Fatty acid composition of some edible dung beetles in Thailand

Bophimai, P. and *Siri, S.

Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

Abstract: Many species of dung beetles are consumed in the Northeastern region of Thailand, but their fatty acid composition has not been reported. In this paper, the fatty acid profiles of six species of edible dung beetles were evaluated, including *Onthophagus seniculus, Onthophagus mouhoti, Onitis spp*., *Copris nevinsoni, Liatongus rhadamitus, and Heliocopris bucephalus*. The lipid contents were similar in most species (12.1-14.0%), except in *Onitis* spp., which contained only 6.6%. In all species, palmitic acid (C16:0) was the major saturated fatty acids, while oleic acid (C18:1n9) and linoleic acid (C18:2n6) were the most abundant monounsaturated and polyunsaturated fatty acids, respectively. The long-chain fatty acids (C20:4n6) was also found in some species. A low ratio of omega-6/omega-3 (about 2/1 to 5/1) in some species is suitable for human diet. The results of this work would possibly be a nutritional reference concerning fat content and composition for local consumers.

Keywords: edible insect, dung beetle, fatty acid, omega-3, omega-6

Introduction

Fats, also known as lipids, are important for health. They provided a source of energy for body processes and a source of essential fatty acids, sometimes referred to as vitamin F. Fats also used for the transportation and absorption of fat-soluble vitamins and nutrients, the synthesis of hormones, cellular membrane, structural elements in cells, and the protection of vital organ (Lee, 1997). Nevertheless, fats are unpopular and avoidable for consumers, being considered as unhealthy. In fact, the consumption of proper quality and quantity of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) in diets may benefit human health. Many evidences showed that some dietary saturated fatty acids and trans fatty acids could raise serum cholesterol concentrations and increase a risk of cardiovascular diseases (CVD), therefore, low SFA and trans-fatty acid uptake was recommended in a nutritionally adequate diet (Grundy, 1997; German and Dillard, 2004; Lefevre *et al*., 2004). On the other hand, a consumption of PUFAs is more benefit to human health. A high intake of long-chain n-3 fatty acids of fish oils could reduce incidence of coronary heart disease, primarily due to its effect on prevention of ventricular arrhythmias (Charnock *et al*.,1991). Regular intakes of certain amounts of alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid were suggested to prevent and treat CVD (Gebauer *et al*., 2006). Docosahexaenoic acid and arachidonic acid were important to nerve functions and the right balance between n-3 and n-6 fatty acids was suggested to be important to mental health (Haag, 2003). In general, SFAs are commonly found in coconut oil, butter fat, and most fats and oils. Major sources of n-6 fatty acids are in vegetable oils such as corn, safflower and soybean oil, whereas n-3 fatty acids are mainly found in fish, such as salmon, trout, and tuna. The studies of fatty acid profiles in conventional meats, such as pork, beef, chicken, and fish have been available for consumers (Wood *et al*., 2008). However, in many countries, insects are considered as abundance alternative sources of nutrients. Although, in some countries, insects could serve as the major nutrients in the areas encountered food deficient problems, in many other countries, insects were just the palatable dishes, regularly consumed by local people. However, their nutritional values, especially in fatty acid compositions, which are important information for consumers, have yet studied.

In Thailand, several hundred species of insects have been consumed on regular meals or snacks. Among these, several species of dung beetles, locally called “Gud-ji”, are one of delicious insects, although, they are unconventional food for people of different regions due to their habitant and feeding behavior. Dung beetles are mostly found in rice fields and cattle farms, where cattle manure is found since their tunnel nests are built underneath. Dung beetle adults feed on fluid extracted from cattle dung, while larvae live on undigested plant fibers in the dung. Although, more than 200 species of dung beetles were identified (Hanboonsong, 2003) only some are

*Corresponding author.
Email: *ssinee@kku.ac.th*
Tel: +66897119112, Fax: +6643342911

edible according to local people. Excluded head and wings, the rest portion of dung beetles can be eaten by various means such as grilling, boiling, and streaming. Although, nutritional values of some species of dung beetles were reported to contain high protein contents (Kloinhom *et al.*,1984; Hanboonsong, 2003) no data on their fatty acid compositions is reported. This work, therefore, is aimed to determine the fatty acid compositions of six species of edible dung beetles commonly consumed in Thailand.

Materials and Methods

Sample collection

Six species of edible dung beetle mostly found in Khon Kaen province, Thailand were collected from their habitants, in a field near or under cattle dung. Their scientific names were identified by Insect Museum, Department of Agriculture, Khon Kaen University. Samples were washed with water and stored at -20°C before analysis. Analyses were conducted in triplicate (n=3).

Crude lipid extraction

Crude lipids were extracted from 2 g of dried, ground samples using Soxtec system HT (Tecator, Hoganas, Sweden) according to the AOAC No. 920.39 standard protocol (AOAC, 2000). A cellulose thimble containing the sample was extracted in the boiling petroleum ether for 30 min and subsequently rinsed with the solvent for one more hour. The extracted solvent containing lipid material was evaporated at 70°C for 30 min in a hot air oven. The amounts of crude lipids were reported per 100g dry weight of the samples.

Fatty acid analysis

Crude lipid of 0.01 g was used for fatty acid composition analysis. Fatty acid methyl esters (FAMEs) of extracted fat were prepared by Ruiz Lopez's method (Ruiz-López *et al.,* 2003). Ruize Lopez solution (Methanol: Toluene: DMP: H_2SO_4 in ratio 39:20:5:2) was used to hydrolyze the fatty acids and yield methyl groups to form FAMEs. The FAMEs (1.5 µl) were analyzed using a gas chromatography 14B and C-R7A (Shimadzu, Tokyo, Japan) equipped with a fused capillary BPX70, SGE column (30 m length x 0.25 mm width x 25 µm diameter) and flame ionization detection using helium as the carrier gas. The temperature programming was from 177°C (hold 1 min) to 192°C (hold 3 min) at 3°C/min, and then to 222°C (hold 3 min) at 5°C/min. Temperatures for the injector and detector were 250OC and 300°C, respectively. Identification of FAMEs was analyzed by comparison to the PUFA No.3 quantitative F.A.M.E. standard and AOCS oil reference standard (Sigma, California, USA), including 4 types of SFAs (14:0, 16:0, 18:0, 20:0, 22:0, and 24:0), 5 types of MUFAs (16:1n7, 18:1n7, 18:1n9, 20:1n9, and 22:1n9), and 11 types of PUFAs (16:2n4, 16:3n4, 18:2n6, 18:3n3, 18:3n4, 18:4n3, 20:4n3, 20:4n6, 20:5n3, 22:5n3, 22:6n3). The amounts of fatty acids were shown in mg per 100 g of dried weight and the percents of total fatty acids.

Statistical analysis

Data at least in triplicate were presented as mean ± standard deviation (SD). Statistical analysis was analyzed by the two-way ANOVA testing by SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL) for windows version 11.5. A p value of ≤ 0.05 was considered statistically significant.

Results and Discussion

Total lipid content of edible dung beetles

There were at least 268 dung beetle species of 26 genera in 15 tribes collected from both cultivated and forested in Thailand (Hanboonsong, 2003) but only some of them are edible according to local people that passed on their knowledge from one generation to the next generations. In Northeastern region of Thailand, at least 22 species of both edible and non edible species of dung beetles have been reported (Pimpasalee, 2000). In this work, six edible dung beetle species commonly found in Khon Kaen, the province in the Northeastern region of Thailand, were collected for the study, including *Onthophagus seniculus, Onthophagus mouhoti, Onitis* spp.*, Copris nevinsoni, Liatongus rhadamitus, and Heliocopris bucephalus*, which are locally called Gudji-Wai, Gudji-Leum, Gudji-Pom, Gudji-Ka, Gudji-Som, and Gudji-Bao, respectively (Figure 1). All collected dung beetles are belonged to family Scarabaeidae, order Coleoptera. Their sizes were varied according to the species from approximately 1.5 cm to 4.5 cm in length, among which *Heliocopris bucephalus* is the largest.

Total lipid content of dung beetles is shown in Table 1. The lipid contents were reported per 100 g dried weight of edible portion of the insects. The lipid contents were similar in most species (12.1-14.0%), except in *Onitis* spp., which contained only 6.6%. Since, all insects were collected in the same vicinity and collected period; its lowest fat content of *Onitis* spp. was hypothesized to due to its capability to produce fat with lesser amount, not from a limitation of food, a different environment, or a different stage of the insects.

Fatty acid composition

Fatty acid compositions of 6 species of dung beetles were presented in Table 2. Total fatty acids of the insects ranged from 3,934.1 ± 67.3 mg/100g (dry weight, edible portion) in *Onitis* spp. to 9,832.4 ± 472.3 mg/100g in *Heliocopris bucephalus*. The concentration of total SFAs ranged from 1,440.9 ± 21.2 mg/100g in *Onitis* spp. to 5,406.3 ± 258.2 mg/100g in *Heliocopris bucephalus*. There were three SFAs (14:0, 16:0, and 18:0) detected in all species. In only 2 species of dung beetles, *Onthophagus mouhoti* and *Onitis* spp., six SFAs (14:0, 16:0, 18:0, 20:0, 22:0, and 24:0) were detected. The most abundance SFA in all 6 species of dung beetles was palmitic acid (16:0), which was similar in many edible insects, such as mole cricket, ground cricket, spur-throated grasshopper, giant water bug, true water beetle, water scavenger beetle, and winged reproductive of termite (Yang *et al.*, 2006; Ekpo and Onigbinde, 2007).

The concentration of MUFAs was detected from 2,109.5 ± 48.3 mg/100g in Onitis spp. to 4,152.9 ± 208.0 mg/100g in Heliocopris bucephalus. High concentrations of 16:1n7 and 18:1n9 were detected in all species of dung beetles, which was also reported in some fresh water insects in the order Coleopera (Ghioni et al., 1996). Among detected MUFAs, oleic acid (18:1n9) was the most abundance. Low concentrations of eicosenoic acid (20:1n9) were also detected in 3 species of dung beetles, Onthophagus mouhoti, Onitis spp., and Liatongus rhadamitus, which might synthesized from 18:1n9 precursor by Δ9 desaturase (Barker et al., 2007).

The concentration of total PUFAs ranged from

Figure 1. Six species of edible dung beetles in this study

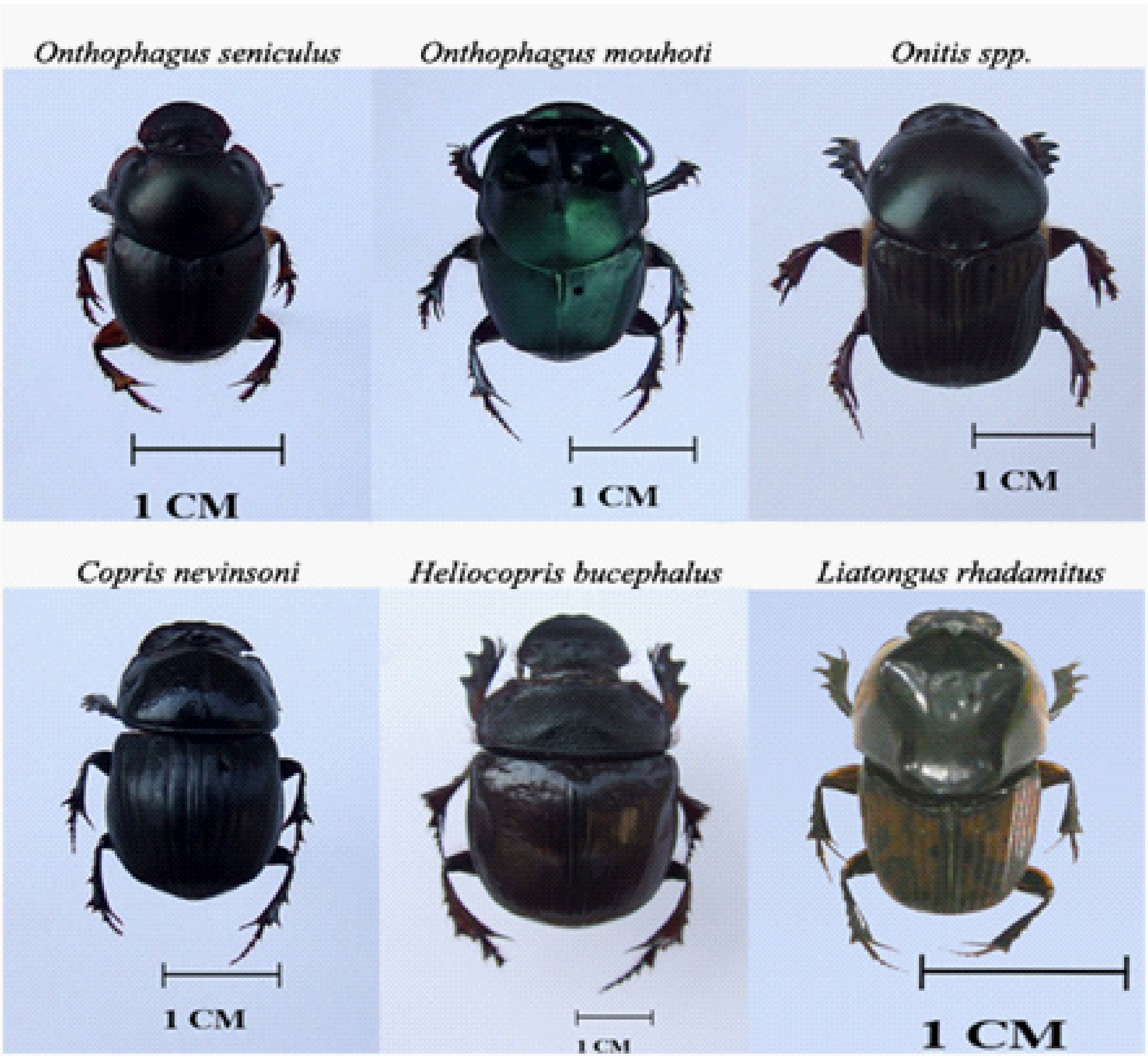

Table 1. Crude fat content (g/100g dry matter) of 6 edible dung beetles (n=6)

Dung beetles	Crude Fat
Onthophagus seniculus (Gudji-Wai)	14.0 ± 0.4
Onthophagus mouhoti (Gudji-Leum)	12.1 ± 0.8
Onitis spp. (Gudji-Pom)	6.6 ± 0.1
Copris nevinsoni (Gudji-Ka)	12.7 ± 0.3
Liatongus rhadamitus (Gudji-Som)	12.2 ± 0.2
Helicopris bucephalus (Gudji-Bao)	13.5 ± 0.6

Table 2. Fatty acid composition of 6 edible dung beetles (mg/100g dry matter) (n=3)

Fatty Acid	*O. seniculus (Gudgi-Wai)*	*O. mouhoti (Gudgi-Leum)*	*Onitis spp. (Gudgi-Pom)*	*C. nevinsoni (Gudgi-Ka)*	*L. rhadamitus (Gudgi-Som)*	*H. bucephalus (Gudgi-Bao)*
C14:0	116.2 ± 4.2	89.3 ± 2.2	42.2 ± 2.1	105.7 ± 1.9	140.7 ± 2.8	89.6 ± 3.6
C16:0	1,666.5 ± 12.8	1,545.2 ± 29.6	734.4 ± 10.2	1,736.8 ± 44.9	1,983.4 ± 45.6	3,965.9 ± 177.8
C18:0	1,244.3 ± 17.4	1,228.2 ± 12.6	510.5 ± 11.9	796.7 ± 21.2	738.4 ± 21.4	1,302.8 ± 68.1
C20:0	42.7 ± 0.5	58.9 ± 1.6	44.3 ± 1.6	nd	34.0 ± 1.6	48.0 ± 8.7
C22:0	41.5 ± 15.9	108.3 ± 6.3	72.4 ± 3.6	nd	nd	nd
C24:0	nd	52.1 ± 2.8	37.1 ± 1.8	nd	nd	nd
SFA	3,111.2 ± 11.6	3,082.1 ± 41.4	1,440.9 ± 21.2	2,639.2 ± 67.7	2,896.6 ± 70.9	5,406.3 ± 258.2
C16:1n7	206.3 ± 31.3	123.3 ± 2.6	91.3 ± 11.8	164.9 ± 5.3	334.3 ± 6.4	158.3 ± 7.7
C18:1n9	3,003.3 ± 33.4	3,218.2 ± 26.5	1,990.2 ± 35.8	2,857.9 ± 82.5	3,307.2 ± 83.4	3,994.6 ± 200.2
C20:1n9	nd	42.9 ± 1.0	28.0 ± 1.0	nd	27.8 ± 7.6	nd
MUFA	3,209.6 ± 2.7	3,384.4 ± 27.6	2,109.5 ± 48.3	3,022.8 ± 87.7	3,669.3 ± 97.0	4,152.9 ± 208.0
C18:2n6	526.3 ± 3.8	611.2 ± 5.9	348.1 ± 8.8	234.9 ± 5.5	266.8 ± 5.0	209.3 ± 9.8
C18:3n3	96.2 ± 1.0	30.5 ± 0.6	20.3 ± 0.3	50.5 ± 3.2	46.4 ± 2.0	63.8 ± 4.4
C20:4n6	41.7 ± 1.1	28.6 ± 0.1	15.3 ± 0.5	48.6 ± 1.8	34.6 ± 1.0	nd
PUFA	663.2 ± 5.5	670.4 ± 5.6	383.7 ± 9.5	334.0 ± 10.5	336.3 ± 17.0	273.1 ± 10.1
Total FA	6,983.9 ± 6.0	7,136.9 ± 72.3	3,934.1 ± 67.3	5,996.1 ± 165.8	6,902.2 ± 155.2	9,832.4 ± 472.3
SFA/UFA	0.8	0.8	0.6	0.8	0.7	1.2
n-6/n-3	5.9	21.0	17.9	5.6	5.5	3.3

nd (not detected); FA (fatty acid); SFA (saturated fatty acid); MUFA (monounsaturated fatty acid); PUFA (polyunsaturated fatty acid); UFA (unsaturated fatty acid = MUFA + PUFA)

273.1± 10.1 mg/100g Heliocopris bucephalus to 670.4 ± 5.6 mg/100g in Onthophagus mouhoti, which accounted for 2.8% and 9.8% of total fatty acids, respectively. The content of PUFAs in all dung beetles was very low compared to the content of SFAs (36.6-55.0%) and MUFAs (42.2-53.6%). Among detected PUFAs, linoleic acid (C18:2n6) was the most abundance. Three PUFAs (18:2n6, 18:3n3, and 20:4n6) were detected in most species, except in Heliocopris bucephalus, whose 20:4n6 was not detected. The presence of the long-chain fatty acids (C20:4n6) was usually found in aquicolous insects, but not in terricolous insects The long-chain fatty acids in aquicolous insects were obtained from their diets of larvae and algae or synthesized by using Δ5 desaturase and Δ6 desaturase enzymes.

Diet with excessive amounts of omega-6 PUFA and a very high omega-6/omega-3 ratio, has been reported to promote the pathogenesis of many diseases, including cardiovascular disease, cancer and inflammatory and autoimmune diseases (Simopoulos, 2006). However, consumption of a lower ratio of omega-6/omega-3 diet resulted in suppressing these effects. Diet with a ratio of omega-6/omega-3 ranging from about 2/1 to 5/1 benefited human health, such as reduction of mortal rate in patients with cardiovascular disease, reduction of colorectal cancer proliferation, repression of inflammation in patients with rheumatoid arthritis, and beneficial effect on patients with asthma (De Lorgeril *et al.*, 1994; Simopoulos, 2006). In this study, a low ratio of omega-6/omega-3 fatty acid was found in 4 species; *Onthophagus seniculus*, *Copris nevinsoni*, *Liatongus rhadamitus* and *Heliocopris bucephalus,* which would be recommended species for consumption.

Conclusions

Fatty acid composition of six edible dung beetles in Thailand was first reported here. Similar pattern of fatty acid composition (6 SFAs, 3 MUFAs, and 3 PUFAs) was found in all species, but differed in amount. Interestingly, long-chain fatty acid was detected in some dung beetles, which was usually found in aquicolous insects, not in terricolous insects. In addition, three PUFAs (C18:2n6, C18:3n3, and C20:4n6), which are important to human diet, were also found in dung beetles, suggesting that dung beetles could be an alternative dietary source of human essential fatty acids.

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