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NUTRITIONAL ANALYSIS AND CHEMICAL FINGERPRINTING OF BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE MEAL NATIVE TO THE PERUVIAN AMAZON

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ABSTRACT

The search for new protein sources for food formulation is increasing. Black soldier fly (*Hermetia illucens*) larvae have high levels of protein, good fat content and are rich in essential amino acids, making them an alternative source of dietary protein. In this work, the nutritional properties and metabolomic characterization of black soldier fly larvae meal native to the Peruvian Amazon reared on three organic waste substrates were carried out. The three meals had a high protein content (38.90-44.68%), lipids (26.96-31.01%), as well as a high potassium, calcium and low sodium content. The larvae meal fed with orange bagasse presented the best content of iron and manganese. The fatty acid profile showed a high content of palmitic, oleic and linoleic acids for the three flours. The most abundant essential amino acids in the three meals were leucine, lysine and valine. A total of 48 compounds were identified in the larvae meal fed with orange bagasse, 24 compounds in the larvae meal fed with oats and 44 compounds in the larvae meal fed with ripe banana pulp. These results indicate that black soldier fly larvae meals native to the Peruvian

Amazon are a good source of proteins, minerals, essential amino acids and unsaturated fatty acids with high nutritional value.

KEYWORDS: nutritional value, animal feed, metabolomic, organic waste processing

ANÁLISIS NUTRICIONAL Y HUELLA QUÍMICA DE LA HARINA DE LARVA DE MOSCA SOLDADO NEGRA (*Hermetia illucens*) NATIVA DE LA AMAZONÍA PERUANA

RESUMEN

La búsqueda de nuevas fuentes de proteínas para la formulación de alimentos es cada vez mayor. Las larvas de la mosca soldado negra (*Hermetia illucens*) tienen altos niveles de proteína, buen contenido de grasa y son ricas en aminoácidos esenciales, lo que las convierte en una fuente alternativa de proteína dietética. En este trabajo se evaluó las propiedades nutricionales mediante la composición proximal, contenido de minerales, perfil de ácidos grasos, aminoácidos y la caracterización metabolómica de harina de larvas de mosca soldado negra nativa de la amazonia peruana criadas en tres sustratos de residuos orgánicos. Las tres harinas presentaron un alto contenido de proteínas (38,90-44,68%), lípidos (26,96-31,01%), así como un alto contenido de potasio, calcio y bajo contenido en sodio. Las harinas de larvas alimentadas con el bagazo de naranja presentaron los mejores contenidos de hierro y manganeso. El perfil de ácidos grasos mostró un alto contenido los ácidos palmítico, oleico y linoleico para las tres harinas. Los aminoácidos esenciales más abundantes en las tres harinas fueron leucina, lisina y valina. Un total de 48 compuestos fueron identificados en la harina de larva alimentada con bagazo de naranja, 24 compuestos en la harina de larva alimentada con avena y 44 compuestos en la harina de larva alimentada con pulpa de plátano maduro. Estos resultados nos indican que las harinas de larva de mosca soldado negra nativa de la Amazonía peruana son una buena fuente de proteínas, minerales, aminoácidos esenciales y ácidos grasos insaturados con alto valor nutricional.

PALABRAS CLAVE: valor nutricional, alimento animal, metabolómica, procesamiento de residuos orgánicos

INTRODUCTION

In recent years, one of the biggest drawbacks in the production of animal feed is the availability of protein sources for the formulation of diets. Protein is one of the most expensive nutrients in the formulation and it must be taken into account that the crude protein requirements in animals (poultry and pigs) range between 16-23% depending on the rearing phase (te Pas *et al.*, 2021).

An alternative for obtaining protein and nutrients are insects. Recently, insects have gained great relevance as an important source of sustainable raw materials for animal feed; especially for fish, poultry and pigs (Sogari *et al.*, 2019). Insect meal, made largely from larvae, is rich in high quality protein (45–68% dry matter basis) and has a high digestibility and good amino profile. This new resource could substitute soybean meal and fishmeal in animal and fish diets (Chaalala *et al.*, 2018).

Insects are consumed whole, crushed or as meal. This last form of preparation is the most used addition to human food and animal diets. Among the most studied insect meals used as protein replacement in human foods are the domestic cricket (*Acheta domesticus*), the larvae of black soldier fly (*Hermetia illucens*) and mealworm (*Tenebrio molitor*). The main nutritional components in insects are protein and fat, followed by fiber, non-protein nitrogen and ash, and the composition depends on the type of insect, the stage of growth, and the diet of the insects (Avendaño *et al.*, 2020).

The black soldier fly (BSF), *Hermetia illucens* (Diptera, Stratiomyidae), is native to the Neotropics, but is now found widely distributed in tropical and temperate regions worldwide (Marshall *et al.*, 2015). Their larvae contain high levels of protein (ranging from 37 to 63% dry matter) and high content of saturated fatty acids (58-72%) (Barragan-Fonseca *et al.*, 2017). The amino acid profiles are particularly rich in lysine and contain high amounts of alanine, methionine, histidine, and tryptophan. BSF larvae contain higher concentrations of minerals such as iron (Fe), cooper (Cu), Manganese (Mn), zinc (Zn), Calcium (Ca) and phosphorus (P) than other insects. Notably, the nutritional value of the BSF larvae depends on the quality and quantity of food ingested (Barragan-Fonseca *et al.*, 2017). The BSF larvae are an alternative replacement for soybean meal or fish meal as a feed protein source (Lu *et al.*, 2022b). In recent years, there has been great interest in using BSF larvae as an organic waste converter. They feed on different types of organic waste, offering an alternative method to transform it into high-value products (Amrul *et al.*, 2022).There are several nutritional studies on BSF larvae and their use for the preparation of animal feed, but no reports on the nutritional value of BSF larvae native to the Peruvian Amazon. Recently, a study exclusively detailed the semi-captive rearing of BSF larvae native to the Loreto, Peru, using citrus residues as a substrate (Morey *et al.*, 2023). Therefore, our present study aims to describe the nutritional properties and conduct chemical fingerprinting by UHPLC–MS analysis of BSF (*H. illucens*) larvae meal. These larvae were sourced from both their native habitat and semi-captive breeding within the Peruvian Amazon, and have been reared on different organic waste substrates.

MATERIAL AND METHODS

REARING AND HARVESTING

First instar *H. illucens* larvae were taken from the BSF parenting module at the Natural Resources Research Center (CIRNA) of the National University of the Peruvian Amazon (UNAP),

which has a semi-captive parenting system from wild females originating from the urban forest at CIRNA. To assess their effects on the nutritional value and chemical composition of BSF larvae, three different substrates were used: orange bagasse (*Citrus sinensis*), ripe banana pulp (*Musa paradisiaca*) and oatmeal. The crushed substrates were anaerobically fermented for 4 days and then mixed with rice dust (*Oriza sativa*) in a 4:1 ratio for each diet. These mixtures were then homogenized with water to reach a humidity of 70%.

Approximately 1000 six to eight day-old BSF larvae were raised in dark plastic-lined wooden boxes measuring 2 x 0.50 x 0.40 m (length x width x depth) and supported by 0.7 m legs. This rearing took place over a 15-day period at a constant temperature of 28 °C and a relative humidity of 75%. Each box had a movable lid covered with a metal mesh to prevent the entry of predators. After 15 days, the larvae developed on the different substrates were manually removed from the residue using metal mesh strainers and then frozen overnight at -20 °C. The orange bagasse and the ripe banana fruit were obtained from Iquitos markets, while oatmeal and rice dust were purchased from commercial food stores.

PREPARATION OF LARVAE MEAL

After the larvae were separated from the residues, they were washed with distilled water and dried in an oven at 50 °C for 48-72 hours until their weight stabilized. Then, the larvae were ground to a powder using a blade mill (Grindomix GM 200, Tesch, Haan, Germany) and hermetically packed and stored at 5 °C prior to analysis.

PROXIMATE ANALYSIS

Procedures established by the AOAC were used in all determinations (Vargas-Arana *et al.*, 2022). The moisture content was determined by oven-drying the sample at 105 °C to reach a constant weight. The crude protein content was measured by the Kjeldahl method (N x 6.25), and the total lipid content was obtained using the Soxhlet procedure using petroleum ether as solvent. Fiber content was determined by gravimetry after hydrolysis of the samples, and ash content was measured by incineration in a muffle furnace at 550 °C for 6 hours. Total carbohydrates were calculated as 100 - (g water + g protein + g fiber + g fat + g ash). The results are expressed in g per 100 g larvae meal (g/100 g).

MINERAL ANALYSIS

The larvae meal was dried to ash at 550 °C, boiled with 10 mL of 20% hydrochloric acid, and then filtered and brought to 100 mL with deionized water. The levels of minerals -- magnesium (Mg), sodium (Na), potassium (K), calcium (Ca), Phosphorus (P), copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) -- were determined using atomic absorption spectroscopy (Varian AA240) calibrated with standard solutions containing known amounts of the minerals being tested. The atomic absorption spectroscopy was accomplished with flames of air-acetylene and nitrous oxide-acetylene, with the latter only being used for calcium analysis. Hollow cathode monometallic lamps were utilized for each element analyzed, and all analyses were performed in triplicate (Vargas-Arana *et al.*, 2022).

FATTY ACID ANALYSIS

The fatty acid (FA) profile was determined according to Vargas-Arana *et al.* (2022), with some modifications, using gas chromatography of fatty acid methyl esters (FAME). The oils were

converted to their corresponding methyl esters, which were prepared by saponification and esterification with KOH in methanol (2 M). FAME were extracted with hexane and processed on a Varian 450-GC gas chromatograph (Varian Inc., Palo Alto, CA, USA). The chromatograph was equipped with a VF-WAXms, 60 m x 0.25 mm ID, 0.25 m capillary column, CP9207, flame ionization detector and Varian CP-8400 autoinjector. Helium was used as carrier gas and the chromatograph was programmed to operate under the following conditions: injector temperature 250 °C, column temperature gradient 100 °C x 4 min, followed by an increase to 200 °C (rate 25 °C/min), 200 °C for 8 min, followed by an increase to 250 °C (rate 5 °C/min), and 250 °C for 6 min. Individual peaks were identified by comparing the FAME retention time with the standard Supelco 37 component FAME mix (Supelco, Bellefonte, PA, USA). FAs were expressed as a percentage of total FAME.

AMINO ACID ANALYSIS

The amino acid profile was determined according by Cobos *et al.* (2020), with some modifications. 25 mg of larvae meal was weighed and hydrolyzed with 2 mL of 6 N HCl at a temperature of 100 °C for 24 h. Then, 50 µL of the hydrolyzed meal was transferred to a test tube with 100 µL of 2.5 mM 2-L-Aminobutyric acid internal standard and 4850 µL of ultrapure water. The solution was filtered through 0.45 µm PTFE syringe filters. Then, 10 µL of the filtered solution was derivatized using the reagent 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate according to the instructions of the AccQ-Fluor reagent kit (Waters Corporation, Milford, MA, USA). 5 µL of the derivatized sample was injected into the HPLC (Hitachi, Japan) with fluorescence detector. Equipment conditions were: Hypersil GOLD C18 5 µm x 4.6 mm x 150 mm column, column temperature 37 °C, excitation of fluorescent amino acid derivatives: 250 nm, detection of fluorescent emission: 395 nm, flow rate 1 mL/min, mobile phase A: water, mobile phase B: sodium acetate pH 5.1 and mobile phase C: acetonitrile, mobile phase ramp was: 0 min (0:100:0), 1 min (0:100:0), 25 min (0:83:17), 33 min (50:0:50), 36 min (0:100:0) and 40 min (0:100:0). Amino acid quantification was performed with a 5 to 50 µM calibration curve.

CHEMICAL FINGERPRINTING

The chemical fingerprinting analysis was carried out by UHPLC-ESI-QTOF-MS on the samples of larvae meal previously defatted with hexane and without defatting. Samples were extracted one time with ethanol using microwaves (Anton Paar monowave 400). The extracts then were filtered, concentrated in vacuo, and stored at -20 °C.

LC PARAMETERS AND MS PARAMETERS

The compounds present in the larvae meal extracts were separated and identified in both negative or positive ESI modes using a UHPLC-ESI-Q-TOF-Mass system. This system consisted of an Ultimate 3000 RS chromatograph managed with Chromeleon 6.8 software (Dionex GmbH, Idstein, Germany) and was coupled to a Bruker maXis ESI-QTOF-MS with the Data Analysis 4.0 software (both Bruker Daltonik GmbH, Bremen, Germany). Each extract (5 mg) was dissolved in 2 mL of ethanol, filtered with a polytetrafluoroethylene (PTFE) filter for ulterior analysis, and 10 µL was injected into the equipment. The chromatographic equipment consisted of a quaternary pump, an autosampler, a thermostatic column compartment, and a photodiode array detector (PDA). Elution was

performed with a binary gradient system consisting of eluent (A) with 0.2% formic acid in water and eluent (B) with 0.2% formic acid in acetonitrile. The gradient sequence employed was as follows: 1% B isocratic (0–3 min), 1–5% B (3–5 min), 5% B isocratic (5–8 min), 5–10% B (8–12 min), 10–35% B (12–35 min), 35–95% B (35–42 min) and 1% isocratic B (42–50 min). Separation was carried out with a Thermo 5 µm C18 80 Å column (150 mm × 4.6 mm) at a flow rate of 1.0 mL/min. ESI-QTOF-MS experiments were recorded in negative ion mode, and the scan range was between 100 and 1200 m/z. Electrospray ionization (ESI) conditions consisted of a capillary temperature of 200 °C, a capillary voltage of 2.0 kV, a dry gas flow rate of 8 L/min, and a nebulizer pressure of 2 bar. The experiments were performed in automatic MS/MS mode. The structural characterization of secondary metabolites relied on HR full MS, analysis of MS fragmentation patterns, and cross-referencing with data available in the literature.

STATISTICAL ANALYSIS

All experiments were repeated at least three times, and the results were expressed as mean ± standard deviation (SD) using SigmaPlot 11.0 software. Significant differences among means were determined using Tukey's comparison, considering p values < 0.05 as significant.

RESULTS AND DISCUSSION

PROXIMAL COMPOSITION OF BLACK SOLDIER FLY LARVAE MEAL

Table 1 displays the proximal composition of black soldier fly (BSF) larvae meal reared on three different substrates, showing varying contents of moisture, ashes, lipids, protein, fiber and carbohydrates. The results of a proximal composition showed that three larvae meal have a high protein content (from 38.90 to 44.68%) and lipids (from 26.96 to 31.01%), alongside very low crude fiber content (from 0.12 to 0.17%). These values are similar to those reported by (Spranghers *et al.*, 2017), who also studied larvae fed with different organic wastes. Notably, larvae reared on restaurant waste showed the highest protein (43.1%) and lipid (38.6%) content in dry matter. These protein and fat contents vary according on the substrate. For example BSF larvae reared on liver and fish are high in protein (62.7 and 57.9% respectively) and fat content (25.1 and 34.6% respectively), and higher than those reared on chicken feed, fruits and vegetables (Barragan-Fonseca *et al.*, 2017). Our study's protein and fat values fall within the typical range reported in the literature for BSF larvae meal, which varies between 31.7% and 47.6% for proteins and between 11.8% and 41.7% for fats in BSF larvae reared on different diets or sources (Wang & Shelomi, 2017).

MINERALS COMPOSITION

Table 2 shows the mineral contents (macro and micronutrients). For macronutrients, the three larvae meal showed a high potassium (from 1531.23 to 1876.79 mg/100 g) and calcium content (from 516.32 to 1787.39 mg/100 g), good magnesium content (from 401.09 to 462.39 mg/100 g) and low sodium content (from 7.86 to 20.12 mg/100 g). The BSF larvae meal reared on orange bagasse was the one that presented the best results in potassium and calcium content. The values of potassium and magnesium are higher than those reported for BSF larvae reared on chicken feed. The phosphorus content is similar, but calcium and sodium are lower (Spranghers *et al.*, 2017). For micronutrients,

Table 1. Proximal composition (%) of BSF larvae meal reared with different substrates.

Parameter	Orange bagasse	Ripe banana pulp	Oatmeal
Humidity	8.44 ± 0.22[a]	7.45 ± 0.30[b]	8.25 ± 0.12[a]
Ashes	8.16 ± 0.10[a]	6.16 ± 0.06[b]	5.65 ± 0.03[c]
Total Lipids	28.53 ± 0.37[a]	31.01 ± 0.83[b]	26.96 ± 0.56[c]
Crude protein	38.90 ± 1.00[a]	44.68 ± 0.47[b]	43.53 ± 0.80[b]
Crude fiber	0.15 ± 0.01[a]	0.12 ± 0.00[b]	0.17 ± 0.01[c]
Carbohydrates	15.81 ± 0.77[a]	10.57 ± 0.20[b]	15.43 ± 1.52[a]

*Tests: n = 3; the means ± standard deviation, assigned different letters on the same row indicate are significant difference at 0.05 level of significance according to Tukey's test.

Table 2. Mineral content (mg/100 g) in BSF larvae meal reared with different substrates.

Element	Orange bagasse	Ripe banana pulp	Oatmeal
K	1876.79 ± 53.52[a]	1782.42 ± 36.19[a]	1531.23 ± 52.46[b]
Na	7.86 ± 0.23[a]	7.90 ± 0.22[a]	20.12 ± 0.37[b]
Ca	1787.39 ± 42.94[a]	520.82 ± 5.89[b]	516.32 ± 5.35[b]
Mg	401.09 ± 6.53[a]	462.39 ± 4.55[b]	422.53 ± 5.30[c]
P	480.38 ± 5.08[a]	530.17 ± 4.01[b]	490.19 ± 4.04[a]
Zn	9.89 ± 0.05[a]	10.18 ± 0.24[a]	8.90 ± 0.13[b]
Cu	0.85 ± 0.02[a]	1.20 ± 0.02[b]	0.70 ± 0.02[c]
Mn	21.76 ± 0.76[a]	15.87 ± 0.50[b]	15.55 ± 0.44[b]
Fe	36.32 ± 1.13[a]	14.68 ± 0.55[b]	15.14 ± 0.33[b]

*Tests: Means ± standard deviations (n = 3). Different letters assigned on the same row indicate a significant difference at $\alpha = 0.05$ according to Tukey's test.

the BSF larvae meal reared on orange bagasse showed the higher iron (36.32 mg/100 g) and manganese content (21.76 mg/100 g), values higher than those reported for larvae of other insects (coleopterans) (Ghosh *et al.*, 2017). In general, all these values are within the range of what is reported in the literature for BSF larvae (Lu *et al.*, 2022b) and in quantities recommended for animal nutrition (Cedeño-López & WingChing-Jones, 2022).

FATTY ACID PROFILE

The fatty acid content is shown in Table 3. The most abundant saturated fatty acids are lauric acid (C12:0), which ranged from 18.16 to 27.62%, and palmitic acid (C16:0), which ranged from 15.47 to 22.25 %. The major monounsaturated fatty acids are oleic acid (C18:1), which ranged from 16.58 to 22.64%, and linoleic acid (C18:2), which ranged from 11.90 to 26.51%. These values coincide with what has been reported in the literature for BSF larvae reared on different organic waste substrates (Barragan-Fonseca *et al.*, 2017, Almeida *et al.*, 2020). For the high concentration of lauric acid, BSF larvae oil could be used in cosmetic formulations for the skin, refining it to improve its color and odor characteristics, and eliminate inappropriate phospholipids and free fatty acids

(Verheyen *et al.*, 2018). In addition, the high content of palmitic, oleic and linoleic acids makes it an alternative for use in the production of soaps and cosmetics (Franco *et al.*, 2022).

AMINO ACID PROFILE

The amino acid profile of BSF larvae meal is shown in Table 4. Despite being reared on different substrates, the differences in amino acid content were small. The values are similar to those reported in the literature by other authors for BSF larvae reared on different substrates (Spranghers *et al.*, 2017, Almeida *et al.*, 2020, Lu *et al.*, 2022b). The most abundant essential amino acids were leucine (from 27.82 to 29.46 mg/g), lysine (from 24.01 to 25.63 mg/g) and valine (from 24.70 to 25.59 mg/g). The content of these three amino acids is slightly higher than those reported for soybean meal produced in different countries for broilers (Ravindran *et al.* 2014) and similar than those reported for fish meal (Kim *et al.*, 2012). The values of histidine, iso-leucine, phenylalanine, threonine and tryptophan are basically the same as soybean meal, but slightly lower than those of fish meal (Kim *et al.*, 2012).

CHEMICAL FINGERPRINTING

In the literature, there are few reports about chemical fingerprinting of BSF larvae. In a study carried out by Hsiao *et al.* (2022), phenylalanine, arginine, oleic acid and 10-hydroxy-8E-octadecenoic acid were identified by LC-MS as the major components in various instars of the BSF larvae reared on sesame (*Sesamum indicum* seed) residues. Other studies report the characterization and quantification of functional proteins and bioactive peptides produced from the enzymatic digestion of BSF larvae fed with food wastes using proteomics-based analysis (Lu *et al.*, 2022a).

Analysis of the BSF larvae meal from three distinct substrates revealed the presence of various compounds. For instance, flavonoids detected in larvae fed with orange bagasse could serve as identifiable chemical markers for *Hermetia illucens* larvae. Despite defatting the sample with hexane, all three BSF larvae meals exhibited the presence of diverse unsaturated fatty acids—essential components in edible fats. This aligns with findings reported by (Li *et al.*, 2021), in their study using an LC-MS-Based Lipidomics Approach, analyzing BSF larvae fed food waste at Different Days of Age. The chromatographic analysis of the ethanolic extracts, previously defatted with hexane (Figure 1A) and without defatting (Figure 1B), of the BSF larvae meal reared on oatmeal by high-resolution mass spectrometric analysis (UHPLC-MS) full scan allowed the identification of 24 compounds which were mainly fatty acids (peak 7, 10, 11, 15, 16, 19, 21-23; as 9-Octadecenedioic acid ($C_{18}H_{32}O_4$), Hydroperoxy-octadecadienoic acid ($C_{18}H_{32}O_4$), eicosanoic, dilinoleic, (R)-2-hydroxystearic, Hexadecylmalonic acid, Heneicosanoic, Tricosanoic and Behenic acids, respectively). Peak 2 was attained as the amino acid histidine ($C_6H_9N_3O_2$), peak 3-4 as simple acids: threonic acid and quinic acids, respectively, while peak 5 as phenolic acid: caffeoyl quinic acid, respectively, peak 6 as the hydrocarbon Xanthoxin, peak 8 as phosphofatty acids derivative compounds, linoleoylglycerophosphoethanolamine ($C_{23}H_{44}NO_7P$), while peaks 12-14 as 1-(9Z-octadecenyl)-sn-glycero-3-phosphoethanolamine, 1-phosphatidyl-1D-myo-inositol and 1-phosphatidyl-1D-myo-inositol derivative, respectively, while peak 24 as 1-stearidonoyl-2-stearoyl-sn-glycero-3-phosphoethanolamine, and peak 25 as Phosphatidylinositol 16:0-18:2. Peak 9 was identified as the amino fatty compounds N-Dodecanoyl-N-methylglycine, peak 16 as N-

Oleoyl-Phenylalanine ($C_{27}H_{43}NO_3$), and peak 17 as N-Oleyl-Leucine ($C_{24}H_{45}NO_3$). Finally, peak 20 was identified as sclareol and peak 18 as Oleic estolide ($C_{36}H_{68}O_4$) (Appendix 1).

In the ethanolic extracts of the BSF larvae meal reared on orange bagasse, previously defatted with hexane (Figure 2A) and without defatting (Figure 2B), , several compounds were identified. Primarily, the compounds consisted mainly of fatty acids, including peaks 4, 6, 8, 12, 15, 16, 21, 27, 29, 30, 34-42, 48 as (11E)-13-Hydroxy-9-methoxy-10-oxo-11-octadecenoic acid, 9-HPODE, Porrigenic acid, (R)-2-hydroxystearic acid, hydroxyStearic acid isomer, Stearic acid, 9-HOTrE, 9-HPODE isomer, Eicosanoic acid, 9-HODE, 12-Hydroxyoleic acid, 17-Hydroxylinolenic acid, Myristic acid, Arachidonic Acid, Tetratriacontatetraenoic acid, FAHFA 18:2/20:4, linoleic acid, Dilinoleic acid, hydroxystearic acid isomer and Docosapentaenoic acid. Additionally, there were some glycerol derivatives present, including peak 10 LPE 18:2 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine, peak 23, 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine, peak 24 1-(9Z-octadecenoyl)-sn-glycero-3-phosphoserine, peak 46 peak 44 Glycerol monooleate, peak 46 1,2-Dipalmitoyl-sn-glycero-3-phosphate, peak 49 1,2-Dilinoleoyl-sn-glycero-3-phosphatidylethanolamine, and some phospho and amino derivative compounds, peak 7 N-Dodecanoyl-N-methylglycine , peak 9 Lysophosphatidylethanolamine 16:0, peak 11 Phosphatidylethanolamine lyso 18:1, peak 13 N-Oleoyl-Phenylalanine, peak 22 N-Dodecanoyl-N-methylglycine, peak 32 LPE O-18:0 1-Octadecyl-sn-glycero-3-phosphoethanolamine, peak 33 LPE O-18:1 1-Palmitoylglycol-2-phosphocholine, peak 43 N-Oleyl-Leucine, peak 47 as [1-hexadecanoyloxy-3-phosphonooxy-55-propan-2-yl]octadec-9-enoate, one flavone nevadensin, beside some phenolic acids, peak 2 as Coumaroyl quinic acid, peak 3 as 2-Isopropylmalic Acid, peak 5 as 4',5,7-trihydroxy-3,6-dimethoxyflavone, and one chomenone, peak 19 as 5,7-dihydroxy-3,6-

Table 3. Fatty acid profile (%) of BSF larvae meal reared with different substrates.

Fatty acids	Orange bagasse	Ripe banana pulp	Oatmeal
Capric (10:0)	0.28 ± 0.01^a	0.48 ± 0.01^b	0.38 ± 0.01^c
Lauric (12:0)	18.16 ± 0.10^a	27.62 ± 0.16^b	23.28 ± 0.20^c
Myristic (14:0)	4.75 ± 0.13^a	6.33 ± 0.15^b	4.93 ± 0.17^c
Palmitic (C16:0)	17.24 ± 0.17^a	22.25 ± 0.22^b	15.47 ± 0.12^c
Palmitoleic (16:1)	1.90 ± 0.02^a	4.27 ± 0.11^b	1.83 ± 0.03^a
Stearic (18:0)	2.82 ± 0.04^a	2.42 ± 0.05^b	2.81 ± 0.04^a
Oleic (18:1)	19.62 ± 0.09^a	16.58 ± 0.12^b	22.64 ± 0.21^c
Linoleic (18:2)	26.07 ± 0.75^a	11.90 ± 0.14^b	26.51 ± 0.32^a
Linolenic (18:3)	0.15 ± 0.00^a	0.16 ± 0.00^b	0.09 ± 0.00^c
Arachidic (20:4)	9.01 ± 0.14^a	1.96 ± 0.03^b	0.68 ± 0.00^c
Eicosapentenoic (20:5)	1.40 ± 0.02^a	6.03 ± 0.10^b	1.38 ± 0.02^a
Saturated FAs	43.25	59.10	46.87
Mono-UFAs	21.52	20.85	24.47
Poly-UFAs	35.23	20.05	28.66

*Tests: Means ± standard deviations (n=3). Different letters assigned on the same row indicate a significant difference at α =0.05 according to Tukey's test.

Table 4. Amino acid composition of BSF larvae meal reared with different susbstrates (mg/g dry matter).

Amino acids	Orange bagasse	Ripe banana pulp	Oatmeal
Indispensable amino acids			
Histidine	8.30 ± 0.12[a]	9.50 ± 0.16[b]	11.30 ± 0.18[c]
Iso-Leucine	18.70 ± 0.10[a]	19.01 ± 0.13[b]	18.58 ± 0.11[a]
Leucine	28.28 ± 0.40[a]	29.46 ± 0.52[b]	27.82 ± 0.33[a]
Lysine	24.01 ± 0.59[a]	25.63 ± 0.36[b]	24.50 ± 0.48[ab]
Phenylalanine	14.56 ± 0.21[a]	15.94 ± 0.36[b]	14.35 ± 0.22[a]
Threonine	16.19 ± 0.29[a]	16.76 ± 0.22[ab]	15.60 ± 0.26[ac]
Tryptophan	18.42 ± 0.30[a]	19.64 ± 0.20[b]	17.61 ± 0.32[c]
Valine	24.70 ± 0.23[a]	25.08 ± 0.42[ab]	25.59 ± 0.30[b]
Dispensable amino acids			
Alanine	37.88 ± 0.66[a]	39.89 ± 1.10[a]	44.13 ± 1.72[b]
Arginine	16.18 ± 0.61[a]	20.28 ± 0.70[b]	19.27 ± 0.65[b]
Aspartic acid	29.00 ± 0.22[a]	29.57 ± 0.30[a]	29.05 ± 0.19[a]
Glutamic acid	52.52 ± 1.14[a]	49.54 ± 0.86[b]	49.10 ± 0.73[b]
Glycine	21.59 ± 0.31[a]	23.26 ± 0.61[b]	21.87 ± 0.45[a]
Proline	27.17 ± 0.34[a]	27.07 ± 0.26[a]	28.98 ± 0.30[b]
Serine	17.51 ± 0.28[a]	18.46 ± 0.32[b]	17.00 ± 0.21[a]

*Tests: Means ± standard deviations (n = 3). Different letters assigned on the same row indicate a significant difference at α = 0.05 according to Tukey's test.

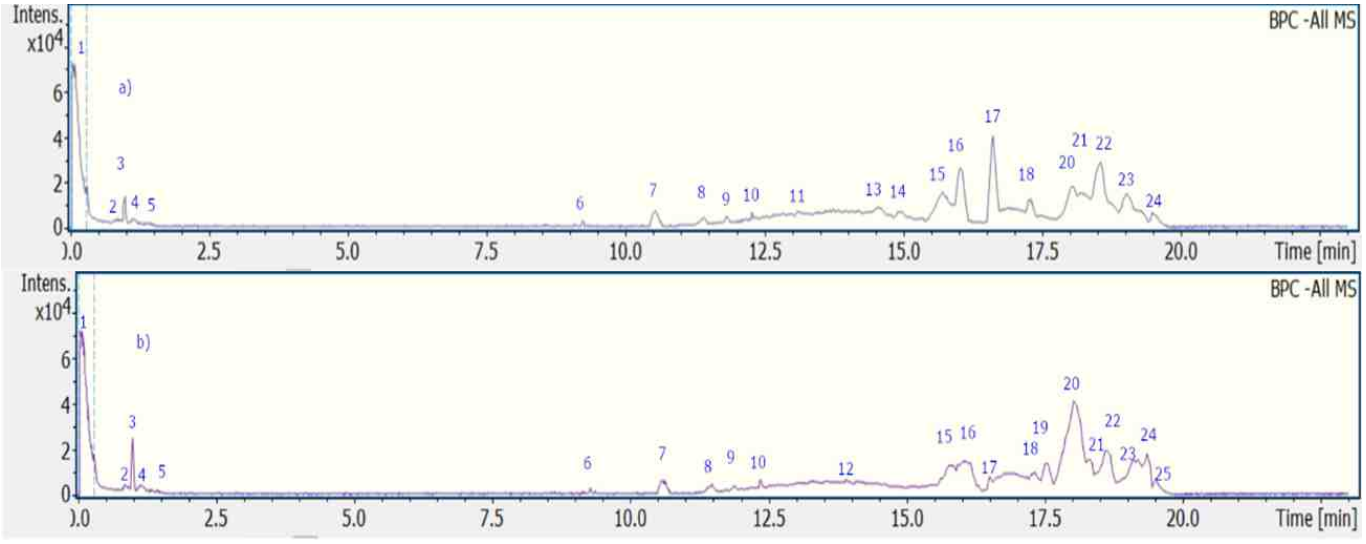

Figure 1. Chromatogram of ethanolic extracts of BSF larvae meal reared on oatmeal: A) previously defatted with hexane; and B) without defatting.

dimethoxy-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one (Appendix 2).

In the ethanolic extracts of the BSF larvae meal reared on ripe banana pulp, both previously defatted with hexane (Figure 3A) and without defatting (Figure 3B), several compounds can be identified, which were also mainly fatty acids (peaks 4-9, 12,13, 17, 21, 27, 29, 31, 34-42, as Dodecanedioic acid, Omega-Hydroxydodecanoic Acid, Porrigenic acid, 9-HPODE, (10E,12E)-9-hydroperoxyoctadeca-10,12-dienoic acid, 10-Hydroxydecanoic Acid, 8,13-dihydroxy-9,11-octadecadienoic Acid, omega-hydroxydodecanoic acid, Porrigenic acid isomer, Eicosanoic acid, 9-Hydroxyoctadecadienoic acid, 12-Hydroxyoleic acid, 17-Hydroxylinolenic acid, Myristic acid, Tetratriacontatetraenoic acid, linoleic acid, Dilinoleic acid and (R)-2-hydroxystearic acid, respectively. Peak 2 was attributed as the amino acid histidine ($C_6H_9N_3O_2$), peak 3 as threonic acid and peak 10 as the benzoic derivative 2-((Decyloxy)carbonyl) benzoic acid. Peak 11 was identified as chromenone cassiaside (5-hydroxy-2-methyl-6-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxybenzo[g]chromen-4-one) and peak 15 as the polyol derivative 1-phosphatidyl-1D-myo-inositol, respectively, while several phosphatidylethanolamines were detected: peak 18, as Lysophosphatidylethanolamine 16:0, peak 19 as 1-linoleoylglycerophosphoethanolamine, peak 20 as Phosphatidylethanolamine lyso 18:1, peak 23 as hexadecanoyl-lysophosphatidylethanolamine, peak 25 as Phosphatidylethanolamine lyso 18:1, and peak 32 as 1-Octadecyl-sn-glycero-3-phosphoethanolamine. Other phosphoamine derivative compounds detected were peak 16 as N-Dodecanoyl-N-methylglycine ($C_{15}H_{29}NO_3$), peak 24 as 1-(9Z-octadecenoyl)-sn-glycero-3-phosphoserine ($C_{24}H_{46}NO_9P$), peak 33 as 1-Palmitoylglycol-2-phosphocholine, and peak 43 as N-Oleoyl-Phenylalanine ($C_{27}H_{43}NO_3$). Peak 30 was identified as the nitrophenol 2,6-Di-tert-butyl-4-nitrophenol ($C_{14}H_{21}NO_3$), peak 28 as Dodecylbenzenesulfonic acid ($C_{18}H_{30}O_3S$), peak 26 as Tetradecylsulfate. Finally, peak 44 was identified as sclareol and peak 45 as Glyceryl monolinoleate (Appendix 3).

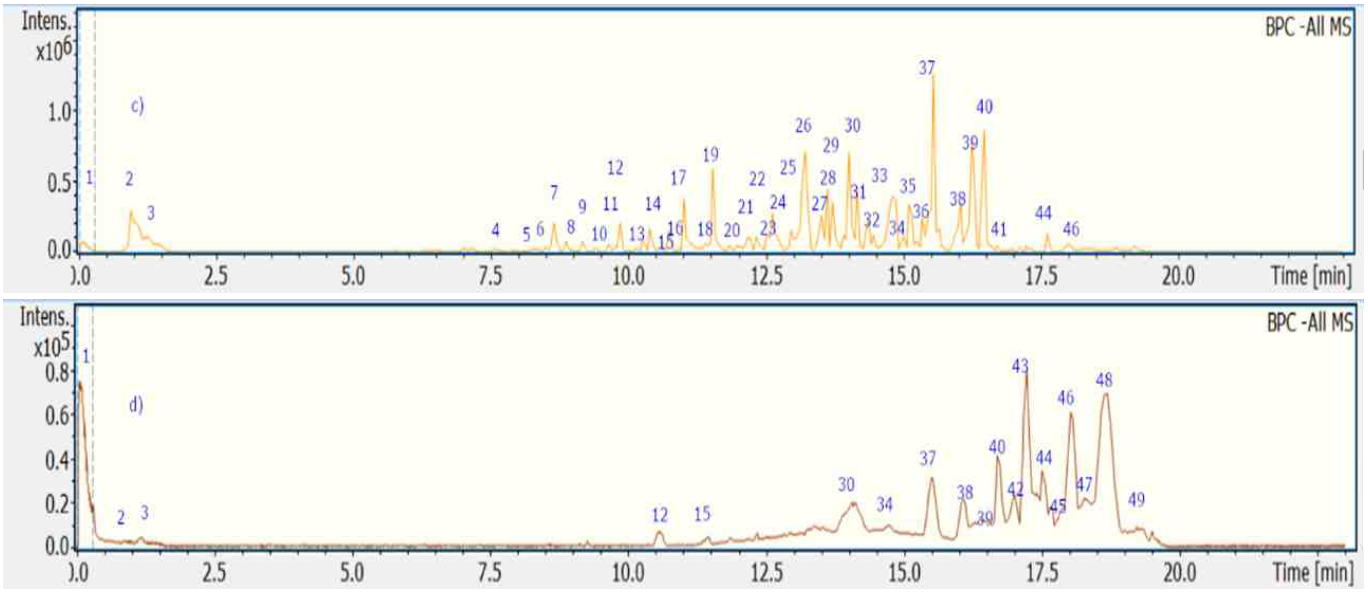

Figure 2. Chromatogram of ethanolic extracts of BSF larvae meal reared on orange bagasse: A) previously defatted with hexane; B) without defatting.

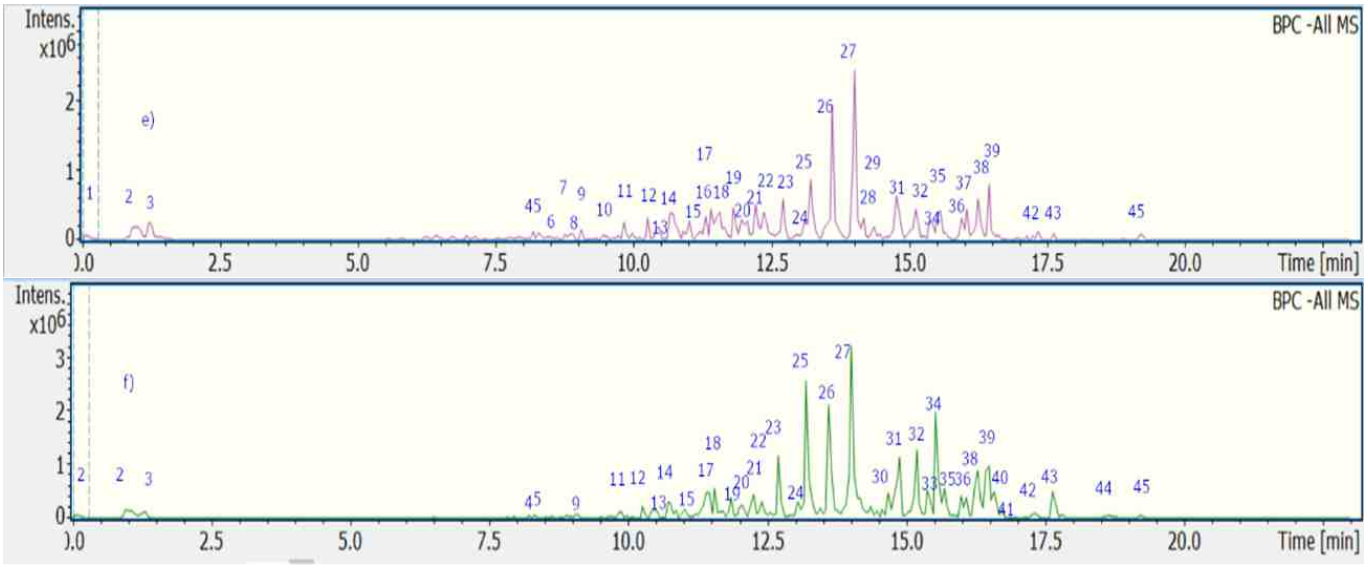

Figure 3. Chromatogram of ethanolic extracts of BSF larvae meal reared on ripe banana pulp: A) previously defatted with hexane; B) without defatting.

CONCLUSION

Our results indicate that the rearing of BSF larvae in semi-captivity using Amazonian fruit waste yields insect meals rich in crude protein, minerals, amino acid profiles, and unsaturated fatty acids of nutritional importance. This study is the first report on the nutritional value of black soldier fly (*Hermetia illucens*) larvae native to the Peruvian Amazon. These larvae meals are a good option for inclusion in the formulation of animal feeds to replace soybean meal or fish meal. In the context of the Peruvian Amazon, these meals could serve as a valuable addition to produce balanced feed for fish and poultry. Such integration could effectively reduce production costs while contributing to regional development through the implementation of a circular economy system.

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Appendix 1. Identification of metabolites in BSF larvae meal reared on oatmeal by UHPLC Q-TOF-MS.

Peak	Retention Time (min.)	Tentative Identification	Molecular formula	Measured Mass (m/z) $[M+H]^+$	Molecular Mass (M)	Accuracy (ppm)	Metabolite Type	MS Ions (ppm)
1	0.37	Na formiate (internal standard)	$C_4H_2O_4$	113.9829	112.9856	3.1	Standard	-
2	0.86	Histidine	$C_6H_9N_3O_2$	154.06189	155.06917	-2.632	Amino acid	
3	0.92	Threonic acid	$C_4H_8O_5$	135.02964	136.03691	-1.76	Carboxilic acid	
4	0.95	Quinic acid	$C_7H_{12}O_6$	191.05598	192.06326	-0.52	Carboxilic acid	
5	1.03	Caffeoyl quinic acid	$C_{16}H_{18}O_9$	353.08802	354.09529	-0.84	Phenolic acid	300.18076
6	9.46	Xanthoxin	$C_{15}H_{22}O_3$	249.1499	250.15627	-2.49	Fatty acid	
7	10.81	9-Octadecenedioic acid	$C_{18}H_{32}O_4$	311.2226	312.22987	-0.431	Fatty acid	
8	11.31	LPE 18:2 1-linoleoylglycerophosphoethanolamine	$C_{23}H_{44}NO_7P$	476.27814	477.28542	0.472		
9	11.55	N-Dodecanoyl-N-methylglycine	$C_{15}H_{29}NO_3$	270.20746	271.21473	0.503		
10	12.45	Hydroperoxy-octadecadienoic acid	$C_{18}H_{32}O_4$	311.22292	312.23023	2.91	Fatty acid	161.64924
11	13.16	Eicosanoic acid	$C_{20}H_{40}O_2$	311.29569	312.30296	-2.115	Fatty acid	294.27885
12	14.03	1-(9Z-octadecenyl)-sn-glycero-3-phosphoethanolamine	$C_{23}H_{48}NO_6P$	464.31335	465.32063	-2.798	Fatty acid	
13	14.45	PI 34:2 1-phosphatidyl-1D-myo-inositol	$C_{43}H_{79}O_{13}P$	833.51655	834.52382	-2.42	Fatty acid	
14	15.27	PI 36:3, 1-phosphatidyl-1D-myo-inositol derivative	$C_{45}H_{81}O_{13}P$	859.53367	860.54095	-0.616	Fatty acid	
15	15.54	Dilinoleic acid 9,12-Octadecadienoic acid	$C_{36}H_{64}O_4$	559.47289	560.48017	-0.525	Fatty acid	279.23346
16	15.56	(R)-2-hydroxystearic acid	$C_{18}H_{36}O_3$	299.25908	300.26636	-0.292	Fatty acid	259.02293
16	16.23	N-Oleoyl-Phenylalanine	$C_{27}H_{43}NO_3$	428.31709	429.32436	0.346	Fatty acid-aminoacid	
17	16.56	N-Oleyl-Leucine	$C_{24}H_{45}NO_3$	394.33239	395.33967	-0.729	Fatty acid-aminoacid	311.31526
18	16.49	Oleic estolide	$C_{36}H_{68}O_4$	563.50338	564.51066	-2.191	Fatty acid	255.23317
19	16.52	Hexadecylmalonic acid	$C_{19}H_{36}O_4$	327.25415	328.26143	0.202	Fatty acid	281.24899
20	16.67	Sclareol	$C_{20}H_{36}O_2$	307.26387	308.27115	-0.096	Sterol	
21	16.67	Heneicosanoic acid	$C_{21}H_{42}O_2$	325.31056	326.31784	-1.28	Fatty acid	
22	16.67	Tricosanoic acid	$C_{23}H_{46}O_2$	353.34256	354.34983	-1.63	Fatty acid	256.23673
23	18.63	Behenic acid	$C_{22}H_{44}O_2$	339.32633	340.33361	-2.53	Fatty acid	324.32711
24	18.88	1-stearidonoyl-2-stearoyl-sn-glycero-3-phosphoethanolamine	$C_{41}H_{74}NO_8P$	738.50574	739.51302	-3.937	Fatty acid	295.26470
25	19.35	Phosphatidylinositol 16:0-18:2	$C_{43}H_{79}O_{13}P$	833.5155	834.52278	-4.08	Fatty acid	283.26834

Appendix 2. Identification of metabolites in BSF larvae meal reared on orange bagasse by UHPLC Q-TOF-MS.

Peak	Retention Time (min.)	Tentative Identification	Molecular formula	Measured Mass (m/z) $[M+H]^+$	Molecular Mass (M)	Accuracy (ppm)	Metabolite Type	MS Ions (ppm)
1	0.37	Na formiate (internal standard)	$C_4H_2O_4$	113.9829	112.9856	3.1	Standard	-
2	1.13	Coumaroyl quinic acid	$C_{16}H_{18}O_8$	337.09277	338.10005	-0.97	Phenolic acid	172.09856
3	1.16	2-Isopropylmalic Acid	$C_7H_{12}O_5$	175.06114	176.06842	-0.63	Carbohydrate carboxilic acid	131.07159
4	7.52	(11E)-13-Hydroxy-9-methoxy-10-oxo-11-octadecenoic acid	$C_{19}H_{33}O_5$	341.23335	342.22422	-16.01	Fatty acid	337.22915
5	8.58	4',5,7-trihydroxy-3,6-dimethoxyflavone	$C_{17}H_{14}O_7$	329.06743	330.07471	3.56	Flavone	300.18076
6	8.97	9-HPODE (10E,12E)-9-hydroperoxyoctadeca-10,12-dienoic acid	$C_{18}H_{32}O_4$	311.2221	312.22937	-2.5	Fatty acid	283.27449
7	11.55	N-Dodecanoyl-N-methylglycine	$C_{15}H_{29}NO_3$	270.20746	271.21473	0.65	Fatty acid -amino acid	270.20768
8	11.68	Porrigenic acid	$C_{18}H_{30}O_4$	309.20654	310.21382	-1.17	Fatty acid	270.20768
9	11.82	Lysophosphatidylethanolamine 16:0	$C_{21}H_{44}NO_7P$	452.27748	453.28475	-1.696	Phospholipid	-
10	11.31	LPE 18:2 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine	$C_{23}H_{44}NO_7P$	476.27814	477.28542	0.47	Fatty acid	367.25965
11	12.12	Phosphatidylethanolamine lyso 18:1	$C_{23}H_{46}NO_7P$	478.29308	479.30036	-1.06	Phospholipid	436.31792
12	16.02	(R)-2-hydroxystearic acid	$C_{18}H_{36}O_3$	299.25898	300.26626	-0.67	Fatty acid	130.08793
13	16.23	N-Oleoyl-Phenylalanine	$C_{24}H_{45}O_5$	428.31709	429.32436	0.34	Fatty acid-aminoacid	256.26723
14	16.27	Oleic estolide	$C_{36}H_{68}O_4$	563.50338	564.51066	-2.19	Fatty acid	368.31678, 281.24899
15	16.27	hydroxyStearic acid isomer	$C_{18}H_{35}O_3$	299.25917	299.26053	2.37	Fatty acid	281.94923
16	16.38	Stearic acid	$C_{18}H_{35}O_2$	283.26435	283.26481	2.37	Fatty acid	255.24127
17	16.50	Oleic estolide	$C_{36}H_{67}O_4$	563.51913	564.52448	25.98	Fatty acid	281.25241
18	1.16	2-Isopropylmalic Acid	$C_7H_{12}O_5$	175.06114	176.06842	-0.63	Carbohydrate carboxilic acid	131.07159
19	9.71	5,7-dihydroxy-3,6-dimethoxy-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one	$C_{20}H_{20}O_9$	403.10286	404.11014	-0.96	Chromenone	312.21663
20	9.93	Nevadensin	$C_{18}H_{16}O_7$	343.08233	344.08961	1.19	Flavone	313.23786
21	11.42	9-hydroxy 10E,12Z,15Z-octadecatrienoic acid (9-HOTrE)	$C_{18}H_{30}O_3$	293.21199	294.21936	-0.68	Fatty acid	241.18112
22	11.55	N-Dodecanoyl-N-methylglycine	$C_{15}H_{29}NO_3$	270.20746	271.21473	0.54	Fatty aminoacid	270.20781
23	12.52	LPE 16:0 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine	$C_{21}H_{44}NO_7P$	452.27748	453.28475	-1.696	LPE	366.24456
24	12.71	1-(9Z-octadecenoyl)-sn-glycero-3-phosphoserine	$C_{24}H_{46}NO_9P$	522.28394	523.29122	-16.32	Fatty acid	311.22333
25	12.82	Phosphatidylethanolamine lyso 18:1	$C_{23}H_{46}NO_7P$	478.29306	479.30033	-1.789	Fatty acid	255.23370

26	13.23	Tetradecylsulfate	$C_{14}H_{30}O_4S$	293.17919	294.18646	-0.45	Sulfate	196.14236
27	13.45	9-HPODE Hydroperoxy-octadecadienoic acid isomer	$C_{18}H_{32}O_4$	311.22292	312.23023	2.91	Fatty acid	161.64924
28	13.57	Dodecylbenzenesulfonic acid	$C_{18}H_{30}O_3S$	325.18434	326.19128	-0.888	Benzenesulfonic acid	295.22838
29	13.66	Eicosanoic acid	$C_{20}H_{40}O_2$	311.29569	312.30296	-2.115	Fatty acid	294.27885
30	14.14	9-HODE 9-Hydroxyoctadecadienoic acid	$C_{18}H_{32}O_3$	295.22784	296.23511	-0.16	Fatty acid	319.24612
31	14.23	2,6-Di-tert-butyl-4-nitrophenol	$C_{14}H_{21}NO_3$	250.14462	251.1519	-1.43	Phenolic acid	337.25863
32	14.38	O-18:0 1-Octadecyl-sn-glycero-3-phosphoethanolamine	$C_{23}H_{50}NO_6P$	466.33781	467.33727	-0.64	LPE	337.25838
33	14.53	O-18:1 1-Palmitoylglycol-2-phosphocholine	$C_{23}H_{48}NO_6P$	464.31335	465.32063	-2.798	LPE	297.24465
34	14.64	12-Hydroxyoleic acid	$C_{18}H_{33}O_3$	297.24876	298.25352	17.6	Fatty acid	271.23873
35	15.12	17-Hydroxylinolenic acid	$C_{18}H_{29}O_3$	293.21222	294.20045	-22.32	Fatty acid	161.05021, 112.98316
36	15.26	Myristic acid	$C_{14}H_{28}O_2$	227.201321	228.20857	0.09	Fatty acid	209.191400
37	15.46	Arachidonic Acid	$C_{20}H_{32}O_2$	303.23323	304.24051	0.303	Fatty acid	299.22285
38	16.03	Tetratriacontatetraenoic acid	$C_{34}H_6O_2$	499.45459	500.45205	5.06	Fatty acid	447.15651
39	16.43	FAHFA 18:2/20:4	$C_{38}H_{62}O_4$	581.45521	582.46228	-4.05	Fatty acid	279.23372
40	16.53	linoleic acid	$C_{18}H_{31}O_2$	279.23346	280.24295	17.6	Fatty acid	89.04548
41	16.74	Dilinoleic acid 9,12-Octadecadienoic acid	$C_{36}H_{64}O_4$	559.47289	560.48017	-0.525	Fatty acid	279.23346
42	16.87	Hydroxystearic acid isomer	$C_{18}H_{36}O_3$	299.25908	300.26636	-0.292	Fatty acid	259.02293
43	17.37	N-Oleyl-Leucine	$C_{24}H_{45}NO_3$	394.33239	395.33967	-0.729	Fatty acid	241.248993
44	17.52	Glycerol monooleate	$C_{21}H_{40}O_4$	355.28465	356.29193	-1.726	Fatty acid	283.23514
45	18.15	3-Hydroxydodecenoyl-3-hydroxydecanoate	$C_{22}H_{40}O_5$	383.24389	384.25117	-3.75	Fatty acid	337.23516
46	18.39	1,2-Dipalmitoyl-sn-glycero-3-phosphate	$C_{35}H_{69}O_8P$	647.46302	648.47029	-4.019	Fatty acid	383.36213
47	18.45	[1-hexadecanoyloxy-3-phosphonooxy55propan-2-yl]octadec-9-enoate	$C_{37}H_{71}O_8P$	673.46884	674.47612	-20.65	Phospho-alkene	582.52567
48	18.75	Docosapentaenoic acid	$C_{22}H_{34}O_2$	329.25936	330.25936	2.99	Fatty acid	112.98197
49	18.88	1,2-Dilinoleoyl-sn-glycero-3-phosphatidylethanolamine	$C_{41}H_{74}NO_8P$	738.50574	739.51302	-3.97	Phosphoethylamine	574.42184

Appendix 3. Identification of metabolites in BSF larvae meal reared on ripe banana pulp by UHPLC Q-TOF-MS.

Peak	Retention Time (min.)	Tentative Identification	Molecular formula	Measured Mass (m/z) $[M+H]^+$	Molecular Mass (M)	Accuracy (ppm)	Metabolite Type	MS Ions (ppm)
1	0.37	Na formiate (internal standard)	$C_4H_2O_4$	113.9829	112.9856	3.1	Standard	-
2	0.86	Histidine	$C_6H_9N_3O_2$	154.06189	155.06917	-0.63	Carbohydrate carboxilic acid	128.04387
3	0.92	Threonic acid	$C_4H_8O_5$	135.02964	136.03691	-1.76		
4	7.52	(Z)-9,12,13-trihydroxyoctadec-15-enoic acid	$C_{18}H_{34}O_5$	329.23312	330.23897	-1.62	Fatty acid	337.22915
5	7.92	Dodecanedioic acid	$C_{12}H_{22}O_4$	229.14405	230.15132	-1.29	Fatty acid	139.10393
6	8.28	Omega-Hydroxydodecanoic Acid	$C_{12}H_{24}O_3$	215.16507	216.17234	-1.32	Fatty acid	283.27449
7	8.51	Porrigenic acid 10E,12E,14S-14-hydroxy-9-oxooctadeca-10,12-dienoic acid	$C_{18}H_{30}O_4$	309.20635	310.21362	-1.43	Fatty acid	172.09303
8	8.97	9-HPODE (10E,12E)-9-hydroperoxyoctadeca-10,12-dienoic acid	$C_{18}H_{32}O_4$	311.2221	312.22937	-2.5	Fatty acid	271.15824
9	9.57	10-Hydroxydecanoic Acid	$C_{10}H_{20}O_3$	187.13372	188.14099	-0.74	Fatty acid	
10	10.55	2-((Decyloxy)carbonyl)benzoic acid	$C_{18}H_{26}O_4$	305.17561	306.18288	-0.747	Fatty acid	
11	9.71	Cassiaside	$C_{20}H_{20}O_9$	403.10286	404.11014	-0.967	Chromenone	
12	10.28	8,13-dihydroxy-9,11-octadecadienoic Acid	$C_{18}H_{32}O_4$	311.22196	312.22924	-4.08	Fatty acid	
13	10.45	Omega-hydroxydodecanoic acid	$C_{12}H_{24}O_3$	215.16517	216.17244	-0.47	Fatty acid	
14	10.49	Lauryl sulfate	$C_{12}H_{26}O_4S$	265.14772	266.15499	-0.706	Fatty acid	
15	14.43	1-phosphatidyl-1D-myo-inositol	$C_{43}H_{79}O_{13}P$	833.51655	834.52382	-2.406	Fatty acid	
16	11.55	N-Dodecanoyl-N-methylglycine	$C_{15}H_{29}NO_3$	270.20746	271.21473	0.65	Fatty acid-aminoacid	270.20768
17	11.68	Porrigenic acid isomer (10E,12E,14S)-14-hydroxy-9-oxooctadeca-9,12-dienoic acid	$C_{18}H_{30}O_4$	309.20654	310.21382	-1.17	Fatty acid	270.20768
18	11.82	Lysophosphatidylethanolamine 16:0	$C_{21}H_{44}NO_7P$	452.27748	453.28475	-1.696	Fosfolypid	-
19	11.31	1-linoleoylglycerophosphoethanolamine	$C_{23}H_{44}NO_7P$	476.27814	477.28542	0.47	Fatty acid	367.25965
20	12.12	Phosphatidylethanolamine lyso 18:1	$C_{23}H_{46}NO_7P$	478.29308	479.30036	-1.06	Fosfolypid	436.31792
21	16.02	(R)-2-hydroxystearic acid	$C_{18}H_{36}O_3$	299.25898	300.26626	-0.67	Fatty acid	130.087932, 81.94923
22	11.55	Tricosanoic acid	$C_{23}H_{46}O_2$	353.34256	354.34983	-1.63	Fatty acid	256.23673
23	11.82	Hexadecanoyl-lysophosphatidylethanolamine	$C_{21}H_{44}NO_7P$	452.27748	453.28475	-1.696	Fatty acid	366.24456
24	11.91	1-(9Z-octadecenoyl)-sn-glycero-3-phosphoserine	$C_{24}H_{46}NO_9P$	522.28394	523.29122	-16.32	Fatty acid	311.22333
25	11.92	Phosphatidylethanolamine lyso 18:1	$C_{23}H_{46}NO_7P$	478.29306	479.30033	-1.789	Fatty acid	255.23370

26	12.23	Tetradecylsulfate	$C_{14}H_{30}O_4S$	293.17919	294.18646	-0.45	Fatty acid	196.14236
27	12.55	Hydroperoxy-octadecadienoic acid (9-HPODE)	$C_{18}H_{32}O_4$	311.22292	312.23023	2.91	Fatty acid	161.64924
28	12.17	Dodecylbenzenesulfonic acid	$C_{18}H_{30}O_3S$	325.18434	326.19128	-0.888	Fatty acid	295.22838
29	13.16	Eicosanoic acid	$C_{20}H_{40}O_2$	311.29569	312.30296	-2.115		294.27885
30	13.43	2,6-Di-tert-butyl-4-nitrophenol	$C_{14}H_{21}NO_3$	250.14462	251.1519	-1.43	Phenolic acid	337.25863
31	13.44	9-Hydroxyoctadecadienoic acid (9-HODE)	$C_{18}H_{32}O_3$	295.22784	296.23511	-0.16	Fatty acid	319.24612
32	13.78	1-Octadecyl-sn-glycero-3-phosphoethanolamine	$C_{23}H_{50}NO_6P$	466.33781	467.33727	-0.64	Fatty acid	337.25838
33	14.03	1-Palmitoylglycol-2-phosphocholine	$C_{23}H_{48}NO_6P$	464.31335	465.32063	-2.798	Fatty acid	297.24465
34	14.04	12-Hydroxyoleic acid	$C_{18}H_{33}O_3$	297.24876	298.25352	17.6	Fatty acid	271.23873
35	14.32	17-Hydroxylinolenic acid	$C_{18}H_{29}O_3$	293.21222	294.20045	-22.32	Fatty acid	161.05021, 112.98316
36	14.86	Myristic acid	$C_{14}H_{28}O_2$	227.201321	228.20857	0.09	Fatty acid	209.191400
38	15.43	Tetratriacontatetraenoic acid	$C_{34}H_6O_2$	499.45459	500.45205	5.06	Fatty acid	447.15651
39	15.53	FAHFA 18:2/20:4 Fatty acid esters of hydroxy fatty acids (Z)-10-[(3Z,6Z,9Z,12Z,15Z)-octadeca-3,6,9,12,15-pentaenoyl]oxyicos-11-enoic acid	$C_{38}H_{62}O_4$	581.45521	582.46228	-4.05	Fatty acid	279.23372
40	15.53	Linoleic acid	$C_{18}H_{31}O_2$	279.23346	280.24295	17.6	Fatty acid	89.04548
41	15.54	Dilinoleic acid 9,12-Octadecadienoic acid	$C_{36}H_{64}O_4$	559.47289	560.48017	-0.525	Fatty acid	279.23346
42	15.56	(R)-2-hydroxystearic acid	$C_{18}H_{36}O_3$	299.25908	300.26636	-0.292	Fatty acid	259.02293
43	16.23	N-Oleoyl-Phenylalanine	$C_{27}H_{43}NO_3$	428.31709	429.32436	0.341	Fatty amino acid	281.24899
44	16.67	Sclareol	$C_{20}H_{36}O_2$	307.26387	308.27115	-0.096	Terpene	281.25204
45	16.12	Glyceryl monolinoleate	$C_{21}H_{38}O_4$	353.26942	354.2767	-0.88	Fatty acid	224.20339