin [13]. It is interesting to note that Yeboah et al. [14] showed earlier that the FA profile of the phane oil was very similar to the fatty acid profile of the lipid fraction from the leaves of the mophane plant, the host of the mophane caterpillar. Table 3 shows that phane oil is composed of 20 TAG classes, of which the most abundant are classes that contain total carbon atoms of C50, C52 and C54. This implies that phane oil is predominantly composed of C18 and C16 FAs, an observation which very much agrees with the FA composition given in Tables 1 and 2. Indeed the predominant TAG classes in the oil from the mophane caterpillar are C54:4 (14.59%), C52:3 (14.71%) and C52:2 (10.49%). Combining the regio-distribution of the acyl groups given in Table 4 and the TAG composition given in Table 3, we can predict the following as some of the likely dominant TAG molecules in the phane oil.

	sn-1	*sn*-2	*sn*-3
C52:4	18:1	18:3	16:0
	18:2	18:2	16:0
C52:3	18:0	18:3	16:0
	18:1	18:2	16:0
C52:2	16:0	18:2	18:0
	18:1	18:1	16:0
C50:3	16:0	18:3	16:0
C54:6	18:2	18:3	18:1
	18:3	18:3	18:0
C54:5	18:2	18:3	18:0
	18:2	18:2	18:1

In agreement with Table 4, the above structures show occupancy of the *sn*-2 position to be dominated by linolenyl, followed by the linoleoyl group, whilst the *sn*-1/3 positions are predominantly occupied by saturated acyl groups of palmitoyl and stearoyl groups.

The presence of TAG classes with total carbon numbers of C60, C62 and C64, shown in Table 3, would tend to suggest that phane oil may contain fatty acids of chain length C20, C22, C24 and possibly C26. However, the

Table 3 Assignment of major mass peaks of triacylglycerols in the positive ion ESI-FTICR mass spectra of test oil fractions

Observed mass (*m*)/*z*	Theoretical mass	TAG CN:DB	Relative intensity	% composition
Phane				
851.7105	851.7105	C50:3	0.4957	7.29
853.7244	853.7256	C50:2	0.2581	3.8
855.7429	855.7412	C50:1	0.3244	4.77
875.7096	875.7099	C52:5	0.3220	4.74
877.7248	877.7256	C52:4	0.9920	14.59
879.7409	879.7412	C52:3	1.0000	14.71
881.7556	881.7569	C52:2	0.7130	10.49
883.7693	883.7725	C52:1	0.2544	3.74
901.7261	901.7256	C54:6	0.3313	4.87
903.7394	903.7412	C54:5	0.3722	5.47
905.7561	905.7569	C54:4	0.3110	4.57
907.7724	907.7725	C54:3	0.3113	4.58
987.8336	987.8351	C60:5	0.0691	1.02
989.8481	989.8508	C60:4	0.0546	0.8
991.8676	991.8664	C60:3	0.1506	2.21
993.8835	993.8821	C60:2	0.0974	1.43
1015.869	1015.8664	C62:5	0.1062	1.56
1019.8965	1019.8977	C62:3	0.1414	2.08
1021.9096	1021.9134	C62:2	0.0989	1.45
1043.8992	1043.8977	C64:5	0.1691	2.49
1047.9290	1047.9290	C64:3	0.2268	3.34

GC-MS analysis detected only 20:2 FA (0.39%), whilst the earlier GC-FID analysis detected only 20:0 FA, Table 1. As mentioned in our previous paper, we again note that the positive ion ESI-FTICR mass spectrometric technique is so powerful that it is able to reveal the possible presence of fatty acids that are not always detected by gas chromatographic techniques [12].

The determination of the minor components in the mophane oil, i.e., sterols, tocopherols and tocotrienols, was carried out using the unsaponifiable matter in the oil. As described in the experimental section, in the determination of the vitamin E compounds—tocopherols and tocotrienols, saponification was carefully carried out under nitrogen and in the presence of added antioxidant, ethanolic pyrogallol, to help preserve the integrity of the tocol molecules in the phane oil. The composition of tocopherols and tocotrienols was determined by analyzing the extracted unsaponifiable matter using normal-phase HPLC with fluorescence detection (HPLC-FLD) and quantified against external standards. Unlike reverse-phase HPLC, normal-phase HPLC is capable of resolving α- and *β*-tocopherols and tocotrienol isomers [15], and hence was the method of choice in this analysis. The tocopherols and tocotrienols were eluted in order of increasing polarity, which corresponded with the decreasing order of methylation on the chromanol ring of the tocols, in agreement with an earlier literature report [16]. Thus the order of elution was α-, followed by *β*-, *γ*- and *δ*-homologues. The quantitative analysis was performed with calibration curves. A linearity test was carried out over the concentration ranges 1–5 ppm from which regression analysis of the plot of area response versus concentration for each isomer gave an excellent relationship with correlation coefficients of 0.9997 for α-tocopherol, and 1.0000 each, for *β*-, *γ*- and *δ*-tocopherols.

Figure 1 shows the HPLC chromatogram of the tocols in the oil of the mophane caterpillar, whilst Table 5 shows the quantitative results of the analysis. Both Fig. 1 and Table 5 show a rather simple tocol profile for the phane oil. The phane oil sample contained α-tocopherol (71.39 μg/g) and *γ*-tocopherol (1.66 μg/g) as its only tocol content. The total tocol content of 73.05 μg/g in the phane oil fell well below the typical total tocol range of 200–800 μg/g for vegetable oils. However in relation to its oxidative stability, Yeboah et al. [2] have previously reported a peroxide value of 0.27 mequiv/kg for the phane oil. Thus the indication is that notwithstanding its low tocol content, the mophane oil may still exhibit good oxidative stability. This observation would tend to uphold reports in the literature which suggest that stability of oils can be influenced by natural antioxidants and synergists other than the vitamin E compounds [17].

The determination of the sterol content in the unsaponifiable fraction of the phane oil was carried out in two separate experiments. The first experiment was a preliminary determination of the relative percent composition of all sterols in the acetylated total unsaponifiable fraction from the phane oil by GC-MS. In this determination, the

Table 4 Positional distribution of fatty acyl chains on the glycerol backbone of triacylglycerols in phane determined by ^{13}C NMR

Sample	*sn*-1,3 composition					*sn*-2 composition				
	Saturated	Oleoyl	Linoleoyl	uk	uk	Saturated	Oleoyl	Linoleoyl	uk	uk
Phane	70.93	17.74	11.33[a]	ND	ND	ND	38.78	61.22[a]	ND	ND

Values are for single run

ND not detected, *uk* unknown acyl chains

[a] Value is for linoleoyl and linolenyl acyl chains

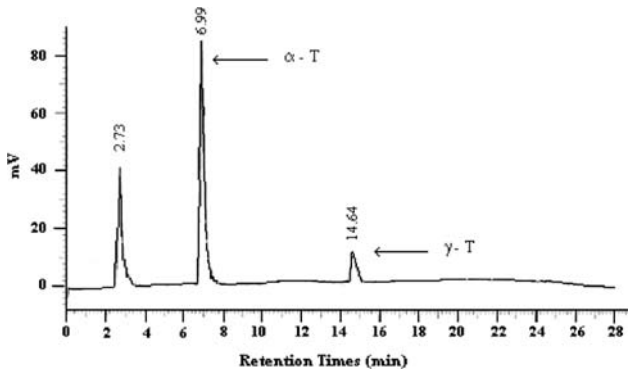

Fig. 1 Chromatogram of tocols in phane oil obtained by HPLC-FLD. *Key*: α-T: α-Tocopherol, γ-T: γ-Tocopherol

components in the region where phytosterols eluted were selected and the identification of the sterols was based on comparison of retention times and reference mass spectra of authentic standards. Figure 2 shows the partial total ion currents (TIC) for the acetylated unsaponifiable matter from phane oil. The relative percent composition of each sterol, shown in Table 6, was calculated as a ratio of its peak area to the total area of all identifiable sterol peaks in the oil sample. Table 6 shows that the sterol content in the oil from the mophane caterpillar consisted to extent of about 98% of 4-desmethylsterols. Hence the second experiment of the sterol analysis involved the determination of the absolute amount of each 4-desmethylsterol in the phane oil. As explained in the experimental section, this was achieved by carrying out a modified Damirchi's SPE method of pre-fractionating the unsaponifiable matter prior to acetylation and subsequent GC-MS analysis [16], using 5α-cholestane as internal standard. The absolute amount of each identifiable 4-desmethylsterol was calculated as μg/g of the phane oil by using the relationship, 4-desmethylsterol $= (A_z \cdot m_{is})/(A_{is} \cdot m)$, where A_z = start peak area, A_{is} = internal standard peak area, m_{is} = mass (μg) of internal and m = mass of oil in g weighed for the analysis [18].

Table 6 shows the results of the preliminary determination of the relative percent composition of all sterols and also the results of the determination of the absolute amount of each 4-desmethylsterol in the phane oil. The two results can be seen to be in excellent agreement, as they indeed reinforce the credibility of each determination. Quite unexpectedly, the cholesterol content constituted up to

Table 5 Tocopherol and tocotrienol content (μg/g) in phane oil obtained by HPLC-FLD

Sample	α-T	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	Unknown
Phane	71.39 ± 1.82	ND	1.66 ± 0.66		ND	ND	ND	ND

Values represent the average of three replicate analyses ± SD

ND not detected

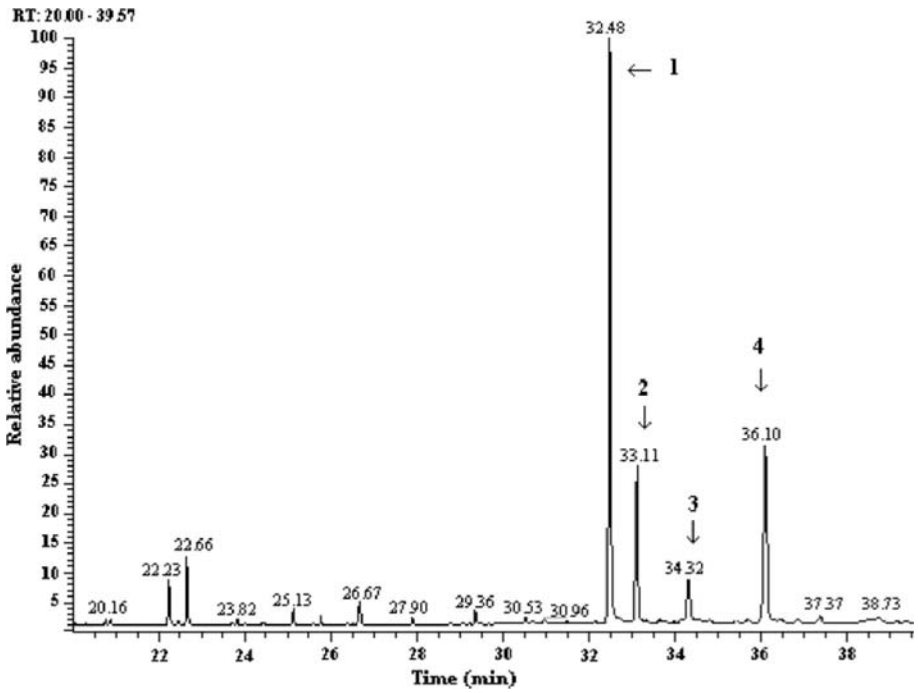

Fig. 2 Partial TIC profiles of the acetylated unsaponifiable matter fraction in phane oil by GC-MS in the full mode scan at 70 eV. *Key*: 1: Cholesterol; 2: 22-dehydrocholesterol; 3: campesterol; 4: sitosterol

Table 6 Relative percent composition of phytosterols and absolute amount of 4-desmethylsterols in Phane oil obtained by capillary GC-MS

Compound	Relative percent composition[a]	4-Desmethylsterols[b] (μg/g of oil)
Cholesterol	53.77 ± 0.45	4482.44
22-Dehydrocholesterol	14.58 ± 0.07	1274.53
24-Me-cholesterol	ND	ND
Campesterol	6.26 ± 0.39	503.83
Campestanol	ND	ND
Stigmasterol	0.08 ± 0.13	21.78
Stigmastanol	ND	ND
Sitosterol	24.16 ± 0.21	1861.95
Δ5-Avenasterol	ND	ND
Sitostanol/Δ5-avenasterol	ND	ND
β-Amyrin	ND	NA
24-Methyllophenol	ND	NA
Cycloartenol	ND	NA
Lupeol	1.16 ± 0.10	NA
22-Dihydrospinasterol	ND	NA
Δ7-Avenasterol	ND	NA
24-Me-cycloartenol	ND	NA
Citrostadienol	ND	NA
Total	100%	8144.53

[a] Values represent the average of three replicate analyses ± SD; [b] Values represent a single SPE run

ND not detected, *NA* not applicable

53.8% of the total sterol content in the oil from the mophane caterpillar. This translated into absolute cholesterol content of 4482.4 μg/g of phane oil, which is much higher than the cholesterol content in raw beef fat (880 μg/g), raw chicken meat with fat (990 μg/g), raw lamb fat (750 μg/g) and raw pork fat (760 *μ*/g) [19]. The measured cholesterol content in the phane oil was comparable only with the cholesterol content in raw lamb kidney (4,000 μg/g), raw ox kidney (4,000 μg/g), raw pig kidney (4,100 μg/g) and raw lamb liver (4,300 μg/g) [19]. Considering the oil content in the mophane caterpillar as approximately 30% and the cholesterol content in the oil as 4.48 mg/g of oil, a 250 g meal of phane would result in cholesterol intake of about 336 mg, which would be higher than the daily recommended intake of cholesterol of 300 mg per day, by the American Heart Association [20] Thus the cholesterol content in phane is rather high and consumers may be advised to eat this delicacy in small portions to avoid the risk of coronary heart disease. Perhaps the mitigating factor on the adverse effect of such high level of cholesterol is that the phane oil also contains substantial amounts of other phytosterols like *β*-sitosterol (1861.95 μg/g), 22-dehydrocholesterol (1274.53 μg/g) and campesterol (503.83 μg/g), which, as mentioned earlier, have been reported to have the property of lowering plasma total cholesterol [7].

This study has shown that the edible mophane caterpillar is a rich source of dietary *α*-linolenic acid, the precursor of the polyunsaturated *ω*-3 essential FAs, eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), which are widely acknowledged to be beneficial in reducing the risk of coronary heart disease and other chronic ailments. In contrast, the study has also revealed that the cholesterol content in phane is several times higher than the cholesterol level in ox, pig or chicken meat and thus chronic hypocholesteremic people may be advised not to include phane in their diet. It has previously been shown that the fatty acid profile of the oil from the mophane caterpillar was very similar to the fatty acid profile of the oil from the leaves of the host plant, *Colophospermum mophane* [14]. This study has clearly shown that it is indeed the sterol profile of the phane oil that truly distinguishes it from oils of plant origin, since oils of plant origin would not be expected to contain such high levels of cholesterol.

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References

1. Sekhwela MBM (1989) The nutritive value of mophane bread-mophane insect secretion (mophote or maboti). Botsw Notes Rec 26:151–154
2. Yeboah SO, Motshegwe SM, Holmback J (1998) General properties and fatty acid composition of the oil from the mophane caterpillar, *Imbrasia belina*. J Am Oil Chem Soc 75(6):725–728
3. Hunter JE (2001) Studies on effects of dietary fatty acids as related to their position on triglycerides. Lipids 36:655–668
4. El-Handy AH, El-Fizga N (1995) Detection of olive oil adulteration by measuring its authenticity factor using reversed-phase high performance liquid chromatography. J Chromatogr 708:351–355
5. Damirchi SA, Savage GP, Dutta PC (2005) Sterol fractions in hazelnut and virgin olive oils and 4,4-dimethylsterols as possible markers for detection of adulteration of virgin olive oil. J Am Oil Chem Soc 82:717–725
6. Awad AB, Fink CS (2000) Phytosterols as anticancer dietary components: evidence and mechanism of action. J Nutr 130:2127–2130
7. Piironen V, Lindsay DG, Miettinen TA, Toivo J, Lampi AM (2000) Plant sterols: biosynthesis, biological function and their importance to human nutrition. J Sci Food Agric 80:939–966
8. Holmback J (2000) Magnetic moments: NMR spectroscopy in lipid science. Dissertation, Royal Institute of Technology, Stockholm, pp 23–54
9. Panfili G, Alessandra F, Mario I (2003) Normal phase high-performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals. J Agric Food Chem 51:3940–3944

10. Wilson PW, Kodicek E, Booth VH (1962) Separation of tocopherols by gas-liquid chromatography. Biochem J 84:524–531
11. Damirchi SA, Dutta PC (2006) Novel solid-phase extraction method to separate 4-desmethyl-, 4-monomethyl-, and 4,4′-dimethylsterols in vegetable oils. J Chromatogr A 1108:183–187
12. Mitei YC, Ngila JC, Yeboah SO, Wessjohann L, Schmidt J (2008) NMR, GC-MS and ESI-FTICR-MS profiling of fatty acids and triacylglycerols in some Botswana seed oils. J Am Oil Soc 85:1021–1032
13. Gunstone FD (1979) Lipids. In: Barton D, Ollis WD, Haslam E (eds) Comprehensive organic chemistry: the synthesis and reactions of organic compounds. Pergamon, Oxford, UK, pp 633–664
14. Yeboah SO, Pharithi MT, Suping SM (2004) Variations of the fatty acid composition in the oil from the larval stages of the emperor moth caterpillar, *Imbrasia belina*. Bull Chem Soc Ethiop 18(1):67–72
15. Bruni R, Medici A, Guerrini A, Scalia S, Poli F, Romagnoli C, Muzzoli M, Sacchetti G (2002) Tocopherol, fatty acids and sterol distributions in wild Ecuadorian *Theobroma subincanum* (Sterculiaceae) seeds. Food Chem 77:337–341
16. Eitenmiller R, Lee J (2004) Analysis of tocopherols and tocotrienols in foods. In: Eitenmiller R, Lee J (eds) Vitamin E—food chemistry, composition and analysis. Marcel Dekker, New York, pp 323–423
17. Eitenmiller R, Lee J (2004) Oxidation and the role of vitamin E as an antioxidant in foods. In: Eitenmiller R, Lee J (eds) Vitamin E - food chemistry, composition and analysis. Marcel Dekker, New York, pp 89–135
18. Rena RJ, White KD, Jahngen EG (1997) Validated method for quantitation and identification of 4,4-desmethylsterols and triterpene diols in plant oils by thin-layer chromatography-high resolution gas chromatography-mass spectrometry. J Assoc Anal Chem Int 80:1272–1280
19. Holland B, Welch AA, Unwin ID, Buss DH, Paul AA, Southgate DAT (1995) McCance and Widdowson's the composition of foods, 5th edn. The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food, Cambridge, pp 124–186
20. American Heart Association (2009) Cholesterol. http://www.americanheart.org/presenter.jhtml?identifier=4488. Accessed Feb 2009