Potential Fatty Acid Composition of *Hermetia illucens* Oil Reared on Different Substrates

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Abstract. *Hermetia illucens* larvae (HiL) has been studied because of its capability to break down organic waste. HiL can convert waste biomass efficiently, that can be used as future feedstuffs. This study aimed to evaluate the fatty acids composition from HiL oil (HiLO) which reared on the different substrates. The experimental was compared of (1) HiL reared on palm kernel meal and (2) HiL reared on 80% industrial waste mix with 20% organic waste. The chemical composition of HiL measured based on the Association of Official Analytical Chemists (AOAC). The *Hermetia illucens* larvae oil (HiLO) was extracted according to the modification of the Soxhlet extraction procedure. Fatty acid compositions were analyzed using gas chromatography (GC). The data collected and analyzed as descriptive. The results of this study ether extract content of HiL (1) 51.46% higher than HiL (2), this is due to substrate types. Fatty acids of both are almost the same and the most dominant are lauric acid 40.54% and 46.72%, oleic acid 17.48% and 15.98%, palmitic acid 14.55% and 12.12% and myristic acid 15.57 and 11.13%, respectively for each HiLO (1) and (2). The conclusion of this study is the HiL reared on different substrates affect lipid content but the fatty acid composition is similar.

1. Introduction
The recent issue of the environmental problem is waste management, especially in the industrial region. Overproduction of waste and limitation recycle process can cause exposure waste to the health environmental. Industrial waste from food industry mostly was organic waste that a non-B3 industrial waste category. Non-B3 industrial waste means if waste does not contain toxicity agent and dangerous things that can directly damage the environment. But if these things not be solved, that can make ecological diseases and can negatively affect to the resident. It is necessary to solve that problem, reduce industrial waste using a biological agent like.

*Hermetia illucens* larvae (HiL) is known as black soldier fly that can degrade organic waste. On the other hand, HiL can produce biomass that can be used as feedstuffs. According to Rozkosný et al. [1], adult HiLs cannot eat because they do not have functional mouths so they depend on reserves that accumulate during the larval period [2]. For this reason, the quality and amount of substrate given to HiL converted well impact on protein and fat content in insects. The recent study reported HiL content high lipid content and great fatty acid composition. Spranghers et al. [3], HiL reared from organic waste has lauric acid (C12:0) highest than other fatty acids component. Other research did by Li et al.
HiL oil does not influence the growth of *Cyprinus carpio* var. Jian but decreased lipid deposition in the intraperitoneal fat tissue by higher gene expression of PPARα. Higher C12:0 components indicate HiLO is saturated oil same as palm kernel oil, its mean HiLO can be used as an energy source in poultry feed. Spranghers *et al.* [3] reported HiL reared on vegetable and restaurant waste highest short chain fatty acid (SFA) 40.68 and 54.05%. Depending on substrate HiL can express different fatty acid composition. From the recent study saturated component domination of HiL fatty acid, HiL as insect either his metabolism or the substrate can dominantly affect the fatty acid composition. This research aims to evaluate fatty acids composition of *Hermetia illucens* larvae that reared on palm kernel meal compare to industrial waste.

### 2. Experimental Details

#### 2.1. Materials

The HiL eggs obtained from PT Sahabat Tani Farm, Bubulak Village, Bogor Regency, West Java in March 2018. Two different substrates were prepared, a substrate (1) was a palm kernel meal and substrate (2) was industrial waste (solid and sludge cake waste from ice cream industry) 80% mix with organic waste 20%. The industrial waste was collected from Cikarang, West Java in March 2018.

#### 2.2. Materials

HiL hatched in started media consists of commercial fish feed, palm kernel meal, soluble sugar, and mineral mix until 7 days in a dark room at temperature 30° C and humidity 75-80%. The ratio of HiL eggs and started media were 1:500 (b/b). In 6th day HiL separate to the experiment substrate with ratio 1:6000 (b/b). HiL reared on the substrate until 11th days, then HiL was harvested. HiL was rinsed using distilled water and dried at 60 °C until 2 days. Then, dried HiL ground and sieved with sieve no.100 mesh. HiLO produces used oil extraction procedure according to AOAC [5], modified. Dried HiL extracted using an n-Hexane solvent with a ratio of 175 gram: 1.5 litres (b/v). Extraction duration was 8 hours to separate rendemen and crude oil. Then the oil was carried out from the solvent using a distillation procedure, finally, HiLO was produced. The yield of oil extract and decrease of ether extract were calculated using the following equation:

\[
Yield \ (\%) = \frac{HiLOil(\text{gram})}{DriedHiL(\text{gram})} \times 100\%
\]

\[
Decrease \ of \ EE \ (\%) = \left( \frac{EEofHiL(\%)}{EEofRendemen(\%)} \right) \times 100\%
\]

note: EE (ether extract measured based on AOAC [5]).

The nutritional composition consists of ash, crude protein (CP), ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) were measured base on AOAC [5], except moisture according to SNI 01.2891 [6]. The HiLO sample sends to PT Saraswanti Indo Genetech and fatty acid profiles were analyzed using AOAC procedure 969.33 [7]. The descriptive analysis performance to the data.

### 3. Results and Discussion

Nutrient compositions listed in Table 1. The moisture, ash, CF, and NFE were similar. Crude protein of HiL (1): reared on palm kernel meal substrate was higher than HiL (2): reared on industrial waste, with values are 37.46 and 24.98%, respectively. This result difference with ether extract component of HiL (1) was lower than HiL (1) with values are 51.46 and 35.08, respectively. These result related to Barragan-Fonseca *et al.* [8], crude protein of *Hermetia illucens* instar 15th was 37% and ether extract was 39%. This is a cause of palm kernel meal as a substrate with higher CP content and the industrial waste used is a substrate with higher EE content. The substrate that contains higher protein express hexamerin-containing genes (HiLSP-1 and HiLSP-2) and fat-related HiLs1 gene. Increasing of that
gene by over-consumption of palm kernel meal can decrease expression of fat in HiL body [9]. But, this mechanism contrast with HiL reared on industrial waste, cause lower protein content that can increase the expression of the fat body.

| Table 1. Nutrient composition of the dried Hermitia illucens larvae |
|---------------------------------------------------------------|
| Nutrient composition (%)                                      |
| Moisture | Ash | CP    | EE    | CF   | NFE   |
| HiL (1)  | 4.78| 9.38  | 37.46 | 35.08| 9.71  | 8.37  |
| HiL (2)  | 7.40| 10.23 | 24.98 | 51.46| 4.02  | 9.31  |
CP: crude protein, EE: ether extract, CF: crude fibre, NFE: non fat extract, HiL (1): reared on palm kernel meal, HiL (2): reared on 80% industrial waste: 20% organic waste

Figure 1. Oil extraction process of Hermitia illucens larvae. A: HiL (1) reared on palm kernel meal, B: HiL (2): reared on 80% industrial waste: 20% organic waste, HiL: ether extract contents in dried HiL (before extraction), EE: ether extract contents of rendemen (after extraction), Decrease of EE: percentages of the effectiveness extraction and Yield: percentage of the oil produced.

Extraction process used n-Hexane can extract EE component as shown in Figure 1. The EE content after extraction HiL (1) and (2) were 17.71 and 13.73%. The EE from rendemen is lower compared to the Mawaddah et al. [10] was 17.95, but higher compare to Schiavone et al. [11] was 4.6%. Using n-Hexan as a solvent to dissolve ether extract or oil component was effective, using a modified Soxhlet procedure. Percentage yield of HiLO (1) was lower than HiLO (2). This is relevant to the decrease of EE of HiL (1) and (2), it cause of content EE from dried HiL (1) was higher than (2).
Table 2: Fatty acid profile of the *Hermetia illucens* larvae oil.

|        | HiLO (1) | HiLO (2) | Δ = HiLO (2) – HiLO (1) |
|--------|----------|----------|-------------------------|
| C 12: 0 (lauric acid), % | 40.54    | 46.72    | 6.19                    |
| C 18: 1W9C (oleic acid), % | 17.48    | 15.95    | 1.53                    |
| C 14: 0 (myristic acid), % | 15.57    | 11.13    | 4.44                    |
| C 16: 0 (palmitic acid), % | 14.55    | 12.12    | 2.44                    |
| Omega 6 fatty acids, %    | 4.47     | 5.77     | 1.30                    |
| C 16: 1 (palmitoleic acid), % | 2.21     | 3.83     | 1.62                    |
| C 18: 0 (stearic acid), % | 2.13     | 1.43     | 0.70                    |
| C 10: 0 (capric acid), %  | 1.27     | 1.44     | 0.17                    |
| Omega 3 fatty acids, %    | 0.32     | 0.12     | 0.20                    |
| C 14: 1 (miristoleic acid), % | 0.22     | 0.60     | 0.38                    |
| C 21: 0 (heneikosanoat acid), % | 0.22     | 0.12     | 0.09                    |
| C 17: 0 (heptadecanoic acid), % | 0.18     | 0.51     | 0.32                    |
| EPA, %                      | 0.15     | 0.00     | 0.15                    |
| C 17: 1 (heptadecanoic acid), % | 0.10     | 0.06     | 0.04                    |
| C 20: 0 (arakidat acid), % | 0.07     | 0.07     | 0.00                    |
| C 20: 1 (eicoseoic acid), % | 0.05     | 0.02     | 0.03                    |
| C13: 0 (tridecanoic acid), % | 0.05     | 0.02     | 0.03                    |
| C 8: 0 (acetic acid), %    | 0.04     | 0.13     | 0.09                    |
| C 23: 0 (tricosanoic acid), % | 0.04     | 0.00     | 0.04                    |
| C 22: 0 (acetic acid), %   | 0.02     | 0.03     | 0.01                    |
| C11: 0 (udekanoic acid), % | 0.02     | 0.02     | 0.00                    |
| DHA, %                      | 0.01     | 0.00     | 0.01                    |
| C 15: 0 (pentadecanoic acid), % | 0.00     | 0.07     | 0.07                    |
| C 4: 0 (butyric acid), %   | 0.00     | 0.04     | 0.04                    |

EPA: eikosanoidpentanoic acid, DHA: docosahexaenoic acid

The fatty acid composition HiLO (1) and (2) are not different. This indicated that the difference of substrate does not affect the fatty acid composition. HiLO has the potential source of lauric, oleic, myristic, palmitic and omega fatty acids as shown in Table 2. This is in accordance with the research of C12: 0 fatty acids, the most abundant component found in HiL fed with fruit waste, C12: 0 was highest at 76.13% fed with fruit waste [12]. Lauric acid combine with lauric acid has antibacterial activity [13]. Oleic fatty acids can function as a stabilizing low-density lipoprotein in the blood. Linoleic acid is precursors of a group of hormone-like eicosanoids, namely prostaglandin, prostacycllin, thromboxane and leukotriene [9].
4. Conclusion
In conclusion, *Hermetia illucens* reared on different substrate affect nutrient composition especially ether extract and crude protein. But the Substrate did not impact the fatty acid composition, with lauric acid as the dominant fatty acid of *Hermetia illucens* larvae.

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