Characteristic of Fatty Acids Biotransfofm from Hermetia Illucens Prepupae Fed with Various Organic Wastes Before Conversion to Methyl Ester Form

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Abstract. Farming of Black solder fly (Hermetia illucens) had received great research interest by scientist to investigate the application of this insect’s larvae as a means of organic waste biotransformation, feed for aquaculture and husbandry as well as for biodiesel feedstock. The compositions of this insect’s larvae is well known to have high content of lipid and protein, this has demonstrates its potential use as animal feed and alternative energy. To the best of our knowledge, this study provides the ultimate investigation on the characteristic of the pre-pupae fatty acids than that of the feeds before conversion to methyl ester for biodiesel production. The study also investigates the impact of different feeds on fatty acid profile of Hermetia illucens. The farming and breeding of Hermetia illucens fly lifecycle was set up and the populations were maintained at Universiti Tunku Abdul Rahman (UTAR), Kampar campus. The newly hatched neonates were fed on fruit waste and food waste, respectively. Continuous feeding was dispensed to the neonates until it became pre-pupae. At this stage, cessations of feeding and self-harvesting by the pre-pupae were observed. The harvesting of the pre-pupae was done at this instar. Thereafter, the harvested live pre-pupae were cleaned and processed into dried pre-pupae. The dried pre-pupae were kept in an air tight container for further use. The characteristic of the feed and the dried pre-pupae were determined in a series of analysis such as crude lipid extraction, free fatty acid, acid value and fatty acid methyl ester analysis. All the analysis was done in accordance to AOAC methods and each analysis was repeated in triplicates. The results from the study shows that, Hermetia illucens pre-pupae crude lipids content as well as fatty acid profile was influenced by types of feeds as their diet. The lipid content found in the larvae biomass were profound regardless the types of feed. Comparatively, the amount of crude lipid obtained from Hermetia illucens pre-pupae was found to be higher than that of all feeds. The amount of crude lipids extracted from feeds of fruit waste and food waste were approximately 1.24 ± 0.06 and 3.27 ± 0.28 wt%, respectively. The amount of pre-pupae’s crude lipid derived from fruit waste and food wastes were higher by 35.85 and 11.48 times, respectively. It is noteworthy that, increase in the lipid accumulation in larvae biomass indicates that the larvae are able to assimilate and bio-convert the feed composition into their own biomass. Consequently, the nutrient composition contains in the types of feed, ingested and digested by the larvae strongly affect the overall lipid accumulation. Moreover, the fatty acid extracted from the Hermetia illucens biomass showed approximately 1.77 times higher in saturated fatty acid (SFA) than that of fruit waste and food waste. Lauric acid is the predominant saturated fatty acid found in the prepupae. The SFA attained by the pre-pupae derived from fruit waste and food wastes were 84.7 and 81.5 wt%, respectively. While the total
unsaturated fatty acids were found to be higher in the feeds than that of the pre-pupae. Meanwhile, the total amount of unsaturated fatty acid from fruit waste and fruit waste were attained at 50.9 and 53.7 wt%, respectively. In conclusion, the next stage of this study will be carried out to evaluate the biodiesel quality of the converted fatty acid methyl ester derived from *Hermetia illucens* fatty acid.

1. Introduction

*Hermetia illucens* larvae (black soldier fly) has been regards as potential prospect for organic waste management that will bring benefits to the environment and socio-economic. These benefits include reduced greenhouse gases (GHG) emissions [1–2], minimizing commercial fertilizer usage, decreased water use, increased soil carbon storage, and decreased soil erosion. The waste biotransform system using the *Hermetia illucens* larvae introduces an alternative concept of waste conversion into valuable products such as protein for animal feed [2], fatty acid for biodiesel [2–4] and frass for fertilisers. Organic waste such as food waste and fruit waste are discarded even before it starts to putrefy. Most of these wastes generated still contain high calorific value as well as nutrient content. If left unmanaged, it is susceptible to environmental pollution and hygiene problem [5]. The *Hermetia illucens* larvae is a decomposer and detritivore. They ingest the organic substance via internal biotransformation process to obtain energy and nutrients for their growth and proliferation. Therefore, it would be an advantage if the organic and the nutrient rich content are transform to higher value product or use as a source for renewable energy. For instance, the grown larvae could be distributed as alternative protein feedstock for chicken, porcine and fish in local farms. This in turn, could increase the yield of local farm animals, improving food and feed security while at the same time reducing environmental pressures and impact [6]. In this manner, it is possible to close the loop and keep valuable nutrients within a cycle. Besides protein, fatty acid is the second most abundant nutrient accumulated in the *Hermetia illucens* larvae. In addition, fatty acids derives from the pre-pupae would be a potential feedstock for biodiesel production. Therefore, in this study, fatty acid derives from pre-pupae insects are used as a starting material to synthesize fatty acid methyl ester (FAME). Comparisons of FAME derived from lipids of the pre-pupae and feeds were further deduced to determine any changes in fatty acid composition. The effect of different types of organic waste on fatty acid profile of *Hermetia illucens* was studied.

2. Materials and methods

The *Hermetia illucens* (*H. illucens*) fly populations was established and the lifecycle were maintained at Universiti Tunku Abdul Rahman (UTAR), Kampar campus. The newly hatched neonates were fed on fruit waste and food waste, respectively. Continuous feeding was dispensed to the neonates until it became pre-pupae. At this stage, cessations of feeding and self-harvesting by the pre-pupae were observed. The harvesting of the pre-pupae was done at this instar. Thereafter, the harvested live pre-pupae were cleaned and processed into dried pre-pupae. The dried pre-pupae were kept in an air tight container for further use.

2.1. Proximate analysis

The characteristic of the feed and the dried pre-pupae were determined in a series of analysis such as crude lipid extraction, free fatty acid (FFA), acid value (AV) and fatty acid methyl ester (FAME) analysis. Soxhlet method was used to determine the crude lipids content for each dried samples. The crude lipid content was determined by measuring the weight difference. The free fatty acids in samples were determined by titrating the supernatant alcohol layer with standard alkali. The free fatty acids were calculated as oleic acid. All the analysis was done in accordance to AOAC methods [7] and each analysis was repeated in triplicates.
2.2. Conversion of fatty acid into FAME
The conversion of fatty acid into FAME was in-situ transesterified via ultra-sonication. The experiment was done in triplicates. The reaction was conducted using methanol to sample mass, reaction time (min), reaction temperature (°C), and catalyst loading (%). An approximately 5 g of the dried pre-pupae were macerated and placed into a screw cap reaction vessel containing mixture of methanol and sulphuric acid. FAME produced was further separated to remove crude glycerol and the remaining upper layer was washed with warm distilled water (70°C) [5]. FAME samples collected were stored in a sample bottle for further analysis. The yield of FAME was determined using equation (1) [8].

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\text{Yield} = \frac{\text{weight of FAME}}{\text{weight of oil}} \times 100\% \tag{1}
\]

2.3. Composition analysis of FAME
The compositions of FAME were analysed using gas chromatography with mass spectrometer (Shimadzu GCMS QP2010) and capillary column packed with 70% of cyanopropyl polysilphenylene-siloxane (60 m × 0.32 mm i.d; 0.25 µm film thicknesses). The oven temperature was set at 115°C, raised to 180°C and held for 10 min and finally raised to 240°C and held for 10 min at a fixed flow rate of 8°C/min. The sample volume was 1µL and split ratio of 1:80. The sample was eluted through helium as carrier gas at rate of 1.6 mL/min. The profiling of FAME was compared between the relative retention times of 37 component FAME mixtures (Sigma Chemical Co., St Louis, MO, USA).

2.4. Statistical analysis
All experiments were conducted in triplicates (n = 3) and were presented as mean ± standard deviation (SD).

3. Results and discussion

3.1. The effect of feed on lipid quantity of H. illucens
The result shows that the feeds consist of nutrients that are essential for the larvae proliferation such as lipids, protein and carbohydrates. The amount of crude lipid obtained from fruit waste and food waste were attained at 1.24 ± 0.06 and 3.27 ± 0.28 %, respectively. Table 1 summarizes the chemical properties of the dried H. illucens pre-pupae’s lipid fed with different feeds of organic waste. Generally, larvae fed on all types of feed showed the accumulation of lipid in their body mass harvest during the pre-pupae stage. Results obtained from table 1, compares the chemical properties of crude lipid produced from the pre-pupae fed with fruit waste and food waste. Crude lipids of H. illucens pre-pupae derived from fruit waste and food waste were approximately 44.46 ± 0.79 and 37.56 ± 0.51%, respectively. Meanwhile, the amount of crude lipids obtained from feeds of fruit waste and food waste were approximately 1.24 ± 0.06 and 3.27 ± 0.28 %, respectively.

Comparatively, the amount of crude lipid obtained from H. illucens pre-pupae was found to be higher than these feeds. The lipid content found in the larvae biomass were profound regardless the types of feed. H. illucens pre-pupae crude lipids content as well as fatty acid profile was influenced by types of feeds as their diet. Highest acid value was achieved at 19.54 ± 4.81 mg KOH/g for pre-pupae derived from food waste and the lowest was achieved at 7.73 ± 1.50 mg KOH/g for pre-pupae derived from fruit waste.
Table 1. Comparison of chemical properties of *H. illucens* fed with various waste.

| Parameter                     | Types of feed                                      |
|-------------------------------|----------------------------------------------------|
|                               | Fruit waste | Food waste (this study) | SRF [4] | Manure [11] |
| Stage of larval               |            |                        |         |
| Pre-pupae                     | 8th day old larvae                                 |
| Crude lipid from *H. illucens*, % | 44.46 ± 0.79 | 37.56 ± 0.51 | 39.2 ± 1.9 | 23.16 |
| Crude lipid from feed, %      | 1.24 ± 0.06 | 3.27 ± 0.28 | Not mention | Not mention |
| AV, mg KOH/g                  | 7.73 ± 1.50 | 19.54 ± 4.81 | 7.1 ± 0.3 | 8.7 ± 0.4 |
| FFA, (% oleic acid)           | 3.89 ± 0.75 | 9.82 ± 2.42 | Not mention | Not mention |

*Values are mean ± SD, n = 3 samples*

The study shows a great significant difference between the crude lipid extracted from feed and pre-pupae (Table 1). Comparatively, the amount of crude lipid obtained from *H. illucens* pre-pupae indicate a remarkable increased in lipid than that of the feeds. The amount of lipid content found in fruit waste and food waste were attained at 1.24 ± 0.06 and 3.27 ± 0.28 %, respectively. Nevertheless, higher amount were achieved by pre-pupae lipid derived from fruit waste and food waste at 44.46 ± 0.79 and 37.56 ± 0.51 %, respectively. Highest pre-pupae lipid was derived from fruit waste. It is noteworthy that, increase in the lipid accumulation in larvae biomass indicates that the larvae are able to assimilate and bio-convert the feed composition into their own biomass.

Presumably, in this study, the high lipids content found in the larvae fed with fruit waste was converted from ingested carbohydrate into energy and the excess of carbohydrate were stored as lipid. The differences in the lipids content accumulated in the larvae biomass maybe contributed by assimilation of other reserved feed composition such as protein. Generally, insect larvae are capable of transforming dietary carbohydrate into fat as storage form and few studies have proven positive correlation between carbohydrate content and lipid deposition [9–10]. Accumulations of lipids in larvae serve as energy storage and used to support metamorphosis, reproduction and flight. Most of the lipid is present as triacylglycerides (TG) and the amount of TG stored varies with the stage of development and state of feeding of the larvae.

3.2. The effect of feed on fatty acid profile of *H. illucens*

The effects of different feeds on fatty acid profile metabolized from the pre-pupae biomasses were investigated. Figure 1 compares between fatty acids of the feeds and the prepupae. Based on figure 1, the composition of fatty acid obtained from the pre-pupae biomass varied among feeds. The major changes with respects to fatty acid compositions derived from pre-pupae of all feeds were predominantly high in lauric acid (C12:0) as compared to fatty acids from feeds.

The amount assimilated by the larvae did not reflect the high percentage of this fatty acid in the feeds (figure 1–2). The most dominance content of lauric acid was observed from the larvae fed with fruit waste attained at 67.84 wt% (figure 1). This was followed by *H. illucens* pre-pupae fed with food waste (55.47 wt%) (figure 2). In contrast, lauric acids obtained from Zheng’s [3–4] studies were lower when fed with solid residual fraction of restaurant waste (SRF) and manure. The values were attained at 23.4 and 35.6 wt%, respectively.

Fatty acids composition of palmitic acid, C16:0 and oleic acid, C18:1n9c were predominant among saturated and unsaturated fatty acids in all feeds but to a much lesser amount in biomasses of the pre-pupae. As shown, biomasses of the pre-pupae did not show an increment of these fatty acids. Comparatively, these fatty acids produced by the pre-pupae were 50 % lesser than that of those feeds. For instance, figure 2 showed the highest compositions of these fatty acids achieved were pre-pupae derived from food waste with palmitic and oleic acid attained at 12.52 and 10.01 wt%, respectively. However, the amount of palmitic acid and oleic acid were higher in feeds at 39.09 and 42.77 wt%, respectively.
**Figure 1.** Comparison of FAME between pre-pupae and feed derived from FrW.
With regards of various feeds, *H. illucens* larvae were perceived to be able to synthesize medium chain of fatty acids. This is further justified based on the high composition of lauric acid of at least 50% from the total fatty acids compositions. Besides C12:0, pre-pupae with mid chain fatty acid of C14:0 were also produced in higher quantity that that of the feeds at most 10 wt% derived from food waste (figure 2). Moreover, the high percentage of these mid chain fatty acids can be further deduced from the DG composition presence in crude lipid derived from the pre-pupae. The predominance contribution of C12:0 was similar amongst the previous study done by Zheng et al. [3–4] and Li et al. [11], although value of lauric acid reported were comparatively low compared to those results in this present study.

**Figure 2.** Comparison of FAME between pre-pupae and feed derived from FW.
4. Conclusion
On the basis of this result, it is conclude that, the bioconversion of waste by the larvae thus effect on the fatty acid composition. In this study, higher content of saturated fatty acid (SFA) were found in the pre-pupae biomasses when fed with fruit waste and food waste. The amounts of SFA attained in this study were 88.06 and 81.56 wt%, respectively. The next stage of this study will be the optimization study for biodiesel production and quality evaluation.

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