Transformational Production of Sustainable Aviation Fuel and Biofertilizer from Black Solider Fly

Toni Wang, Charles E. Wharton Institute Professor, Food Science
Sindhu Jagadamma, Associate Professor, Biosystems Engineering and Soil Science

Goal, Justification, and Significance

Our goal is to use chemistry-based approaches to study the separation and properties of lipid and protein of black soldier fly larvae (BSFL), so their high-value and superior-performance applications can be created using feasible and scalable technologies. We intend to use insect as a vector to address the challenges of environmental sustainability, renewable energy, and biobased products, achieving a more circular economy.

Black soldier fly larvae (BSFL, *Hermetia illucens***) can transform wastes to high-value and high-quality biomass.** BSF has been regarded as the "crown jewel" of insects for its inherent ability to convert organic wastes and food processing byproducts into protein and lipid-rich biomass. It is considered an ideal tool to establish a circular bioeconomy (Bosch et al, 2019; Chen et al, 2019; Pang et al, 2020 a, b; Magee et al, 2021). It has high bioconversion performance (72% substrate reduction), minimum requirement for land and water, and significant reduction of greenhouse gas emissions (Ebeneezar et al, 2021; Kristiansen et al, 2021; Pang et al, 2020b). The insect farming industry is expanding rapidly in Europe and the U.S. Global black soldier fly market is valued at $175 million in 2021, and it is anticipated to attain a value of $1.4 billion by 2028, at a CAGR of 35% over 2022-2028. The U.S. has already approved the use of BSFL for meat animal production (US Regulations, 2022), and NSF has funded a Center for Environmental Sustainability through Insect Farming, with which PI Wang has collaborated on BSF compositional analysis. The center is focused on insect rearing, but the downstream processing, and identification for application beyond feed are lacking and much of the fundamental properties are yet to be fully studied.

Two unique applications are proposed that have not been reported in the literature. BSFL lipid is unique due to its richness in lauric acid (C12:0), irrespective of the diet. **The lipids can be converted to sustainable aviation fuel (SAF)** with more desirable properties than the other current oil sources. The International Air Transportation Association established the goal of 50% CO_2 reduction by 2050 and one solution is to use alternative jet fuels derived from renewable sources. The hydrotreated esters and fatty acids (HEFA) is one of the 5 SAF that meet the ASTM standards, and it can be blended with commercial jet fuel up to 50%. HEFA does not contain aromatics (~25% in petroleum-based jet fuel) that create incomplete combustion, thus the release of soot and particulate matters in the environment (Zheng et al, 2021). BSFL lipid has been reported as biodiesel feedstock (Hasnol et al, 2020), but HEFA is a superior fuel that is free of oxygen and sulfur, and it has a high cetane number. The SAF derived from Camelina seed oil and tallow (C18 based fatty acids) contains the C14 isoparaffins (~ 40% of the fuel, Pires et al, 2018). However, there has been no SAF from medium chain FA, such as BSFL fat, which should have more desirable properties and a composition more comparable to the commercial jet fuel with shorter chain length.

The other novel application of BSF is its protein hydrolysates as antioxidant and biofertilizer. The uniqueness of this insect protein is its low extractability, possibly due to the high concentration of mineral ions that crosslink the protein molecules (Leyva-Gutierrez et al, 2022; Caligiani et al. 2018), and the presence of chitin that maybe tightly bound to proteins to form highly insoluble scroloprotein protein (Tan et al, 2021; Oonincx and Finke, 2021). Many researchers have shown low protein extraction yield, and protein fractions having much lower than expected protein contents (Rumpold et al, 2017; Queiroz et al, 2021; Smets et al, 2020) compared to that from defatted oilseed meal. This challenge can be addressed by applying enzymatic hydrolysis that shortens the chains of protein and chitin, leading to a more soluble product with unique properties of antioxidant activity and promoting plant and soil health.

Alternative biofertilizer based on waste streams and with additional functional properties, in addition to providing major plant nutrients such as nitrogen, phosphorus and potassium, holds exciting potentials. Using peptides as plants' biostimulants can mitigate injuries caused by abiotic stresses, and regulate nitrogen uptake and root development. The application of protein hydrolysates is also expected to enhance populations and activities of beneficial soil microorganisms, such as ammonifying, nitrifying and nitrogen-fixing bacteria and inorganic phosphate-solubilizers (Khan et al, 2019; Daniel et al, 2022; Atieno et al, 2020). In addition, soil application of peptides can result in increased soil organic carbon accumulation. Such beneficial effects on soil nutrients, organic carbon, and microbial populations can have a strong positive effect on soil health, thus contributing to sustainable and climate resilient arable lands. However, **how the chemical nature of the hydrolysate (peptide molecular size and conjugation with chitin as in BSL biomass) impact plant and soil health is not well known**.

Another uniqueness of the project is the study of the spent adult fly, which is currently a waste but it may be converted to protein hydrolysate with high chitin content, as shown by PI Wang's team (the only report on adult fly's composition). Chitin and chitosan have antimicrobial properties (Guarnieri et al, 2022), can destroy cuticle of nematodes and their eggs, and act as plant defense activator (Chitosanlab, 2023). **This work will demonstrate a 100% utilization of the BSF biomass.**

The BSF industry is likely to be on the forefront of the most promising technologies for recovering resources from diverse organic wastes. This study addresses the essential need for providing higher value and novel applications of BSF products, and the discoveries will help transform BSF industry from its infancy state to one driven by science-based novel applications. Ultimately, this research will contribute to a greater circular bioeconomy and environmental sustainability by creating biobased aviation fuel and agriculture products. The proposed processes are scalable that can lead to deployment and commercialization of UT technologies.

Major Activities, Timeline, and Outcomes (scope for 2-year work)

Objective 1: BSFL oil and protein fractionation and oil purification (quarter 1-2)
Hypothesis: Lipid extracted with different solvent from biomass with different moisture content can have different degree of impurity contamination that negatively impacts fuel conversion. The impurities in the fuel can lead to thermal instability and fuel oxidation. The reactants are believed to be certain N- and/or S-containing compounds and organic acids (Chevron, 2007). BSFL lipids being the fractionated product from the larval protein may contain these impurities that need to be removed before the hydrotreatment. We have shown that using the standard analytical extraction by chloroform:methonol, there was significant level of non-lipid compounds in the oil. The solvent of commercial importance, hexanes, should be used to extract the total lipids. Extraction optimization and absorptive treatment can remove most of the impurities so the lipid can be readily converted to SAF.

Lipid extraction will be conducted using the standard Soxhlet extraction (a simulation of commercial operation) with hexanes for about 10 cycles. BSFL of 3 degrees of drying will be used at solvent:solid ratio of 2:1. Lipid characterization and purity determination will be performed by using a preparative TLC and GC for separation and quantification of lipid classes using our established procedures. Oil yield and purity data will be obtained.

For absorptive purification of the lipid, commercial absorbents designed for polar compound removal will be tested, such as silica hydrogel SorbsilR92, used 0.1-0.5% in oil-hexane miscella (AOCS, 2023). S and N trace and lipid purity before and after purification treatment will be analyzed using Elementar Vario Max Cube, LC-MS, and the lipid assays as above. The effect of absorbent dosage and FFA content on refining effectiveness will be better understood.

Objective 2: Making and evaluating functional peptides from BSFL protein
Hypothesis: After the extraction of alkaline soluble proteins, the biomass residue that is still protein and chitin-rich can be enzymatically treated to produce peptides that can be used as antioxidants in food or feed systems, and as plant growth and soil quality enhancer. BSF adult that is currently landfilled may demonstrate excellent activities upon hydrolysis.

Enzymatic treatments will be conducted to produce a range of products with different molecular size (quarter 2-6). After the alkaline soluble protein is extracted (at pH 9, 30 min solubilization and centrifugation to collect the soluble protein), hydrolysis using protease and cellulase will be performed on the precipitate. Alcalase will be applied at the optimal conditions (pH 8.5, 55 °C) for various lengths of times to solubilize the cross-linked proteins; then, cellulase will be applied (pH 5.0, 50°C) for 0-4 hr to shorten the chain length of chitin. The entire hydrolysate will be used for the following studies. The same hydrolysis procedure will be done on a common soy protein concentrate (~70% purity) and BSF adult, and these hydrolysates will be used as a control and a comparison. Protein and peptide solubility profile changes will be determined using the typical pH solubilization method, and SDS-PAGE and HPLC size exclusion will be used to characterize the hydrolysate molecular size and distribution. These data will be used to correlate with the bioassay results.

Peptides' antioxidation potential will be evaluated (quarter 4-5). Since the main mechanisms by which antioxidant peptides inhibit oxidation are through inactivation of reactive oxygen species, scavenging free radicals, and chelating prooxidative transition metals, methods of DPPH free radical quenching, ORAC assay, and ferrous chelating ability will be performed. An oxidative stability study using an oil-in-water emulsion may be conducted if time allows to simulate performance in food and feed systems.

Protein hydrolysates' function of promoting plant and soil health will be evaluated (quarter 4-7) by conducting greenhouse experiment using a cash crop (e.g., corn, wheat). The protein hydrolysates will be added at different rates as treatments along with a commercial fertilizer treatment as positive control. Plant growth attributes such as plant height, above-ground biomass and grain yield will be measured when the crop reaches maturity. Soil samples will be collected before the experiment and at 1 month, 6 month and 12-month intervals from 0-5 and 5-15 cm depths for the analysis of soil macro- and micronutrients, soil organic carbon, and microbial community composition. The expected outcomes are increased plant and soil health attributes from the protein hydrolysate added plots compared to control, and understanding how molecular characteristics of the hydrolysates affect these properties.

At least two peer-reviewed publications are expected.

Future Directions, Team Building, and Proposal Submission Plan (quarter 8)

For future study of using purified BSFL lipid in SAF fuel, the Washington State University's ASCENT Center of Excellence team will be invited to collaborate on the chemical conversion and fuel quality evaluation (Pires et al, 2018). Dr. Tim Rials, Associate Dean of UTIA AgResearch is part of the group, and formal relationship will be established with possible preliminary data collection during this project period. Future federal grant application (DOE and NSF, >$3 million) with a team of entomologists, chemists, processing engineers, and soil scientists will be prepared as a multi-institutional collaboration. Industry partners, such as the Israeli startup BugEra and companies affiliated with the Aviation Sustainability Center will be invited to participate. In addition, technoeconomic and life cycle analyses will be performed when we have the unit operations and conditions examined as shown in this study. Environmental impact and circularity analyses will also be performed. A detailed plan to develop the external proposal will be made once uniquely defined UTK scopes are identified. IP disclosures are expected from this work.

References cited:

Atieno, M., Herrmann, L. et al, Lesueur, D. 2020. Assessment of biofertilizer use for sustainable agriculture in the Great Mekong RegionJournal of Environmental Management, 275:111300.

AOCS, accessed July 2023. https://lipidlibrary.aocs.org/edible-oil-processing/silica-hydrogel-and-its-use-in-edible-oil-processing.

Bosch, G.; van Zanten, H. H. E.; Zamprogna, A.; Veenenbos, M.; Meijer, N. P.; van der Fels-Klerx, H. J.; van Loon, J. J. A., Conversion of organic resources by black soldier fly larvae: Legislation, efficiency and environmental impact. J. Clean. Prod. 2019, 222, 355-363.

Caligiani, A., Angela Marseglia, Giulia Leni, Stefania Baldassarre, Lara Maistrello, Arnaldo Dossena, Stefano Sforza. 2018. Composition of black soldier fly prepupae and systematic approaches for extraction and fractionation of proteins, lipids and chitin. Food Research International 105: 812–820.

Chen, J.; Hou, D.; Pang, W.; Nowar, E. E.; Tomberlin, J. K.; Hu, R.; Chen, H.; Xie, J.; Zhang, J.; Yu, Z.; Li, Q., Effect of moisture content on greenhouse gas and NH3 emissions from pig manure converted by black soldier fly. Sci. Total Environ. 2019, 697, 133840.

Chevron Products Company. 2007. Aviation Fuels: Technical Review. https://www.chevron.com/productsservices/aviation.

Chitosanlab, 2023 July accessed. https://chitosanlab.com/en/biopesticide/.

Daniel, A. I. et al, Klein, A. 2022. Biofertilizer: The Future of Food Security and Food Safety.

Daniel, A.I.; Fadaka, A.O.; Gokul, A.; Bakare, O.O.; Aina, O.; Fisher, S.; Burt, A.F.; Mavumengwana, V.; Keyster, M.; Klein, A. Biofertilizer: The Future of Food Security and Food Safety. Microorganisms 2022, 10, 1220. https://doi.org/10.3390/microorganisms10061220.

Ebeneezar, S. Linga Prabu D., Tejpal C.S. et al. 2021. Nutritional evaluation, bioconversion performance and phylogenetic assessment of black soldier fly (Hermetia illucens, Linn. 1758) larvae valorized from food waste. Environmental Technology & Innovation 23:101783.

Guarnieri, A., Triunfo, M., Scieuzo, C. et al. 2022. Antimicrobial properties of chitosan from different developmental stages of the bioconverter insect Hermetia illucens. Sci Rep 12, 8084. https://doi.org/10.1038/s41598-022-12150-3.

Hasnol, S., et al, Lim, J.W. 2020. A Review on Insights for Green Production of Unconventional Protein and Energy Sources Derived from the Larval Biomass of Black Soldier Fly. Processes 2020, 8(5), 523; https://doi.org/10.3390/pr8050523.

Khan, S., Yu, H., et al, Jiang, W. 2019. Exogenous Application of Amino Acids Improves the Growth and Yield of Lettuce by Enhancing Photosynthetic Assimilation and Nutrient Availability. MDPI Agronomy Agronomy 9, 266; https://doi.org/10.3390/agronomy9050266.

Kristiansen, S.; Painter, J.; Shea, M., Animal agriculture and climate change in the US and UK elite media: Volume, responsibilities, causes and solutions. Environ. Commun. 2021, 15 (2), 153-172.

Leyva-Gutierrez, FMA., Fomich, M., Metzcar, C., Saad, J., Dia, VP., Cammack, JA. Tomberlin, JK., and Wang, T. 2022. Comprehensive chemical analysis of black soldier fly (Hermetia illucens L.) larvae and adults. Journal of Insect for Food and Feed. 8(12): 1411-1429. DOI 10.3920/JIFF2021.0187.

Magee, K.; Halstead, J.; Small, R.; Young, I. Valorisation of OrganicWaste By-Products Using Black Soldier Fly (Hermetia illucens) as a Bio-Convertor. Sustainability 2021, 13, 8345. https://doi.org/10.3390/su13158345.

Oonincx, D.G.A.B. and Finke, M.D. 2021. Nutritional value of insects and ways to manipulate their composition. *Journal of Insects as Food and Feed, 2021; 7(5): 639-659.*

Pang, W.; Hou, D.; Chen, J.; Nowar, E. E.; Li, Z.; Hu, R.; Tomberlin, J. K.; Yu, Z.; Li, Q.; Wang, S., Reducing greenhouse gas emissions and enhancing carbon and nitrogen conversion in food wastes by the black soldier fly. J. Environ. Manage. 2020a, 260, 110066.

Pang, W.; Hou, D.; Ke, J.; Chen, J.; Holtzapple, M. T.; Tomberlin, J. K.; Chen, H.; Zhang, J.; Li, Q., Production of biodiesel from CO2 and organic wastes by fermentation and black soldier fly. Renew. Energy 2020b, 149, 1174-1181.

Pires, A.P.P., Han, Y., Kramlich, J., and Garcia-Perez, M. 2018. Chemical composition and fuel properties of alternative jet fuels. BioResources 12: 2632-2657.

Queiroz, L.S., Regnard, M., Jessen, F. etc, Casanova, F. 2021. Physico-chemical and colloidal properties of protein extracted from black soldier fly (*Hermetia illucens*) larvae. International Journal of Biological Macromolecules. 186: 714-723.

Rumpold, B.A., Bubler, S., Joger, H., Schluter, O. 2017. Insect processing. In Insects as food and feed: from production to consumption, Editors Arnold van Huis and Jeffery K. Tomberlin, Published: 2017. Page 319-342. https://doi.org/10.3920/978-90-8686-849-0.

Smets, R., Verbinnen, B., Van De Voorde, I., Aerts, G., Claes, J., and Van Der Borght, M. 2020. Sequential extraction and characterisation of lipids, proteins, and chitin from black soldier fly (Hermetia illucens) larvae, prepupae, and pupae. Waste Biomass Valor. 11, 6455–6466.

Tan, Y.N., Chin, Y.L., and Chen, W.N. 2021. Comparison of Sustainable Lipid and Protein Removal Methods for the Isolation of Insect Chitin from Black Soldier Fly Exoskeleton. ACS Food Sci. Technol. 2021, 1, 698−706.

US Regulations, 2022 (accessed). The future of edible insects. https://thefutureofedibleinsects.com/us-regulations/.

Zheng, L., Singh, P., Cronly, J. et al. (5 more authors). 2021. Impact of aromatic structures and content in formulated fuel for jet engine applications on particulate matter emissions. Journal of Energy Resources Technology, 143 (12). 122301. ISSN 0195-0738.