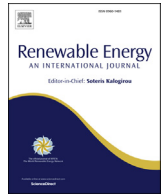




Contents lists available at ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Bioconversion of organic wastes into biodiesel and animal feed via insect farming

K.C. Surendra ^a, Robert Olivier ^b, Jeffery K. Tomberlin ^c, Rajesh Jha ^d,
Samir Kumar Khanal ^{a,*}

^a Department of Molecular Biosciences and Bioengineering, University of Hawai'i at Mānoa, Honolulu, HI, 96822, USA

^b Prota Culture, LLC, Honolulu, HI, USA

^c Department of Entomology, Texas A&M University, College Station, TX, USA

^d Department of Human Nutrition, Food and Animal Sciences, University of Hawai'i at Mānoa, Honolulu, HI, 96822, USA

ARTICLE INFO

Article history:

Received 10 January 2016

Received in revised form

2 March 2016

Accepted 4 March 2016

Available online xxx

Keywords:

Organic wastes

Insect farming

Black soldier fly

Biodiesel

Animal feed

ABSTRACT

Approximately one-third of all food produced for human consumption worldwide is wasted. The current waste management practices are not only costly but also have adverse impact on environment. In this study, black soldier fly (BSF) (*Hermetia illucens*) larvae were grown on food wastes to produce fat and protein-rich BSF prepupae as a novel strategy for efficient organic waste management. The lipid content in BSF prepupae was characterized for fatty acids profile. Whole BSF prepupae, pressed cake, and meal were analyzed for important animal feed characteristics. BSF-derived oil has high concentration of medium chain saturated fatty acids (67% total fatty acids) and low concentration of polyunsaturated fatty acids (13% total fatty acids), which makes it potentially an ideal substrate for producing high quality biodiesel. BSF (prepupae, pressed cake, and meal) has feed value comparable to commercial feed sources. Thus, the bioconversion of organic waste into BSF prepupae has significant potential in generating high-value products with simultaneous waste valorization.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Meeting an ever increasing demand for the food, feed, and fuel and managing waste, especially the organic waste, has become a major global challenge. The situation is projected to be aggravated with the rapidly increasing global population, which is estimated to increase from 7.3 billion in 2015 to 9.7 billion in 2050 [1]. Currently, about one-third of food produced, which is equivalent to 1.3 billion metric tons is wasted or lost and has significant environmental (i.e., greenhouse gas (GHGs) emissions) and economic footprints [2]. For example, in India alone, the postharvest loss of agricultural produce is estimated to be about 92 million tons (Mton) per year [3]. Without considering the land use change, the annual GHGs emissions equivalent of food wastage is estimated to be 3.3 billion metric tons of CO₂ equivalent GHGs [2]. Similarly, the economic footprint of the food produced but not consumed accounts for the

loss of nearly \$750 billion per year [2]. Additionally, waste management, especially in developing and underdeveloped countries, has become a serious issue. The management of organic wastes, among different wastes, is more challenging due to its bulky nature and rapid degradability [4]. The current organic wastes management practices, namely land fill and waste treatment/stabilization via anaerobic digestion and composting to meet environmental regulations, are not only costly but also have adverse impacts on environment such as ground and surface water contaminations and GHGs emissions among others [4,5].

On the other hand, both rapid increase in global population and associated affluence are believed to have caused significant increase in demand for food, feed, and fuel, and waste generation [5]. For example, global energy demand is estimated to increase from 524 Quadrillion Btu (QBtu) in 2010 to 820 QBtu by 2040, a 56% increase compared to 2010 [6]. Similarly, the global demands for food and animal products are projected to increase by 70–100% [7] and 50–70% [8], respectively, by 2050. To cope up with the demand for animal products, a substantial increase in nutritious animal feedstuffs is needed. On one hand, the production of conventional feedstuffs such as soybean meal is reported as the major

* Corresponding author.

E-mail addresses: surendra@hawaii.edu (K.C. Surendra), robert@protaculture.com (R. Olivier), jktomberlin@tamu.edu (J.K. Tomberlin), rjha@hawaii.edu (R. Jha), khanal@hawaii.edu (S.K. Khanal).

<http://dx.doi.org/10.1016/j.renene.2016.03.022>

0960-1481/© 2016 Elsevier Ltd. All rights reserved.

contributor to land occupation, climate change, and water and energy consumption [9,10]. Additionally, such conventional animal feedstuffs are not only limited in supply but also are becoming more expensive over the years. On the other hand, the growing concern of climate change, which is expected to bring extreme climate variability (e.g., long drought, heavy winter storm, and occurrence of frequent floods among others), the situation is likely to aggravate further the food security situation due to its impact on agriculture-based food and feed production systems [11]. Moreover, there is already a strong competition for resources such as food, feed, and biofuel productions, and the competition is projected to be even fiercer with increased demand for food, feed, and fuel. Thus, there is a pressing need for identifying and exploring the potential of alternative non-conventional sources of food, feed, and fuel, which are economically viable, environmentally friendly, and socially acceptable.

The black soldier fly (BSF) (*Hermetia illucens*), a detritivorous insect belonging to order Diptera and family Stratiomyidae, is native to tropical, subtropical, and warm temperate zone of America [12]. The insect is now distributed, due to global trade and business, in tropical to warm temperate regions in Europe [13], North [14] and South America, Australia [15], and Asia [16,17]. Due to its widespread distribution and ease with which it can be maintained in a colony [14], there is substantial global interest in the mass production of the BSF as a means to produce protein [15].

The BSF lifecycle consists of four stages, namely; egg, larvae, pupae, and adult. Adults typically mate for two days after emergence [18]. Females lay a single clutch of eggs for two days after mating [18] and resulting eggs hatch in approximately four days [12]. Within natural environments (e.g., livestock operations), eggs are deposited in cracks and crevices near to food sources [19]. Larvae pass through five instars. Larvae are quite omnivorous, as they feed on a variety of materials ranging from animal [20] and human feces [21], kitchen waste [22] to vertebrate remains (e.g., decomposing swine carcasses) [23]. Depending on the size of the larvae, type of the substrate available, and environmental conditions (e.g., moisture, temperature, and air supply), the larvae consume from 25 to 500 mg of organic matter per larva per day [24]. Similarly, depending on the substrate type, the larvae are reported to reduce the waste by about 39% (pig manure) [25] and 50% (chicken manure) [26] to 68% (municipal organic waste) [27] and have a food conversion ratio (FCR) of about 10 to 15 [25–27]. The larval stage is usually 14 days or longer depending on availability of food [12], and appropriate environmental conditions [28,29]. During the later larval stage; the stage prior to pupation termed as prepupae [26], larvae get rid of their digestive track and migrate away from their food sources in search of dry and protected place to pupate [26]. Since adults are non-feeding, BSF larvae consume organic matter as much as possible and store fat and protein in their body to support their metabolism during pupal and adult stages [25]. By using a specially designed bioreactor, the typical migrating behavior of prepupae can be exploited for self-harvesting of prepupae [26] for extracting fat and protein for value-added products generation. The pupation stage usually lasts for two weeks under ideal environmental conditions [12,30,31].

In the context of growing demand for food, feed, and fuel, as well as a need for managing organic wastes, the use of insect, to efficiently convert organic wastes into fuel, food, and feed appears to be innovative and promising. Thus, this study aims to farm a unique insect, the BSF on such organic waste as a novel strategy in managing organic wastes and producing high-value fat- and protein-rich insect biomass. Thereafter, the produced fat- and protein-rich insect biomass, in downstream processing, can be fractionated into fat (i.e., raw material for biodiesel production) and protein-rich insect meal (for animal feed applications). The specific

objectives of this study were to (i) characterize fatty acids profile of the insect fat as a potential substrate for biodiesel production, and (ii) analyze the proximate composition and amino acids profile of insect biomass and defatted insect cake and meal for animal feed application.

2. Materials and methods

2.1. Black soldier fly rearing and oil extraction

BSF was grown in the food wastes collected from school cafeteria (Clemson University, Clemson, SC USA and Pearl City High School, Honolulu, HI USA) by using specially designed reactor for self-harvesting of prepupae. The schematic of bioconversion of food waste to biofuel and animal feed is presented in Fig. 1. Harvested larvae were dried using conveyor oven (Despatch Oven Co., Minneapolis, MN USA) at 60 °C to the moisture content of around 5–8%. The dried BSF prepupae grown in Clemson University and Pearl City High School were mixed to make a composite sample. The mixed dried BSF prepupae were fractionated into crude oil and cake/meal using both mechanical and chemical means. Mechanical extraction was conducted using a lab-scale Taby Press Type 20 (Skeppsta Maskin AB, Sweden). The extracted crude oil was collected, centrifuged, and the mass of the supernatant oil was recorded. The pressed cake following mechanical extraction was further chemically extracted using a Soxhlet apparatus (Ace Glass Incorporated, NJ USA) for four hours using petroleum ether as a solvent. Afterwards, solvent was removed using a rotary evaporator (Buchi, Flawil, Switzerland) at 50 °C. BSF prepupae, pressed cake, and pressed and solvent extracted meal (termed as “meal” hereafter) were sent to the Department of Aquatic Feeds and Nutrition at the Oceanic Institute (Waimanalo, HI, USA) for complete characterization (i.e. fatty acids composition and nutritional profile).

2.2. Analytical methods

The nutritional profile of the BSF prepupae, pressed cake, and meal were characterized following the Association of Official Analytical Chemists (AOAC) standard procedures [32] with specific methods as follows: dry matter (DM) (oven drying at 105 °C to constant weight), ash (ignition at 550 °C in an electric furnace, AOAC 942.05), crude fiber (AOAC 962.09), crude protein by determining N by dry combustion using LECO analyzer (LECO FP-528 Nitrogen determiner, Leco Corp., St. Joseph, MI) (AOAC 976.05, crude protein = N × 6.25), crude lipid (AOAC 920.39), and carbohydrate by subtracting the crude protein, crude lipid and ash contents from total dry matter. Gross energy content was determined using an oxygen bomb calorimeter (Parr Bomb Calorimeter 6200, Parr Instrument Co., Moline, IL). Amino acids (both essential and non-essential) was determined using High Performance Liquid Chromatography (Agilent 1200, Agilent Technologies Inc., Santa Clara, CA) using AOAC method no 982.30 E (a,b,c). Fatty acids including EPA and DHA were analyzed using a gas chromatograph (Varian 3800 GC; Varian Analytical Instrument, Walnut Creek, CA) equipped with a flame ionization detector (AOAC 996.06). During fatty acid profile analysis, the C12:0 in BSF prepupae derived oil was determined by assigning the peak before C14:0 as a peak for C12:0 based on literature review [33–35].

2.3. Statistical analyses

The experiments and sample analyses for all important parameters discussed above were performed in triplicates. The statistical analysis was conducted using Statistical Analysis System (SAS) software (SAS 9.2, SAS Institute Inc., Cary, NC USA). The

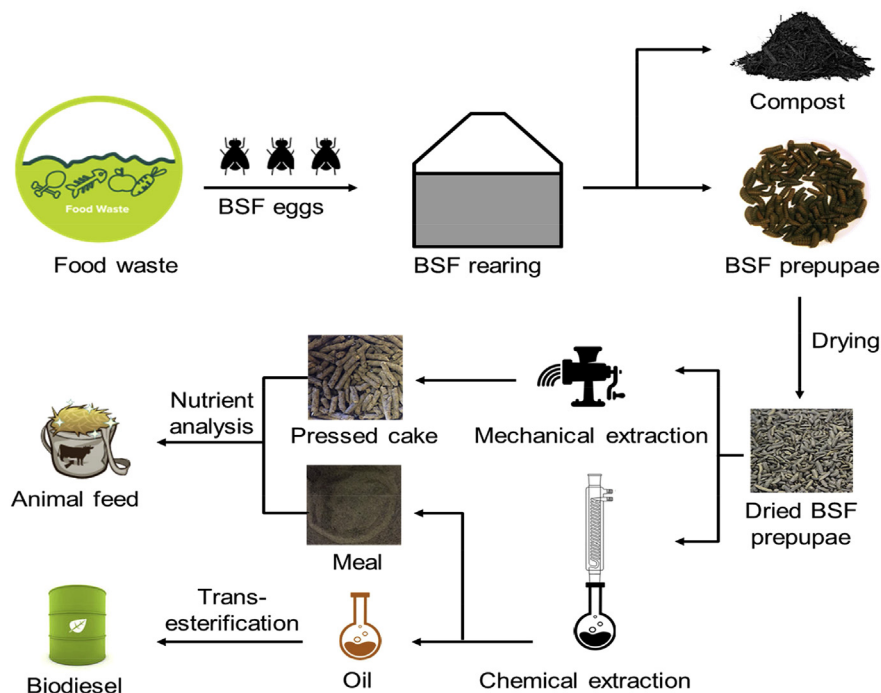


Fig. 1. Schematic presentation of bioconversion of food waste to biofuel and animal feed.

statistical differences were determined by a one-way analysis of variance (ANOVA) followed by Tukey's T-test with a threshold value (α) of 0.05.

3. Results and discussion

3.1. Yield and fatty acids composition of BSF derived oil

The mechanical pressing of 100 g of prepupae resulted about 15–20 g of crude oil and 80–85 g of pressed cake (data not presented). The mechanical pressing extracted nearly 40% of oil content in the insect biomass. The solvent extraction of the pressed and milled BSF prepupae, however, removed about 90% of total oil content in the BSF prepupae.

The low oil yield during mechanical pressing could be mainly because of the low crude fiber content (~10%) in BSF prepupae, which is not sufficient to create enough back pressure during pressing. The low oil yield during pressing could also be because of the equipment used in this study. The Taby Press Type 20 (Skeppsta Maskin AB, Sweden) used in this study was a lab scale/kitchen press mainly designed for extracting oil from oil crops (usually rich in fiber content) and was not optimized for the feedstock such as insect biomass. The large scale mechanical press especially designed for extracting oil from low fiber containing feedstocks could improve the oil extraction from BSF prepupae. Further, the extraction efficiency of BSF prepupae by mechanical means could potentially be improved by co-pressing BSF prepupae with fiber rich substrates such as sunflower seeds and macadamia nuts. Since fatty acid composition varies with substrate (e.g., BSF derived oil has high saturated fatty acids (~70%) and low in polyunsaturated fatty acids (~12%) while sunflower oil is rich in polyunsaturated fatty acids (~65%) and poor in saturated fatty acids (~10%)) (Table 1), such co-extraction could improve not only the oil extraction efficiency, but also the fatty acid profile in the extracted oil for high quality biodiesel production.

3.2. Fatty acid composition

The composition of the fatty acids derived from BSF is summarized in Table 1. It was found that lauric (C12:0), palmitic (C16:0), and oleic (C18:1) acids were the most dominating fatty acids in the oil derived from BSF prepupae. The relative percentage of the lauric, palmitic, and oleic acids were about 45, 14, and 12%, respectively, followed by linoleic (C16:2) (10%) and myristic (C14:0) (8%) acids. The relative concentration of the saturated, monounsaturated, and polyunsaturated fatty acids in the BSF derived oil were about 70, 15, and 13% of total fatty acids, respectively (Table 1). The relative dominance of lauric, palmitic, and oleic acids in the BSF prepupae derived oil were also reported by others [33–35]. The dominance of the lauric acid in BSF prepupae derived oil is unique compared to the oil derived from the crops which is most commonly used for biodiesel production (e.g., soybean, sunflower, and palm) [36]. The relative concentration of the medium chain saturated fatty acids (C12:0 – C16:0) in the BSF prepupae derived oil (67% of total fatty acids) is higher compared to the soybean (11% of total fatty acids) and palm oil (37% of total fatty acids) (Table 1). In addition, the relative concentration of unsaturated (i.e., mono- and polyunsaturated) fatty acids in the oil derived from BSF prepupae (28% of total fatty acids) is less than in the soybean oil (85%) and palm oil (55%). The fatty acids composition of the substrate (i.e., oil) has been stated to have significant effect on the quality of the produced biodiesel [36]. The biodiesel production process from vegetable oils and animal fats has been discussed elsewhere [37,38]. In general, high concentrations of long chain saturated fatty acids in the substrate have been reported to produce biodiesel with poor cold flow property (i.e., low cold plugging point). Similarly, the biodiesel derived from the oil rich in polyunsaturated fatty acids have been found to have poor oxidative stability, which means the low storability of the produced biodiesel [36]. Thus, the relatively high concentration of medium chain saturated fatty acids (i.e., 67%) and low concentration of the polyunsaturated fatty acids (i.e., 13%) makes the BSF prepupae derived oil potentially an ideal substrate for producing high quality biodiesel (i.e., lower viscosity and higher

Table 1
Fatty acid composition of BSF prepupae derived oil and biodiesel feedstocks (% w/w of total fatty acids) (n = 3, for the BSF sample), data presented as mean ± standard deviation.

Fatty acids	BSF prepupae oil	Soybean oil [36]	Palm oil [36]
Lauric (C12:0)	44.9 ± 1.5	0.0	0.1
Myristic (C14:0)	8.3 ± 0.6	0.0	0.7
Palmitic (C16:0)	13.5 ± 0.7	11.3	36.7
Palmitoleic (C16:1n-7)	2.4 ± 0.2	0.1	0.1
Stearic (C18:0)	2.1 ± 0.2	3.6	6.6
Oleic (C18:1n-9)	12.0 ± 0.7	24.9	46.1
Linoleic (C18:2n-6)	9.9 ± 0.5	53.0	8.6
Linolenic (C18:3n-3)	0.1 ± 0.0	6.1	0.3
Saturated Fatty Acids (SFA)	69.9 ± 1.4	15.3	44.7
Monounsaturated Fatty Acids (MUFA)	14.9 ± 0.8	25.6	46.4
Polyunsaturated Fatty Acids (PUFA)	12.5 ± 0.4	59.1	8.9

Table 2
Proximate composition of the black soldier fly (prepupae, pressed cake, and meal) and commercial animal feed ingredients (n = 3, for the BSF sample), data presented as mean ± standard deviation.

Parameters	BSF prepupae	BSF prepupae pressed cake	BSF prepupae meal	Soybean meal (IFN: 5-04-602) [39]	Fishmeal (IFN: 5-01-977) [39]
Dry Matter (%)	93.9 ± 0.7 ^a	94.3 ± 0.3 ^a	96.7 ± 0.25 ^b	88.8 ± 0.7	93.7 ± 2.4
Crude Protein (%)	43.7 ± 0.6 ^a	53.1 ± 0.2 ^b	63.9 ± 0.2 ^c	43.9 ± 2.0	63.3 ± 4.7
Crude Lipid (%)	31.8 ± 0.3 ^a	19.7 ± 0.6 ^b	3.4 ± 0.1 ^c	1.2 ± 0.3	9.7 ± 1.3
Ash (%)	6.0 ± 0.0 ^a	8.5 ± 0.2 ^b	10.7 ± 0.1 ^c	6.4 ± 0.2	16.1 ± 3.2
Crude Fiber (%)	10.1 ± 0.2 ^a	10.9 ± 0.4 ^a	13.2 ± 0.3 ^b	6.6 ± 0.0	0.2 ± 0.2
Carbohydrates (%)	12.3 ± 0.3 ^a	12.9 ± 0.5 ^a	18.7 ± 0.3 ^b	NA	NA
Gross Energy (Kcal/kg)	5751.7 ± 52.4 ^a	5233.0 ± 40.6 ^b	4614.3 ± 12.7 ^c	4257.0 ± 168.0	4496.0 ± 84.4

Note: Mean with the same letter in the same row are not significantly ($\alpha = 0.05$) different.
NA: Not available.

oxidative stability). Additionally, Li et al. [33] mentioned that the important biofuel properties, namely density, viscosity, flash point, and Cetane index, of biodiesel derived from BSF larva were within the recommended range of international standard of biodiesel.

3.3. Proximate composition BSF prepupae, pressed cake, and meal

The proximate composition of the BSF prepupae, pressed cake, and meal are summarized in Table 2. There was significant ($\alpha = 0.05$) difference in the crude protein, crude lipid, ash, carbohydrate, gross energy, and crude fiber content among the BSF prepupae, pressed cake, and meal. The crude protein, ash, carbohydrate, and crude fiber content were highest in the meal and lowest in BSF prepupae. The gross energy content decreased significantly ($\alpha = 0.05$) with significant ($\alpha = 0.05$) removal of the crude lipid followed by mechanical pressing, and mechanical pressing and solvent extraction.

The proximate composition of BSF prepupae obtained in this study was within the range of reported studies [25,35]. The proximate composition of BSF prepupae, especially crude protein and crude fat contents, however, differs with the substrate type and growth stage (e.g., larvae, prepupae, and pupae) [25,40]. When BSF larvae were fed with cow manure and mixture of cow manure and fish offal at different ratios, St-Hilaire et al. [40] observed variation in not only the total lipid content but also in the composition of fatty acids. The authors reported an increase in lipid content from 21% (fed with cow manure) to 30% as well as substantial enrichment (2.5–3.8% of total lipid) of omega-3 fatty acids in the prepupae fed with mixture of fish offal and cow manure. The majority of the lipid stored in the insect body is triglyceride, which is synthesized from carbohydrates, fatty acids, or proteins [41,42]. Diglyceride is the immediate precursor for triglyceride formation. The esterification of diglyceride in the presence of diacylglycerol acyltransferase as a catalyst leads to the formation of triglyceride [42,43]. The conversion of carbohydrates present in insect diet into lipid in the insect fat body has been reported elsewhere [42,44,45].

The capacity of fat body for lipid synthesis from carbohydrate is higher than for glycogen synthesis, thus insect fat body contains higher amount of lipid than glycogen [42].

The crude protein content of the BSF prepupae, pressed cake, and meal were compared with the most common protein source in animal feeds such as soybean meal and fishmeal. The crude protein content of the BSF prepupae meal (63.9%) was higher than crude protein content of solvent extracted soybean meal (43.9%) and was comparable with the crude protein content of fish meal (63.2%). The crude protein content of BSF prepupae pressed cake (53.1%), however, was in between crude protein content of soybean meal and fishmeal (Table 2). Though the BSF prepupae, pressed cake, and meal are treated as protein source, the energy content of the BSF prepupae, pressed cake, and meal (i.e., 5752, 5233, and 4614 kcal/kg DM, respectively) are higher than the reference protein sources (i.e., 4257 and 4496 kcal/kg DM for soybean meal and fish meal, respectively). The energy content of the BSF prepupae, pressed cake, and meal are comparable or higher than the most common energy source in animal diets such as corn (4508 kcal/kg DM) and wheat (4063 kcal/kg DM). Additionally, the crude fiber content in BSF prepupae (10.1%), pressed cake (10.9%), and meal (13.2%) were higher than the reference protein sources (6.6% in soybean meal and 0.2% in fishmeal), but were in range with reference energy sources (2.4 and 1.7% in corn and wheat, respectively). Thus BSF prepupae, pressed cake, and meal have potential to serve as both protein and energy source in animal diets.

The removal of crude fat from BSF prepupae not only improved the crude protein content of the pressed cake and meal but also the removal of crude fat from high fat containing feedstuffs is essential for efficient mixing of feed ingredients while processing animal feeds. Further, the fat removal is necessary to improve the storability, especially the oxidative stability, of the feed. In addition, fat removal from the BSF prepupae is believed to increase the protein digestibility of the BSF prepupae derived feed.

Table 3

Amino acid content of BSF prepupae, pressed cake, and meal (Unit % w/w) (n = 3, for the BSF sample), data presented as mean ± standard deviation.

	BSF prepupae	BSF prepupae pressed cake	BSF prepupae meal	Soybean meal, (IFN: 5-04-602) [39]	Fishmeal (IFN: 5-01-977) [39]
Essential AA					
Arginine	2.22 ± 0.08 ^a	2.78 ± 0.03 ^b	3.25 ± 0.03 ^c	3.17 ± 0.19	3.84 ± 0.48
Histidine	1.69 ± 0.04 ^a	1.99 ± 0.07 ^b	2.13 ± 0.08 ^b	1.26 ± 0.14	1.44 ± 0.29
Isoleucine	1.51 ± 0.06 ^a	1.97 ± 0.02 ^b	2.37 ± 0.02 ^c	1.96 ± 0.19	2.56 ± 0.31
Leucine	2.34 ± 0.05 ^a	2.92 ± 0.01 ^b	3.68 ± 0.03 ^c	3.43 ± 0.26	4.47 ± 0.50
Lysine	2.19 ± 0.07 ^a	2.56 ± 0.04 ^b	3.23 ± 0.10 ^c	2.76 ± 0.24	4.56 ± 0.90
Methionine	0.88 ± 0.02 ^a	0.97 ± 0.01 ^b	1.04 ± 0.03 ^c	0.60 ± 0.06	1.73 ± 0.45
Phenylalanine	1.50 ± 0.07 ^a	1.84 ± 0.05 ^b	2.27 ± 0.03 ^c	2.26 ± 0.16	2.47 ± 0.22
Threonine	1.48 ± 0.07 ^a	1.84 ± 0.02 ^b	2.15 ± 0.02 ^c	1.76 ± 0.13	2.58 ± 0.33
Tryptophan	NA	NA	NA	0.59 ± 0.26	0.63 ± 0.10
Valine	2.42 ± 0.06 ^a	2.98 ± 0.01 ^b	3.68 ± 0.11 ^c	1.93 ± 0.35	3.06 ± 0.45
Non-essential AA					
Alanine	2.68 ± 0.19 ^a	3.11 ± 0.10 ^b	3.85 ± 0.09 ^c	1.92 ± 0.18	3.93 ± 0.54
Asparagine + Aspartate	2.64 ± 0.06 ^a	3.35 ± 0.03 ^b	4.16 ± 0.08 ^c	4.88 ± 0.73	5.41 ± 1.18
Cysteine	1.12 ± 0.05 ^a	1.98 ± 0.05 ^b	1.60 ± 0.07 ^c	0.68 ± 0.20	0.61 ± 0.20
Glutamate + Glutamine	2.85 ± 0.02 ^a	3.69 ± 0.10 ^b	4.48 ± 0.09 ^b	7.87 ± 1.15	7.88 ± 1.18
Glycine	2.46 ± 0.10 ^a	3.11 ± 0.12 ^b	3.70 ± 0.17 ^c	1.89 ± 0.20	4.71 ± 0.98
Proline	2.11 ± 0.02 ^a	2.60 ± 0.07 ^b	3.20 ± 0.07 ^c	2.43 ± 0.46	2.89 ± 1.07
Serine	1.53 ± 0.01 ^a	2.03 ± 0.05 ^b	2.21 ± 0.03 ^c	2.14 ± 0.28	2.43 ± 0.59
Tyrosine	2.37 ± 0.02 ^a	2.97 ± 0.01 ^b	3.49 ± 0.03 ^c	1.55 ± 0.21	1.88 ± 0.38
Total Essential AA	16.06 ± 0.49 ^a	19.86 ± 0.09 ^b	23.79 ± 0.17 ^c	NA	NA
Total Non-essential AA	17.76 ± 0.30 ^a	22.83 ± 0.42 ^b	26.70 ± 0.44 ^c	NA	NA
Total AA	33.82 ± 0.73 ^a	42.70 ± 0.38 ^b	50.49 ± 0.49 ^c	NA	NA

Note: Mean with the same letter in the same row are not significantly ($\alpha = 0.05$) different.

AA: Amino acid, NA: Not available.

3.4. Amino acid content

Though crude protein content gives the approximation of the protein content in the feedstuffs, the amino acids composition of the protein shows the quality of the protein. The amino acid content of the BSF prepupae, pressed cake, and meal along with the reference protein sources (soybean meal and fishmeal) are summarized in Table 3. The BSF prepupae, pressed cake, and meal show the good amino acids profile with the presence of the both essential and nonessential amino acids. Similar amino acids profile was reported by Newton et al. [25] in BSF prepupae grown on swine manure.

The removal of the fat from BSF prepupae increased the concentrations of all amino acids (except cysteine) in BSF prepupae pressed cake and meal. Thus the concentration of all amino acids (except cysteine) in the BSF prepupae meal was significantly ($\alpha = 0.05$) higher than both among BSF prepupae and pressed cake. The essential amino acids content in the BSF prepupae meal is comparable with the soybean meal and fishmeal. More importantly, the concentration of the lysine, methionine, tryptophan, threonine, cysteine, and valine, which are the more frequent limiting amino acids in animal diets (e.g., pig and poultry) were higher in BSF prepupae meal than the solvent extracted soybean meal. The lysine, methionine, leucine, and threonine content in the BSF prepupae derived meal, however, was slightly lower compared to their respective concentration in the fishmeal. The presence of most common limiting amino acids in the concentration higher than or comparable with the commercial animal feed ingredients (i.e., soybean meal and fishmeal) makes BSF prepupae, pressed cake, and meal potentially an ideal protein source in animal diets.

4. Conclusions and future recommendations

Overall, BSF is capable of utilizing food wastes to support its lifecycle. BSF prepupae derived oil has a good fatty acids profile, which could potentially produce a high quality biodiesel. Additionally, BSF prepupae, pressed cake, and meal has feed value comparable to commercial feed ingredients. In-depth study,

however, is required to (i) account and improve the organic waste to insect biomass conversion efficiency, (ii) trans-esterify the insect oil into biodiesel and characterize thus produced biodiesel for fuel properties, and (iii) test nutrients digestibility and palatability of insect biomass before such insect biorefinery could be successfully implemented as an efficient technology for generating biofuel and animal feed with simultaneous organic waste valorization.

Acknowledgments

This study was funded by United States Department of Agriculture (USDA) HATCH grant from the College of Tropical Agriculture and Human Resources (CTAHR), University of Hawai'i at Mānoa, and USDA-National Institute of Food and Agriculture (NIFA)-Small Business Innovative Research (SBIR) grant. The authors would like to thank Dr. Zhi Yong Ju, Oceanic Institute of Hawaii Pacific University, Honolulu, HI for his help in analyzing samples, David Thornton, Clemson University, Clemson, SC, USA and Pearl City High School, Honolulu, HI, USA for their support in collecting insect biomass samples.

References

- [1] United Nations Department of Economics and Social Affairs/Population Division World Population Prospects: The 2015 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP.241.
- [2] J. Gustavsson, C. Cederberg, U. Sonesson, R. Van Otterdijk, A. Meybeck, Global Food Losses and Food Waste, Food and Agriculture Organization of the United Nations, Rome, 2011.
- [3] D. Cardoen, P. Joshi, L. Diels, P.M. Sarma, D. Pant, Agriculture biomass in India: part 2. Post-harvest losses, cost and environmental impacts, Resour. Conserv. Recycl. 101 (2015) 143–153.
- [4] J. Yin, K. Wang, Y. Yang, D. Shen, M. Wang, H. Mo, Improving production of volatile fatty acids from food waste fermentation by hydrothermal pretreatment, Bioresour. Technol. 171 (2014) 323–329.
- [5] K.C. Surendra, C. Sawatdeenarunat, S. Shrestha, S. Sung, S.K. Khanal, Anaerobic digestion-based biorefinery for bioenergy and biobased products, Ind. Biotechnol. 11 (2015) 103–112.
- [6] United States Energy Information Administration (U.S. EIA), Annual Energy Outlook 2013, US Energy Information Administration, Washington, DC, 2013.
- [7] H.C.J. Godfray, J.R. Beddington, I.R. Crute, L. Haddad, D. Lawrence, J.F. Muir, J. Pretty, S. Robinson, S.M. Thomas, C. Toulmin, Food security: the challenge of feeding 9 billion people, Science 327 (2010) 812–818.

- [8] Food and Agricultural Organization of the United Nations (FAO), World Livestock 2011 – Livestock in Food Security, Italy, FAO, Rome, 2011.
- [9] R. Mungkung, J. Aubin, T.H. Pihadi, J. Slembrouck, H.M. van der Werf, M. Legendre, Life cycle assessment for environmentally sustainable aquaculture management: a case study of combined aquaculture systems for carp and tilapia, *J. Clean. Prod.* 57 (2013) 249–256.
- [10] M.J. Sánchez-Muros, F.G. Barroso, F. Manzano-Agugliaro, Insect meal as renewable source of food for animal feeding: a review, *J. Clean. Prod.* 65 (2014) 16–27.
- [11] Intergovernmental Panel on Climate Change (IPCC), Technical summary, in: T.F. Stocker, D. Qin, D. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, P.M. Midgley (Eds.), *Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK, and New York, Cambridge University Press, NY, USA, 2013, 30–254.
- [12] J.K. Tomberlin, D.C. Sheppard, J.A. Joyce, Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets, *Ann. Entomol. Soc. Am.* 95 (2002) 379–386.
- [13] H. Čičková, G.L. Newton, R.C. Lacy, M. Kozánek, The use of fly larvae for organic waste treatment, *Waste Manage* 35 (2015) 68–80.
- [14] D.C. Sheppard, J.K. Tomberlin, J.A. Joyce, B.C. Kiser, S.M. Sumner, Rearing methods for the black soldier fly (Diptera: Stratiomyidae), *J. Med. Entomol.* 39 (2002) 695–698.
- [15] J.K. Tomberlin, A. van Huis, M.E. Benbow, H. Jordan, D.A. Astuti, D. Azollini, I. Banks, V. Bava, C. Borgemeister, J.A. Cammack, R.S. Chapkin, H. Čičková, T.L. Crippen, A. Day, M. Dicke, D. Drew, C. Emhart, M. Epstein, M. Finke, C.H. Fischer, D. Gatlin, N.T. Grabowski, C. He, L. Heckman, A. Hubert, J. Jacobs, J. Joseph, S.K. Khanal, J.K. Kleinfinger, G. Klein, C. Leach, Y. Liu, G.L. Newton, R. Olivier, J.L. Pechal, C.J. Picard, S. Rojo, A. Roncarati, C. Sheppard, A.M. Tarone, B. Verstappen, A. Vickerson, H. Yang, A. Yen, Z. Yu, J. Zhang, L. Zheng, Protecting the environment through insect farming as a means to produce protein for use as livestock, poultry, and aquaculture feed, *J. Insect. Food. Feed* 1 (2015) 307–309.
- [16] J. Zhang, L. Huang, J. He, J.K. Tomberlin, J. Li, C. Lei, M. Sun, Z. Liu, Z. Yu, An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*, *J. Insect Sci.* 10 (2010) 202.
- [17] G. Yu, P. Cheng, Y. Chen, Y. Li, Z. Yang, Y. Chen, J.K. Tomberlin, Inoculating poultry manure with companion bacteria influences growth and development of black soldier fly (Diptera: Stratiomyidae) larvae, *Environ. Entomol.* 40 (2011) 30–35.
- [18] J.K. Tomberlin, D.C. Sheppard, Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony, *J. Entomol. Sci.* 37 (2002) 345–352.
- [19] D.C. Booth, D.C. Sheppard, Oviposition of the black soldier, *Hermetia illucens* (Diptera: Stratiomyidae): eggs, masses, timing and site characteristics, *Environ. Entomol.* 13 (1984) 421–423.
- [20] H.M. Myers, J.K. Tomberlin, B.D. Lambert, D. Kattes, Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure, *Environ. Entomol.* 37 (2008) 11–15.
- [21] I.J. Banks, W.T. Gibson, M.M. Cameron, Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation, *Trop. Med. Int. Health* 19 (2014) 14–22.
- [22] T.T. Nguyen, J.K. Tomberlin, S. Vanlaerhoven, Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste, *Environ. Entomol.* 44 (2015) 406–410.
- [23] J.K. Tomberlin, D.C. Sheppard, J.A. Joyce, Black soldier fly (Diptera: Stratiomyidae) colonization of pig carrion in South Georgia, *J. Forensic Sci.* 50 (2005) 152–153.
- [24] H.P. Makkar, G. Tran, V. Heuzé, P. Ankers, State-of-the-art on use of insects as animal feed, *Anim. Feed Sci. Tech.* 197 (2014) 1–33.
- [25] L. Newton, C. Sheppard, D.W. Watson, G. Burtle, R. Dove, Using the Black Soldier Fly, *Hermetia illucens*, as a Value-added Tool for the Management of Swine Manure. Animal and Poultry Waste Management Center, North Carolina State University, Raleigh, NC, 2005.
- [26] D.C. Sheppard, G.L. Newton, S.A. Thompson, S. Savage, A value added manure management system using the black soldier fly, *Bioresour. Technol.* 50 (1994) 275–279.
- [27] S. Diener, N.M.S. Solano, F.R. Gutiérrez, C. Zurbrügg, K. Tockner, Biological treatment of municipal organic waste using black soldier fly larvae, *Waste Biomass Valoriz.* 2 (2011) 357–363.
- [28] J.K. Tomberlin, P.H. Adler, H.M. Myers, Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature, *Environ. Entomol.* 38 (2009) 930–934.
- [29] L. Holmes, Role of Abiotic Factors on the Development and Life History of the Black Soldier Fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae). M.S., University of Windsor Windsor, 2010.
- [30] L.A. Holmes, S.L. Vanlaerhoven, J.K. Tomberlin, Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae), *Environ. Entomol.* 41 (2012) 971–978.
- [31] L.A. Holmes, S.L. Vanlaerhoven, J.K. Tomberlin, Substrate effects on pupation and adult emergence of *Hermetia illucens* (Diptera: Stratiomyidae), *Environ. Entomol.* 42 (2013) 370–374.
- [32] AOAC, Official Methods of Analysis, eighteenth ed., Association of Official Analytical Chemists, Gaithersburg, MD, USA, 2006.
- [33] Q. Li, L. Zheng, H. Cai, E. Garza, Z. Yu, S. Zhou, From organic waste to biodiesel: Black soldier fly, *Hermetia illucens*, makes it feasible, *Fuel* 90 (2011) 1545–1548.
- [34] L. Zheng, Q. Li, J. Zhang, Z. Yu, Double the biodiesel yield: Rearing black soldier fly larvae, *Hermetia illucens*, on solid residual fraction of restaurant waste after grease extraction for biodiesel production, *Renew. Energy* 41 (2012) 75–79.
- [35] S. St-Hilaire, C. Sheppard, J.K. Tomberlin, S. Irving, L. Newton, M.A. McGuire, E.E. Mosley, R.W. Hardy, W. Sealey, Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*, *J. World Aquac. Soc.* 38 (2007) 59–67.
- [36] M.J. Ramos, C.M. Fernández, A. Casas, L. Rodríguez, A. Pérez, Influence of fatty acid composition of raw materials on biodiesel properties, *Bioresour. Technol.* 100 (2009) 261–268.
- [37] L.C. Meher, D.V. Sagar, S.N. Naik, Technical aspects of biodiesel production by transesterification—a review, *Renew. Sustain. Energy Rev.* 10 (2006) 248–268.
- [38] J.M. Marchetti, V.U. Miguel, A.F. Errazu, Possible methods for biodiesel production, *Renew. Sustain. Energy Rev.* 11 (2007) 1300–1311.
- [39] Nutrient NRC, Requirement of Swine, eleventh ed., National Academy Press, Washington, DC, 2012.
- [40] S. St-Hilaire, K. Cranfill, M.A. McGuire, E.E. Mosley, J.K. Tomberlin, L. Newton, W. Sealey, C. Sheppard, S. Irving, Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids, *J. World Aquac. Soc.* 38 (2007) 309–313.
- [41] L.E. Canavoso, Z.E. Jouni, K.J. Karnas, J.E. Pennington, M.A. Wells, Fat metabolism in insects, *Annu. Rev. Nutr.* 21 (2001) 23–46.
- [42] E.L. Arrese, J.L. Soulages, Insect fat body: energy, metabolism, and regulation, *Annu. Rev. Entomol.* 55 (2010) 207–225.
- [43] K. Athenstaedt, G. Daum, The life cycle of neutral lipids: synthesis, storage and degradation, *Cell. Mol. Life Sci.* 63 (2006) 1355–1369.
- [44] W.J.W. Hines, M.J.H. Smith, Some aspects of intermediary metabolism in the desert locust (*Schistocerca gregaria* Forskål), *J. Insect Physiol.* 9 (1963) 463–468.
- [45] S. Inagaki, O. Yamashita, Metabolic shift from lipogenesis to glycogenesis in the last instar larval fat body of the silkworm, *Bombyx mori*, *Insect Biochem.* 16 (1986) 327–331.