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Research Note

Fatty Acid Composition and Volatile Constituents of *Protaetia brevitarsis* Larvae

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ABSTRACT: A total of 48 different volatile oils were identified form *P. brevitarsis* larvae by gas chromatography/mass spectrometry (GC/MS). Acids (48.67%) were detected as the major group in *P. brevitarsis* larvae comprising the largest proportion of the volatile compounds, followed by esters (19.84%), hydrocarbons (18.90%), alcohols (8.37%), miscellaneous (1.71%), aldehydes (1.35%) and terpenes (1.16%). The major volatile constituents were 9-hexadecenoic acid (16.75%), 6-octadecenoic acid (14.88%) and *n*-hexadecanoic acid (11.06%). The composition of fatty acid was also determined by GC analysis and 16 fatty acids were identified. The predominant fatty acids were oleic acid ($C_{18:1}$, 64.24%) followed by palmitic acid ($C_{16:0}$, 15.89%), palmitoleic acid ($C_{16:1}$, 10.43%) and linoleic acid ($C_{18:2}$, 4.69%) constituting more than 95% of total fatty acids. The distinguished characteristic of the fatty acid profile of *P. brevitarsis* larvae was the high proportion of unsaturated fatty acid (80.54% of total fatty acids) versus saturated fatty acids (19.46% of total fatty acids). Furthermore, small but significant amounts of linoleic, linolenic and γ-linolenic acids bestow *P. brevitarsis* larvae with considerable nutritional value. The novel findings of the present study provide a scientific basis for the comprehensive utilization of the insect as a nutritionally promising food source and a possibility for more effective utilization.

Keywords: *Protaetia brevitarsis*, fatty acid, volatile oil, simultaneous distillation extraction (SDE), GC, GC/MS

INTRODUCTION

Over 1,000 species of insects are widely consumed as an important source of nutrition in Asia, Africa, South America and Australia (1,2). Most of the edible insects are rich in proteins with essential amino acids comparable to the major sources of protein such as meat products (3). In general, consumption of edible insects may contribute to not only the total intake of protein but also significant nutritional value such as fat, minerals and vitamins (4). The fat contents of edible insects varied with species, which contained diverse fatty acids including essential fatty acids such as linoleic ($C_{18:2}$) and linolenic acid ($C_{18:3}$) (1). In addition, edible insects have higher feed conversion efficiencies than conventional livestock (5).

The order Coleoptera (beetles) contains edible insects and the larvae and/or adult beetles were used as an important food source in Asian countries due to their nutritional values (6). Pemberton (7) reported an overview of insects as traditional medicine, which showed evidence that larvae belonging to the Scarabaeidae family is one of the most important traditional medicine in Korea. Despite the fact that edible insects possess both various nutrients and bioactivities, the studies of insects as food sources and/or functional foods have been very limited compared with those of plants and animals due to their repulsive appearance. Only grasshopper and silkworm are approved as edible insects in Korea (4,8).

The white spotted flower chafer, *Protaetia brevitarsis* (*P. brevitarsis*), part of the family of Scarabaeidae belonging to the order Coleoptera, are primarily found in Eastern Asia and the larvae stage have been used as a traditional medicine (9). *P. brevitarsis* larvae possess therapeutic effects in the treatment and prevention of breast cancer, inflammatory disease and liver-related diseases such as hepatic cancer, liver cirrhosis, and hepatitis (10,11). High antioxidant effect of *P. brevitarsis* at different growth stages were reported (9) and the antimicrobial peptides protaetins have been purified from *P. brevitarsis* larvae

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(12). Despite the fact that *P. brevitarsis* larvae demonstrated various biological activities, composition analysis of *P. brevitarsis* larvae have not been studied yet. Furthermore, the majority of the previous studies on *P. brevitarsis* larvae were mainly focused on the biochemistry and the physiology of the insect itself (13-17).

Our preliminary analyses suggest that *P. brevitarsis* larvae possess significant amounts of unsaturated fatty acids. However, only limited studies on nutritional characteristics and profiles of lipid soluble components of *P. brevitarsis* larvae have been studied. Therefore, in the present study, we have analyzed both the composition of the essential oils and fatty acid profiles of *P. brevitarsis* larvae to give the possibility for application as a valuable lipid source and also providing a scientific basis for further comprehensive utilization of insects as well.

MATERIALS AND METHODS

Sample preparation

P. brevitarsis larvae were supplied by World Way Corp (Chungnam, Korea) in February, 2012. In brief, massive growth of *P. brevitarsis* larvae were randomly collected and washed with distilled water. After freeze dried at −20°C, the samples were milled using a food grinder and passed through a 30-mesh sieve. The powder of *P. brevitarsis* larvae was sealed in airtight containers and stored at −70°C before analysis.

Proximate composition

The proximate composition (ash, moisture, lipid, protein and carbohydrate) of *P. brevitarsis* larvae was determined by following standard methods of the Association of Official Analytical Chemists (AOAC) (18). Briefly, the crude protein content was determined by the Kjeldahl method and calculated by multiplying the nitrogen content using a factor of 6.25. Crude fat was extracted from *P. brevitarsis* larvae powder in a Soxhlet apparatus with ethyl ether as the solvent. Moisture content was determined by drying the sample in an oven at 105°C until a constant weight was obtained. Ash content was determined by dry-ashing in a furnace at 550°C for 5 hr. Carbohydrate content had been determined by subtracting the sum of the weights including ash, moisture, protein and lipid.

Fatty acid analysis

Fatty acid composition was determined using GC of fatty acid methyl esters (FAME). The preparation of FAME was slightly modified according to Van Wijngaarden (19). Briefly, 3 g of sample was mixed with 5 mL of tetrahydrofuran (THF) and 30 mL of 1 N ethanolic potassium hydroxide solution for saponification in a flask. Reflux condenser was attached to the flask and heated at 85°C for 90 min on the heating mantle. The mixture was cooled to room temperature and filtered using Whatman filter paper (No. 2). The filtered solvent acidified with HCl and petroleum ether:distilled water (40:60 v/v) were added. The ether extract layer was collected and dried over Na_2SO_4. After Na_2SO_4 was filtered out, solvent was removed by using a sand bath. For the methylation of sample, approximately 40 mL of methanol and 0.5 mL of H_2SO_4 were added and the mixture was refluxed for 3 hr. After cooling, diethyl ether (30 mL) and distilled water (50 mL) were added. The ether layer, which contained fatty acid methyl esters, was drawn off and the aqueous layer was backwashed with another 30 mL of ether. The ether was removed by using a rotary vacuum evaporator, and the methyl esters were dissolved in methylene chloride for GC. Analysis of FAME was performed on a HP 6890N GC-FID (Hewlett-Packard Co., Wilmington, DE, USA), equipped with a Supelco™ SP-2560 capillary column (100 m×0.25 mm×0.20 μm) (Sigma-Aldrich, St. Louis, MO, USA). The split ratio was 50:1 and 1 μL of solution was injected into the column. Helium was used as the carrier gas with flow rate of 1 mL/min. The oven temperature was kept at 140°C for 5 min, increased at a rate of 3°C/min to 240°C, and held at 240°C for 10 min. The injector and detector temperatures were maintained at 260°C. The fatty acids were identified by comparing their retention times with those of the FAME standards under the same conditions.

Simultaneous distillation extraction (SDE) of volatile compounds

The volatile compounds were extracted with a modified Likens-Nickerson apparatus as described by Schultz (20). Ten grams of sample was placed in a 2 L round bottom flask with 1 L of deionized water. Sample was extracted using 100 mL of redistilled *n*-pentane : diethyl ether (1:1, v/v). Extraction time was set for 4 hr after the distilled water started to boil in the sample flask. The extract was dehydrated by anhydrous sodium sulfate at −4°C for 24 hr and concentrated to a final volume of approximately 0.5 mL using a rotary vacuum evaporator (N-1100, EYELA, Tokyo, Japan).

Analysis of volatile compounds by GC/MS

The analysis of volatile compounds was carried out on an Agilent 7890A gas chromatograph equipped with a HP-5MS capillary column (30 m×0.25 mm id, film thickness 0.25 μm) (Aglient Technologies Inc., Santa Clara, CA, USA). The capillary column was directly coupled to an Agilent 5975C mass spectrometer (Agilent Technologies Inc.). The carrier gas was helium with a flow rate of 1 mL/min. The sample was injected with a

split ratio of 1:10 into the capillary column. Injector and detector temperatures were set at 250°C and 230°C, respectively. The GC oven temperature was held at 80°C for 2 min, and then programmed from 60 to 300°C for 10 min at a rate of 5°C/min.

Identification of fatty acids and volatile compounds

The volatile compounds were identified by comparing their retention time with those of known compounds and also by comparing their mass spectra with those stored in the National Institute of Standards and Technology 11 (NIST 11) Mass Spectral Library. Some of the identification was confirmed by injecting the chemical standards into the GC/MS system. The fatty acids were identified by comparing their retention times with those of the FAME standards under the same conditions.

RESULTS AND DISCUSSION

Proximate composition of *P. brevitarsis* larvae

Table 1 shows the results of the proximate composition analysis of *P. brevitarsis* larvae. *P. brevitarsis* larvae contained 54.25±1.22% crude protein, 26.70±1.77% crude fat, 10.61±1.22% carbohydrate, 4.45±0.03% crude ash and 3.99±0.16% moisture. The crude protein content was similar to other edible insects such as *Oecophylla smaragdina* (53.46±0.98%) and *Copris nevinsoni* (54.43±0.26%) (21). *P. brevitarsis* larvae with high protein might be used as a valuable alternative dietary source in developing countries faced with a nutritional imbalance. The crude fat content of *P. brevitarsis* larvae was comparable to those of *Brachytrupes portentosus* (20.60±0.60%) and *Tessaratoma papillosa* (23.55±0.78%) (21). The overall results demonstrated that *P. brevitarsis* larvae are a good nutritional source especially for fat and protein.

Table 1. Proximate composition of *Protaetia brevitarsis* larvae

Component	Composition (%)
Moisture	3.99±0.16
Crude protein	54.25±1.22
Crude fat	26.70±1.77
Crude ash	4.45±0.03
Carbohydrate	10.61±1.22

Data are expressed as mean±SD (n=3) on a dry weight basis.

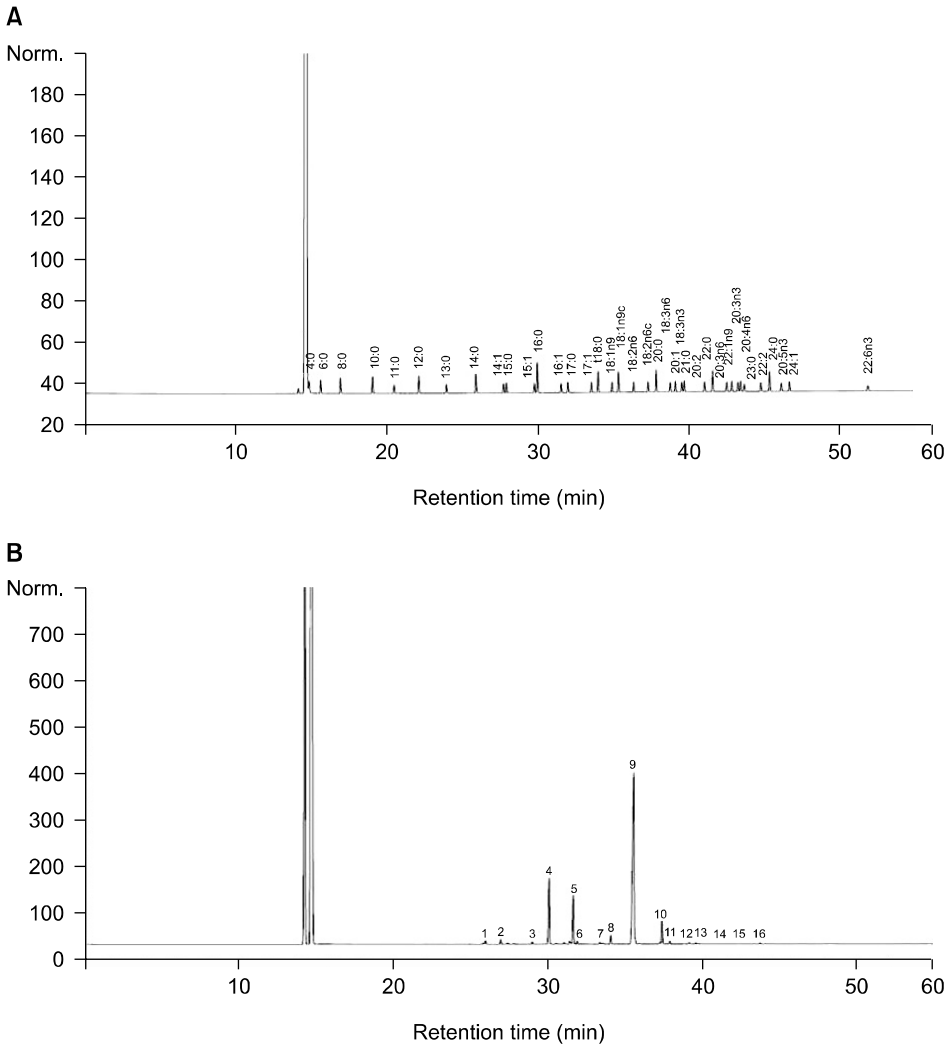

Fig. 1. Gas chromatogram of standard fatty acids (A) and fatty acids of *Protaetia brevitarsis* larvae (B). Identification of major peaks: 4, palmitic acid; 5, palmitoleic acid; 9, oleic acid; 10, linoleic acid. For peak number, see Table 3.

Fatty acids composition of *P. brevitarsis* larvae

Our preliminary study suggested that *P. brevitarsis* larvae contained relatively high content of unsaturated lipid and therefore gas chromatography (GC) analysis was performed in order to investigate the fatty acid profile of *P. brevitarsis* larvae. The chromatograms shown in Fig. 1 correspond to the standard fatty acid methyl ester (A) and fatty acids in *P. brevitarsis* larvae (B). The detailed fatty acid composition and its retention time of *P. brevitarsis* larvae are presented in Table 2, in which the resulting data were expressed as both grams of each fatty acid per 100 grams of *P. brevitarsis* larvae on a dry weight basis and percentage of total fatty acids.

The main characteristic of the fatty acid composition in *P. brevitarsis* larvae was the high proportion of unsaturated fatty acids (80.54% of total fatty acids) versus saturated fatty acids (19.46% of total fatty acids). The predominant fatty acid was monounsaturated fatty acids (MUFA, 75.57%), such as oleic acid ($C_{18:1}$), myristoleic acid ($C_{14:1}$), palmitoleic acid ($C_{16:1}$) and eicosenoic acid ($C_{20:1}$), followed by saturated fatty acids (SFA, 19.46%) and polyunsaturated fatty acids (PUFA, 4.97%). Overall, a total of 16 fatty acids were identified and oleic acid ($C_{18:1}$) was the most predominant fatty acid in *P. brevitarsis* larvae, which accounted for 64.23% of total fatty acids. The other major fatty acids were palmitic acid ($C_{16:1}$, 15.89% of total fatty acids), palmitoleic acid ($C_{18:2}$, 10.43% of total fatty acids), linoleic acid ($C_{18:0}$, 4.69% of total fatty acids) and stearic acid ($C_{14:0}$, 1.81% of total fatty acids), comprising more than 95% of total fatty acids in *P. brevitarsis* larvae. Oleic acid was also reported to be the primary fatty acid in the larvae of *Imbrasia belina*, *Oryctes rhinoceros* and *Rhynchophorus phoenicis* (21,22) are well known for decreasing risk of cancer, heart attack, atherosclerosis and dementia (23,24).

Essential fatty acids such as linoleic acid ($C_{18:1}$, ω6) and linolenic acid ($C_{18:3}$, ω3) were found as 4.69% and 0.23% of total fatty acids, respectively, in *P. brevitarsis*

Table 2. Profile of the fatty acids from *Protaetia brevitarsis* larvae

No.	Components	Fatty acid	Retention time (min)	Amount (g/100 g)	Content (% of total fatty acids)
1	Myristic acid	$C_{14:0}$	25.90	0.10	0.70
2	Myristoleic acid	$C_{14:1}$	27.73	0.02	0.15
3	Pentadecanoic acid	$C_{15:0}$	27.93	0.02	0.11
4	Palmitic acid	$C_{16:0}$	30.01	2.33	15.89
5	Palmitoleic acid	$C_{16:1}$	31.57	1.53	10.43
6	Heptadecanoic acid	$C_{17:0}$	31.99	0.01	0.09
7	*cis*-10-heptadecanoic acid	$C_{17:1}$	33.45	0.04	0.30
8	Stearic acid	$C_{18:0}$	34.00	0.27	1.81
9	Oleic acid	$C_{18:1}$	35.48	9.44	64.24
10	Linoleic acid	$C_{18:2}$	37.30	0.69	4.69
11	Arachidic acid	$C_{20:0}$	37.82	0.08	0.54
12	γ-Linolenic acid	$C_{18:3}$	38.75	0.01	0.05
13	*cis*-11-Eicosenoic acid	$C_{20:1}$	39.09	0.07	0.45
14	Linolenic acid	$C_{18:3}$	39.51	0.03	0.23
15	Heneicosanoic acid	$C_{21:0}$	39.70	0.01	0.09
16	Tricosanoic acid	$C_{23:0}$	43.64	0.03	0.23

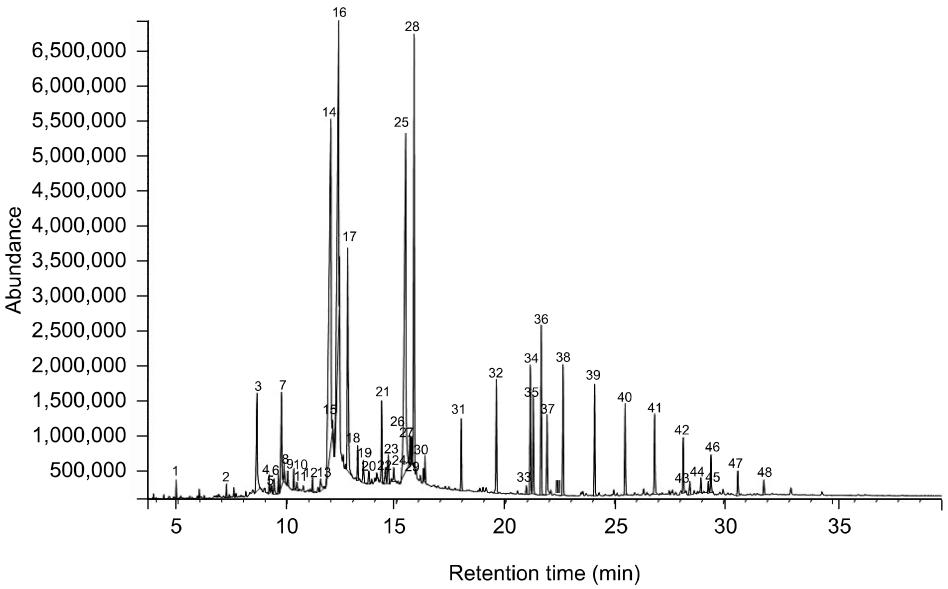

Fig. 2. GC/MS Chromatogram of volatile compounds *in Protaetia brevitarsis* larvae.

larvae (Table 2). γ-Linolenic acid ($C_{18:3}$, ω6), commonly derived from evening primrose oil, borage and black currant, was also identified in *P. brevitarsis* larvae (0.05% of total fatty acids). Small but significant amounts of linoleic, linolenic and γ-linolenic acid bestow *P. brevitarsis* larvae with considerable nutritional value. Several odd-chain fatty acids including pentadecanoic acid ($C_{15:0}$), heptadecanoic acid ($C_{17:0}$), heneicosanoic acid ($C_{21:0}$), tricosanoic acid ($C_{23:0}$) were identified from *P. brevitarsis* larvae, all of which are rarely detected in insect fatty acids.

Volatile oil composition of *Protaetia brevitarsis* larvae

The identified volatile compounds in *P. brevitarsis* larvae analyzed by GC/MS are shown in Fig. 2. A total of 48 compounds were identified (in parenthesis, the number of chemical compounds identified), which included hydrocarbons (12), esters (11), acids (7), alcohols (6), ter-

Table 3. Relative content of functional groups in identified volatile compounds from *Protaetia brevitarsis* larvae

Functional groups	Number	Relative area %
Acids	7	48.67
Alcohols	6	8.37
Aldehydes	3	1.35
Esters	11	19.84
Hydrocarbons	12	18.90
Miscellaneous (including unknowns)	5	1.71
Terpenes	4	1.16
Total	48	100

Table 4. Volatile compounds of *Protaetia brevitarsis* larvae

No	Compound name	Retention time	Relative peak area %
1	Butylated hydroxytoluene	4.913	0.28
2	Cyclododecene, 1-methyl-	7.221	0.25
3	Tetradecanoic acid	8.626	2.9
4	Tetradecanoic acid, ethyl ester	9.187	0.33
5	*cis*-11-Hexadecenal	9.405	0.34
6	Tetradecanal	9.596	0.33
7	Dodecanoic acid	9.741	2.39
8	Pentadecanoic acid	9.867	0.43
9	2,4-Diphenyl-4-methyl-2(E)-pentene	10.038	0.34
10	Pentadecanoic acid, ethyl ester	10.308	0.37
11	Undecanoic acid, 2,8-dimethyl-, methyl ester	10.454	0.23
12	4-t-Butyl-2-(.alpha.,.alpha.dimethylbenzyl)phenol	11.166	0.25
13	Hexadecanoic acid, methyl ester	11.542	0.37
14	9-Hexadecenoic acid	12.004	16.75
15	Hexadecenoic acid, Z-11-	12.083	0.26
16	*n*-Hexadecanoic acid	12.36	11.06
17	Hexadecanoic acid, ethyl ester	12.776	5.76
18	Octadecanal	13.237	0.68
19	Phenol, 2-(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl)-	13.495	0.59
20	10,18-Bisnorabieta-8,11,13-triene	13.745	0.37
21	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl)-	14.332	1.63
22	Heptadecanoic acid, ethyl ester	14.504	0.4
23	8-Octadecenoic acid, methyl ester, (E)-	14.642	0.75
24	Unknown	14.893	0.35
25	6-Octadecenoic acid, (Z)-	15.434	14.88
26	Unknown	15.632	0.55
27	Linoleic acid ethyl ester	15.704	0.45
28	Ethyl oleate	15.823	9.16
29	Octadecanoic acid, ethyl ester	16.252	0.22
30	Heptadecane	16.324	0.44
31	Eicosane	17.98	1.44
32	Heneicosane	19.596	2.45
33	2,4-Diphenyl-4-methyl-1-pentene	20.968	0.2
34	Tricosane	21.146	2.68
35	Phenol, 2,4-bis(1-methyl-1-phenylethyl)-	21.285	2.05
36	2,4-Bis(dimethylbenzyl)-6-t-butylphenol	21.648	3.51
37	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	21.918	1.8
38	Hexacosane	22.65	2.67
39	Heptacosane	24.095	2.35
40	Tetracosane	25.493	1.96
41	Octadecane	26.846	1.88
42	Triacontane	28.158	1.22
43	Phenol, 2,4,6-tris(1-methyl-1-phenylethyl)-	28.462	0.34
44	Unknown	28.963	0.3
45	Unknown	29.306	0.23
46	Heptadecane, 8-methyl-	29.425	0.88
47	Tetratriacontane	30.658	0.61
48	Tetratetracontane	31.85	0.32

penes (4), aldehydes (3) and miscellaneous including unknowns (5) (Table 3, 4). Acids (48.67%) were the largest group of volatile compounds in *P. brevitarsis* larvae, followed by esters (19.84%), hydrocarbons (18.90%), alcohols (8.37%), miscellaneous (1.71%), aldehydes (1.35%) and terpenes (1.16%). The major compounds belonging to acids of *P. brevitarsis* larvae ranged in chain-length of carbons from C_{12} to C_{18}. The major acid compounds were 9-hexadecenoic acid (16.75%), 6-octadecenoic acid (14.88%) and *n*-hexadecanoic acid (11.06%).

The ester group derived from the esterification of alcohols with fatty acids was characterized as the second major chemical group. The major constituents of the esters were ethyl oleate (9.16%), hexadecanoic acid, ethyl ester (5.76%) and 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester (1.80%). Ethyl oleate and hexadecanoic, ethyl ester were also identified from edible black ants (*Polyrhachis vicina* Roger) (25).

Among the hydrocarbons group, tricosane (2.68%), hexacosane (2.67%), heneicosane (2.45%), heptacosane (2.35%), tetracosane (1.96%), octadecane (1.88%) and eicosane (1.44%) were identified. Six alcohol compounds were determined (8.37% of the total volatile components) including 2,4-bis(dimethylbenzyl)-6-t-butylphenol (3.51%), phenol, 2,4-bis(1-methyl-1-phenylethyl) (2.05%), phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl)- (1.63%).

In the present study, we investigated the composition of fatty acids and volatile compounds of *P. brevitarsis* larvae. The results may provide a scientific basis for the comprehensive utilization of insects, as well as to give the possibility for application as a food source. The novel findings of the present study suggest that *P. brevitarsis* larvae could be used as a nutritionally promising food source in virtue of its high content of proteins and lipids and, furthermore, might be a starting point for encouraging insects as a useful food source. Studies in protein characterization of *P. brevitarsis* larvae will be needed to further understanding of the larvae, although the study of bioactive components in *P. brevitarsis* larvae is currently underway.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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