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Bioconversion of Seasonal Vegetable By-Products into Nutrient-Rich Biomass Using Black Soldier Fly Larvae

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Abstract

Agro-industrial processes generate large volumes of by-products rich in proteins, lipids, and bioactives, yet their valorization remains limited. Black soldier fly larvae (BSFLs) offer a sustainable route to convert these residues into nutrient-rich biomass. We evaluated six seasonal by-product diets (pea–chickpea, chickpea–green bean, wheat–green bean, spinach–chickpea, tomato–chickpea, tomato–wheat) and profiled diets and larvae for tocopherols, carotenoids, fatty acids, and amino acids; principal component analysis assessed assimilation patterns. Larvae did not mirror diets but clustered into two compositional regimes, indicating selective metabolism. Tomato-based diets enhanced larval α -tocopherol (22.54 mg/kg dw) and lycopene (6.87 mg/kg dw), while spinach-based diets contributed higher lutein and other xanthophylls. Significant diet–larvae correlations were observed for lycopene ($r = 0.6719$) and β -cryptoxanthin ($r = 0.5845$). Across treatments, lauric (C12:0) and palmitic (C16:0) acids remained dominant, confirming the conserved BSFL lipid hierarchy (SFA > MUFA > PUFA). Amino acid profiles were relatively stable, with lysine and glutamic acid prevailing among essential and non-essential classes. Overall, BSFLs enriched with tocopherols and provitamin A carotenoids offer functional benefits for oxidative stability and micronutrient restoration, underscoring their dual role in waste valorization and nutritional enhancement within circular food and feed systems.

Keywords: vegetable residues; valorization; *Hermetia illucens*; feed; bioactive compounds



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1. Introduction

Across Western countries, demand for plant-based foods (including vegetables and frozen varieties) has risen significantly, driven by the growing adoption of flexitarian, vegetarian, and vegan diets and broader sustainability initiatives [1]. The European Union is the primary producer and importer of frozen vegetables, with approximately 90% of its trade activity concentrated within the internal market [2]. These vegetables retain their nutritional quality due to minimal processing and the absence of additives [3]. Common vegetables, including legumes, leafy greens, and tomatoes, are processed year-round and

typically undergo several preparatory steps before freezing [2,4]. These processes generate substantial by-products (peels, trimmings, seeds, blanching water residues, damaged or misshapen produce), which are often treated through anaerobic digestion to produce biogas [5]. However, due to their high organic content, these by-products can also serve as alternative sources of bioactive compounds [6–8]. Vegetable by-products such as spinach (rich in lutein and violaxanthin) and tomatoes (abundant in lycopene and β -carotene) are valuable sources of antioxidants [9,10]. Given the antioxidant qualities of some vegetable by-products and their valuable macronutrients, insect-based bioconversion may be a practical method to restore these nutrients into the food and feed supply chain. Incorporating insects as an alternative protein source in animal feed has the potential to enhance global food security [11,12]. Moreover, the industry may benefit from effective economic sustainability when the organic wastes are used to feed insects, which are then used to feed poultry [13–15], fish [16–18], and pigs [19,20].

Hermetia illucens (Linnaeus, 1758) (Diptera: Stratiomyidae), also referred to as black soldier fly (BSF), is increasingly recognized for its capacity to thrive on diverse organic substrates, including by-products from industrial vegetable and legume processing [21,22]. The larvae (BSFL) provide a sustainable source of high-quality protein with a generally favorable amino acid profile and rich content of essential nutrients. Because they can convert low-value organic substrates into biomass and have demonstrated strong digestibility and growth performance in animal feed trials, they are well-suited candidates for inclusion in livestock, aquaculture, and poultry feeds [23,24]. Conversely, BSFL has a suboptimal fatty acid profile, characterized by a greater proportion of saturated fatty acids compared to unsaturated fatty acids. For instance, it is essential to address this imbalance when formulating feed for aquaculture given that optimal fish growth and development necessitate significant levels of polyunsaturated fatty acids [25].

The use of insects as feed ingredients is increasingly supported by regulatory developments that ensure their safety and traceability. Within the European Union, the inclusion of insect protein in aquaculture feed was authorized under Commission Regulation (EU) 2017/893 [26], later extended to poultry and pig feeds in 2021 [27]. These frameworks stipulate that only insect species approved for feed use—such as *Hermetia illucens*, *Musca domestica*, *Tenebrio molitor*, *Alphitobius diaperinus*, *Acheta domesticus*, *Gryllobius sigillatus*, *Gryllus assimilis*, and *Bombyx mori*—may be reared on substrates of plant or plant-derived origin deemed safe for farmed animals. Food and feed safety considerations, including control of microbial hazards, heavy metals, and pesticide residues, remain central to ensuring compliance with EU hygiene and feed regulations [28,29]. Incorporating such safeguards underlines the feasibility of using vegetable processing by-products for insect rearing within a secure and sustainable production framework.

Recent research has increasingly explored the bioaccumulation of vitamins and antioxidants in BSFL to improve the nutritional value of insect-based feeds. BSFL can accumulate carotenoids (provitamin A precursors) from their diet, and these carotenoids have been shown to be bioavailable in animal models, offering a promising route for sustainably recycling vitamin A into feed and thereby into the broader food chain (though the effectiveness depends heavily on substrate composition and conversion efficiency) [25,30,31]. These carotenoids have been shown to improve immunity and antioxidant levels in livestock [32,33]. This study investigates the use of BSFL to bioconvert selected vegetable processing by-products into nutrient-rich biomass for incorporation into animal feed, as a potential alternative or complement to anaerobic digestion. Rearing diets were formulated by blending by-products with overlapping seasonality to align with residue generation at the producer level. Collected materials include damaged portions, trimming residues, and post-blanching discards, combined to reflect actual industrial conditions. We hypothesize

that feeding BSFL with blended vegetable by-products will enhance the accumulation of beneficial micronutrients (particularly carotenoids and tocopherols) in the larvae while maintaining favorable amino acid and fatty acid profiles, thus supporting their application as a sustainable feed source and a circular alternative to anaerobic digestion. The resulting insect biomass was chemically characterized for its suitability as a feed ingredient, assessing amino-acid and fatty-acid profiles and concentrations of carotenoids and tocopherols.

2. Materials and Methods

2.1. Materials

Standards for carotenoids and tocopherols (purity > 95%), L-amino acid analytical standards, and DL-norvaline (used as an internal standard, purity 98.5%), derivatization reagent, MTBSTFA (purity > 99%), HPLC-grade solvents (acetone, hexane, and water; purity > 95%), hydrochloric acid (37%), and boron trifluoride in methanol (14% BF₃-MeOH) were also supplied by Merck (Darmstadt, Germany). Fatty acid methyl esters (FAME) mix standard ranging from C4 to C24 (Supelco 37 Component FAME Mix) was obtained from Sigma-Aldrich (Bellefonte, PA, USA). Anhydrous sodium sulfate (Na₂SO₄) was obtained from ITW Company, Darmstadt, Germany.

2.2. Agro-Industrial By-Products Used for BSFL Feeding

By-products were sourced at various stages of vegetable processing, including de-bunching, washing, pneumatic and optical sorting, manual sorting, snipping, trimming, destemming, and blanching. Spinach, peas, green beans, and tomato by-products were supplied by Orto Verde s.c.a.p.a., Senigallia, Italy. Chickpea residues obtained from Società Produttori Sementi s.p.a. in Macerata, Italy, and wheat residues from a grain distributor in Ancona, Italy.

2.3. Rearing of BSFL

Experimental diets used as rearing substrates for BSFL were formulated by combining vegetable by-products with overlapping seasonality to streamline residue management. The binary diets—pea with chickpea, chickpea with green bean, wheat with green bean, spinach with chickpea, tomato with chickpea, and tomato with wheat—were mixed at equal ratios (1:1, *w/w*, as-fed basis)

The control diet followed the standard Gainesville formulation, consisting of wheat bran, alfalfa, and corn meal in a ratio of 50:30:20, respectively, as described by Hogsette [34]. All diets were stored at 2–5 °C for seven days before administration to BSFL. Additionally, a portion of each diet was freeze-dried (Superco engineering, CryoDryer 5, Langweid am Lech, Germany), vacuum-sealed (Electrolux vacuum sealer drawer KBV4X, Dubai, United Arab Emirates), and stored at −20 °C for subsequent analysis. Three replicates of the control diet and nine replicates for each by-product-based diet were prepared.

BSFL were reared at the Entomology Laboratory of the Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, Italy. Larvae were fed on the previously described by-product-based diets and maintained in a climate-controlled chamber set at 27 ± 1 °C with a relative humidity of 65 ± 5% under continuous darkness. For each diet, including the control, six replicates were prepared using 100 larvae per replicate, following the methodology of Meneguz et al. [35]. Each replicate comprised a plastic container (24 × 16 × 13 cm) lined with a finely woven cotton mesh (30 × 30 cm), following the method of Sideris & Tsagkarakis [36], and sealed with a lid containing a single ventilation hole (4.5 cm diameter) as described by Sprangers et al. [37]. Larvae were reared at a density of 0.3 larvae per cm [38] and fed daily at a rate of 100 mg/larvae, amounting to a total of 70 g of feed per replicate per week [39]. When approximately

40% of the larvae within a replicate exhibited the onset of the prepupal stage, as indicated by a change in cuticle coloration from white to black, all larvae were extracted from the substrate, thoroughly rinsed with water to eliminate any residual feed, dried, enumerated, and weighed. The average rearing period was 14 days. Larvae were euthanized by freezing at -20°C , followed by freeze-drying, pulverization into a fine powder, and kept at -20°C prior to analysis.

Three replicates of control larvae and nine replicates from each dietary treatment group were used for subsequent analyses. A summary of diet and BSFL codes is provided in Table 1.

Table 1. Sample codes.

Samples	Diets (By-Products)	Insects (BSFL)
Control	D_CTRL	I_CTRL
Spinach: Chickpea	D_SC	I_SC
Tomato: Chickpea	D_TC	I_TC
Tomato: Wheat	D_TW	I_TW
Pea: Chickpea	D_PC	I_PC
Green bean: Chickpea	D_BC	I_BC
Green bean: Wheat	D_BW	I_BW

2.4. Extraction of Total Lipids and Fatty Acid Profiling in Diets and BSFL

Total lipids were extracted following the protocol Ismaiel et al. [40] described. Briefly, 1 g of ground, freeze-dried diet or larvae sample was placed into a test tube, and 20 mL of chloroform: methanol mixture (2:1, *v/v*) was added. The mixture was shaken for 5 min and centrifuged (Remi Elektrotechnik Ltd., Neya 16R, Mumbai, India) at 3000 rpm for 10 min at 4°C . The resulting organic phase was washed with 2.5 mL of distilled water and filtered through Whatman Grade 4 filter paper containing 3 g of Na_2SO_4 . The solvent was then evaporated at 30°C using a rotary evaporator (Rotavapor R-210, Buchi, Switzerland), and lipid yield was determined gravimetrically.

Fatty acid methyl esters (FAMES) were prepared from the extracted lipids via alkaline transmethylation, following the method of Nartea et al. [41]. FAMES were analyzed using gas chromatography with a flame ionization detector (GC-FID; TRACE 1300, Thermo Scientific, Waltham, MA, USA) and a capillary column (Rt-2560, 100 m $\times$ 0.25 mm, 0.2 μm film thickness; Restek). Helium served as carrier gas at a 1.6 mL/min flow rate. The oven temperature program was as follows: initial isothermal hold at 140°C for 5 min, ramping to 240°C at $4^{\circ}\text{C}/\text{min}$ over 20 min, followed by a final isothermal hold at 240°C for 15 min, totaling 45 min/run. Samples were injected in splitless mode. Fatty acids were identified by comparison with retention times of a commercial FAME standard mixture. Chromatographic data was processed and visualized using Chromeleon software (version 7.2.10). The fatty acid composition was expressed as mg/100 g dw of total fatty acids.

2.5. Amino Acid Determination in Diet and BSFL

The amino acid (AA) composition of insect samples and their corresponding diets was determined by UPLC after acid hydrolysis and derivatization. Briefly, 20 mg of freeze-dried samples were hydrolyzed with 500 μL of 6 M HCl at 110°C for 24 h. Hydrolysates were dried in a vacuum concentrator (1 h), reconstituted in 500 μL of 0.1 M HCl, and centrifuged at 6000 rpm for 5 min at 4°C . The supernatant was diluted with 0.1 M HCl (1:10), and 50 μL of the diluted extract was mixed with 50 μL of DL-norvaline (0.05 mg/mL in 0.1 M HCl) as an internal standard. Samples were dried and derivatized using the AccQ•Tag™ Ultra Derivatization Kit (Waters Corporation, Milford, MA, USA).

Chromatographic analysis was performed on a Waters ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) equipped with a photodiode array (PDA) detector and an AccQ•Tag Ultra C18 column (2.1 × 100 mm, 1.7 µm; Waters) maintained at 49 °C. The mobile phases were Eluent A and B (supplied with kit), run at 0.7 mL/min with the gradient. Detection was by PDA at 260 nm. Quantification was based on external calibration with a Waters amino acid standard mixture (AccQ•Tag Amino Acid Standard H) prepared in 0.1 M HCl at concentrations from 1 to 25 pmol/µL. Calibration curves for all amino acids showed excellent linearity ($R^2 \geq 0.99$).

2.6. Carotenoid Determination in Diets and BSFL

Carotenoids were extracted from freeze-dried diet and larval samples following the protocol of Nartea et al. [42], with minor modifications. Briefly, 100 mg of freeze-dried powder was extracted twice with 5 mL of cold acetone (4 °C). Each extraction involved vortexing, incubation at 4 ± 1 °C for 15 min, shaking for 5 min, and centrifugation at 1370 rpm for 10 min at 4 °C. The combined supernatants were filtered through a 0.45 µm regenerated cellulose filter (Sartorius), evaporated to dryness at 30 °C under reduced pressure, and reconstituted in 0.5 mL acetone.

Carotenoids were analyzed on a UPLC-PDA (Waters Corporation, Milford, MA, USA) and controlled by Empower v3.0. Separations were performed on an ACQUITY UPLC C18 column (2.1 × 100 mm, 1.7 µm) held at 35 °C; the sample manager was maintained at 20 °C. Mobile phase A was acetonitrile/dichloromethane/methanol (75:10:15, v/v/v) and mobile phase B was 0.05 M ammonium acetate in water. The flow rate was 0.4 mL/min and 2 µL injection volume. Detection was at 450 nm with full PDA spectral acquisition from 210–500 nm for peak identification. The quantification was based on external calibration with authentic standards of zeaxanthin and β-carotene. Calibration curves showed excellent linearity ($R^2 \geq 0.999$). Limits of detection (LOD) and quantification (LOQ) were 5 and 16 ng/mL for lutein, and 5 and 18 ng/mL for β-carotene, respectively.

2.7. Tocopherol Determination in Diets and BSFL

Tocopherols were extracted from 100 mg of freeze-dried diet and larval samples following the procedure of Nartea et al. [42], with slight modifications. Samples were extracted twice with 5 mL hexane, vortexed for 5 min, and centrifuged at 3500 rpm for 2 min. The combined supernatants were filtered through a 0.45 µm regenerated cellulose filter (Sartorius), evaporated to dryness under nitrogen, and reconstituted in 0.5 mL hexane for chromatographic analysis.

Chromatographic separation was performed on a Waters ACQUITY UHPLC system (Waters Corporation, Milford, MA, USA) equipped with a fluorescence detector (FLD) and an Ascentis Express HILIC column (150 × 2.1 mm, 2.7 µm; Sigma-Aldrich, St. Louis, MO, USA). Detection was set at excitation 290 nm and emission 330 nm. Tocopherols were identified by comparing retention times to authentic standards of α-, β-, and γ-tocopherol ($\geq 95\%$ purity, Sigma-Aldrich, St. Louis, MO, USA) and quantified by external calibration curves (0.01–10 µg/mL, $R^2 \geq 0.986$). The instrumental limits of detection (LOD)/limits of quantification (LOQ) were 4/14 ng/mL for α-tocopherol, 2/7 ng/mL for β- and γ-tocopherol. Only α-, β-, and γ-tocopherols were detected in the samples.

2.8. Statistical Analysis

Data were presented as mean ± standard deviation from nine replicates. One-way ANOVA was applied to determine significant differences among treatments, followed by Tukey's post hoc test for multiple comparisons ($p < 0.05$). ANOVA was selected as it is suitable for comparing mean values across multiple groups under parametric conditions. Principal component analysis (PCA) was used to visualize compositional patterns and

identify key discriminant variables based on metabolite profiles. Statistical analyses were performed using IBM SPSS Statistics version 23 and R version 3.5.0, while PCA, boxplots, and variable importance in projection (VIP) scores were generated with the MetaboAnalyst 5.0 platform.

3. Results

3.1. Overall Characterization of Diets and BSFL

The experimental diets and the resulting BSFL were comprehensively characterized. This included analysis of fatty acids, amino acids, tocopherols, and carotenoids. Principal Component Analysis (PCA) was applied to all measured variables to visualize the differences in chemical profiles among the diets, as seen in Figure 1 and the BSFL in Figure 2.

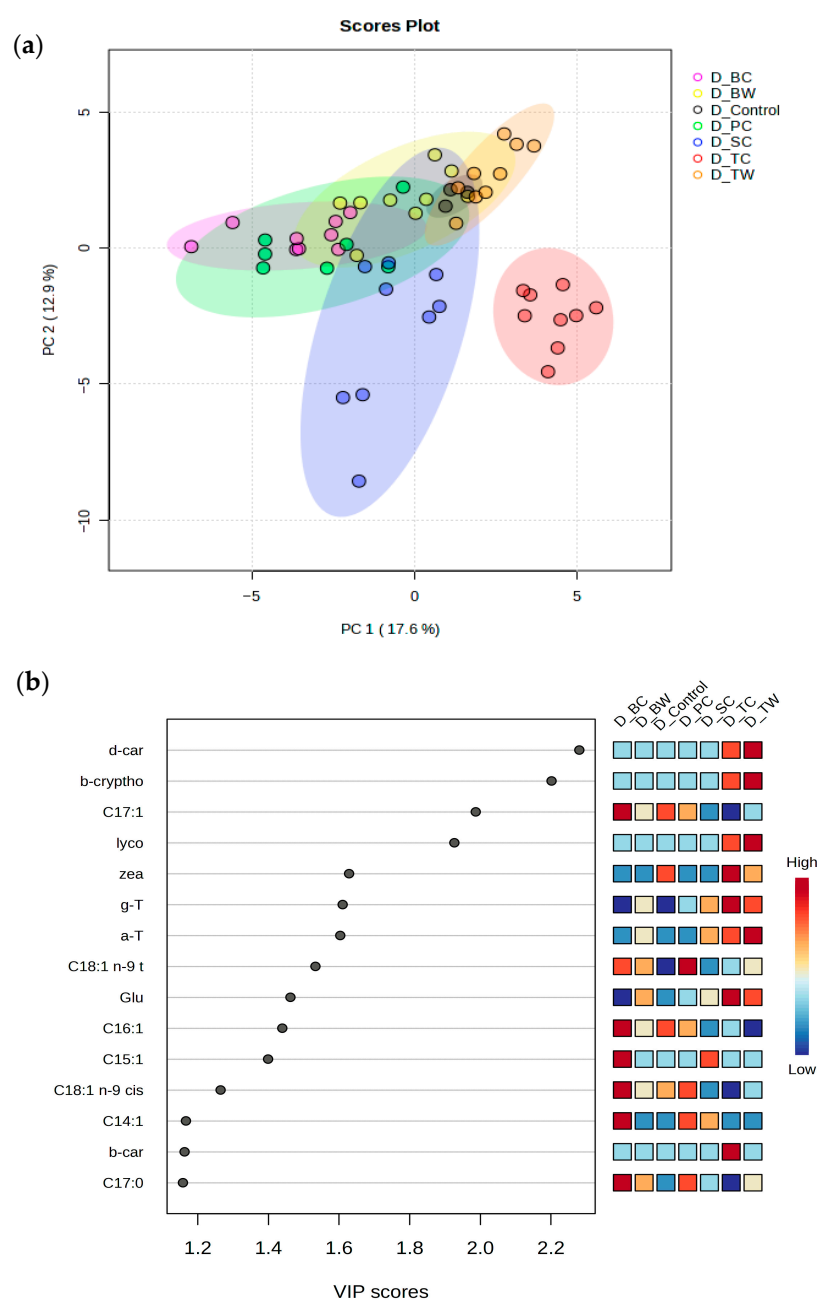


Figure 1. Diets (autoscaled dataset included all variables, i.e., tocopherols, carotenoids, fatty acids, amino acids). **(a)** PCA. **(b)** VIP score. Abbreviations: carotenoids: d-car: δ -carotene; b-crypto: β -cryptoxanthin; lyco: lycopene; zea: zeaxanthin; b-car: β -carotene; tocopherols: g-T, γ -tocopherol; a-T: α -tocopherol; amino acid: glu: glutamic acid; fatty acids.

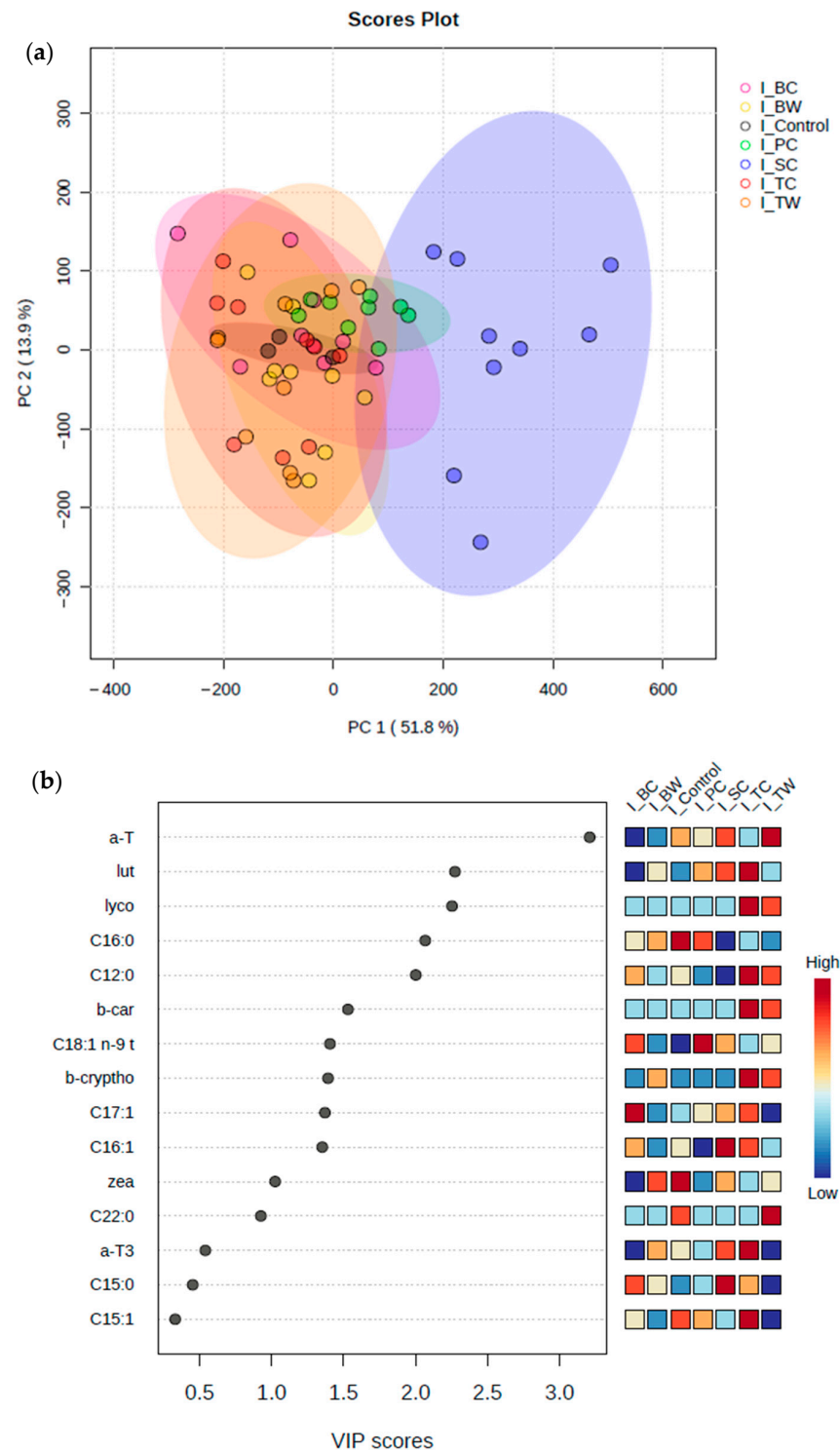


Figure 2. BSFL (Pareto-scaled dataset included all variables, i.e., tocopherols, carotenoids, fatty acids, amino acids). **(a)** PCA. **(b)** VIP score. Abbreviations: tocopherols: a-T: α -tocopherol; a-T3: α -tocotrienol; carotenoids: lut: lutein, lyco: lycopene; b-car: β -carotene; b-crypto: β -cryptoxanthin; zea: zeaxanthin; fatty acids.

The PCA of the diets in Figure 1a revealed clear distinctions, particularly for diets enriched with tomato-based ingredients (D_TW, D_TC) and spinach (D_SC). These differences were primarily attributed to variations in bioactive compounds, especially carotenoids and, to a lesser extent, tocopherols (Figure 1b). Diets containing legume by-products, such as peas, chickpeas, and green beans, formed a distinct cluster.

Despite the compositional variability in the diets, BSFL grouped into two main clusters: one comprising larvae reared on the spinach–chickpea substrate (I_SC) and another consisting of larvae reared on the remaining diets (I_TW, I_CTRL, I_TC, I_PC, and I_BW) as seen in Figure 2a. This separation was mainly driven by the concentrations of α -tocopherol, lutein, lycopene, and specific saturated fatty acids, notably palmitic acid (C16:0), and lauric acid (C12:0), as indicated by the Variable Importance in Projection (VIP) scores in Figure 2b. The influence of these variables stems from both dietary composition and larval metabolic regulation: tomato and spinach residues contribute to elevated carotenoid and tocopherol levels, whereas palmitic and lauric acids are dominant endogenous fatty acids synthesized or accumulated during larval lipid metabolism. Together, these metabolites represent the key biochemical markers distinguishing the diet treatments.

3.2. Fatty Acid Composition

Notable variations were detected in dietary fatty acid (FA) composition, with MUFAs showing the greatest between-diet variability in Table 2.

Table 2. Fatty acid profile of diets expressed as mg/100 g dry weight.

	D_CTRL	D_PC	D_TC	D_SC	D_BC	D_BW	D_TW
C16:0	0.16 ± 0.00 ^{ab}	0.17 ± 0.02 ^{ab}	0.11 ± 0.02 ^d	0.11 ± 0.01 ^{cd}	0.17 ± 0.04 ^a	0.15 ± 0.02 ^{ab}	0.14 ± 0.01 ^{ac}
C18:0	ND	0.03 ± 0.01 ^b	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.02 ± 0.01 ^{ab}	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a
C18:1	0.09 ± 0.00 ^a	0.23 ± 0.05 ^d	0.12 ± 0.00 ^{ab}	0.12 ± 0.05 ^{ab}	0.22 ± 0.01 ^d	0.17 ± 0.01 ^c	0.16 ± 0.01 ^{bc}
C20:3	0.02 ± 0.00 ^{ab}	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.04 ± 0.03 ^b	0.01 ± 0.00 ^a	0.02 ± 0.01 ^a	0.02 ± 0.00 ^a
ΣSFA	0.17 ± 0.00 ^{abc}	0.23 ± 0.04 ^a	0.13 ± 0.01 ^b	0.15 ± 0.02 ^{bc}	0.23 ± 0.06 ^a	0.18 ± 0.03 ^{abc}	0.19 ± 0.03 ^{ac}
ΣMUFA	0.10 ± 0.00 ^a	0.24 ± 0.06 ^d	0.13 ± 0.00 ^{ab}	0.12 ± 0.05 ^{ab}	0.24 ± 0.01 ^d	0.18 ± 0.01 ^c	0.16 ± 0.01 ^{bc}
ΣPUFA	0.02 ± 0.00 ^{ab}	0.02 ± 0.00 ^a	0.01 ± 0.00 ^a	0.04 ± 0.03 ^b	0.01 ± 0.00 ^a	0.02 ± 0.01 ^a	0.02 ± 0.00 ^a

Results are reported as the mean ± standard deviation. The different letters in the same row indicate significant differences ($p < 0.05$). ND = not detected.

Figure 3 presents box plots illustrating the distribution of fatty acid groups across different diet formulations and corresponding insect samples. Each plot displays the median, interquartile range, and variability within treatments, allowing visualization of both central tendency and dispersion before comparing differences determined by ANOVA. Across diets, FA classes followed the order SFA > MUFA > PUFA, with palmitic (C16:0) and oleic (C18:1) as the most abundant species and dihomo- γ -linolenic acid (C20:3) as the predominant PUFA (Figure 3a). In BSFL, lauric (C12:0), myristic (C14:0), and palmitic (C16:0) dominated the SFA fraction; oleic acid was the principal MUFA, and linoleic acid (C18:2) the main PUFA (Table 3). The spinach–chickpea and pea–chickpea diets yielded the highest larval PUFA contents. Correlation analysis in Table 4 showed strong positive diet–larva associations for palmitic acid (C16:0; $r = 0.93$) and dihomo- γ -linolenic acid (C20:3; $r = 0.83$); total PUFAs in larvae were also positively associated with dietary PUFAs ($r = 0.80$), indicating partial dietary reflection of unsaturated lipids.

Table 3. Fatty acid profile of BSFL (insects) expressed as mg/100 g dry weight.

	I_CTRL	I_PC	I_TC	I_SC	I_BC	I_BW	I_TW
C10:0	10.02 ± 0.99 ^b	12.72 ± 6.24 ^b	11.23 ± 1.59 ^b	1.73 ± 1.16 ^a	11.61 ± 2.04 ^b	10.60 ± 1.61 ^b	12.81 ± 0.95 ^b
C12:0	826.80 ± 94.86 ^{ab}	692.98 ± 95.94 ^b	894.15 ± 108.41 ^a	383.48 ± 52.70 ^c	842.30 ± 165.60 ^{ab}	825.92 ± 93.85 ^{ab}	882.24 ± 124.73 ^a
C14:0	128.42 ± 12.87 ^{ab}	101.63 ± 14.63 ^{bc}	131.84 ± 21.18 ^a	86.16 ± 15.80 ^c	125.84 ± 26.57 ^{ab}	130.23 ± 18.14 ^a	114.45 ± 20.32 ^{abc}
C14:1	3.52 ± 1.09	2.94 ± 0.34	3.52 ± 1.28	3.41 ± 0.76	3.29 ± 0.85	2.59 ± 0.45	2.64 ± 0.53
C15:0	7.02 ± 1.70	9.94 ± 1.47	9.74 ± 2.33	14.45 ± 16.47	12.17 ± 2.48	9.12 ± 6.99	8.65 ± 1.02
C15:1	5.44 ± 1.36 ^{ab}	4.82 ± 0.80 ^{ab}	6.25 ± 3.27 ^a	3.08 ± 1.11 ^b	5.03 ± 0.73 ^{ab}	0.66 ± 0.99 ^c	0.34 ± 0.51 ^c
C16:0	202.65 ± 6.73 ^{bc}	169.21 ± 7.83 ^{bc}	200.55 ± 22.33 ^c	126.50 ± 26.88 ^a	186.11 ± 37.74 ^{bc}	162.84 ± 18.00 ^b	183.38 ± 13.91 ^{bc}
C16:1	46.52 ± 5.91 ^{ab}	45.49 ± 1.40 ^{ab}	52.50 ± 11.21 ^{ab}	57.16 ± 11.61 ^a	46.72 ± 13.12 ^{ab}	41.10 ± 6.76 ^b	48.11 ± 7.43 ^{ab}
C17:0	5.75 ± 0.40 ^{ab}	6.15 ± 1.38 ^{ab}	6.96 ± 1.00 ^a	3.47 ± 0.63 ^b	6.69 ± 1.14 ^a	6.04 ± 4.14 ^{ab}	6.75 ± 1.04 ^a
C17:1	10.45 ± 1.13 ^{ab}	13.76 ± 1.82 ^{ab}	13.82 ± 2.89 ^{ab}	16.78 ± 0.81 ^{ab}	20.84 ± 7.58 ^a	11.81 ± 11.31 ^b	11.63 ± 2.64 ^b

Table 3. Cont.

	I_CTRL	I_PC	I_TC	I_SC	I_BC	I_BW	I_TW
C18:0	23.37 ± 0.93 ^{ab}	22.39 ± 1.00 ^{ab}	24.89 ± 2.21 ^b	18.43 ± 3.38 ^a	24.29 ± 5.22 ^b	21.42 ± 3.59 ^{ab}	25.36 ± 4.53 ^b
C18:1	140.30 ± 4.93 ^c	222.30 ± 13.49 ^a	175.67 ± 12.84 ^{bc}	243.81 ± 25.66 ^a	209.20 ± 28.97 ^{ab}	179.64 ± 38.86 ^{bc}	178.25 ± 33.25 ^{bc}
C18:2	35.06 ± 2.19 ^{abc}	83.14 ± 54.19 ^a	42.08 ± 6.50 ^c	81.27 ± 20.37 ^{ab}	39.99 ± 9.07 ^c	42.47 ± 3.50 ^c	46.17 ± 18.76 ^{bc}
C20:3	13.76 ± 0.50 ^{ab}	11.74 ± 1.62 ^{ab}	11.86 ± 0.98 ^{ab}	14.71 ± 4.65 ^b	10.63 ± 2.75 ^a	12.46 ± 1.16 ^{ab}	12.08 ± 0.56 ^{ab}
C20:0	22.98 ± 4.28 ^{ab}	15.38 ± 0.55 ^b	19.28 ± 4.27 ^{ab}	28.07 ± 4.01 ^a	17.67 ± 7.58 ^b	15.78 ± 10.74 ^b	11.14 ± 7.33 ^b
C20:1	2.97 ± 2.58 ^a	nd	6.09 ± 1.75 ^a	5.57 ± 8.59 ^a	nd	6.40 ± 6.06 ^a	nd
ΣSFA	1227.15 ± 114.38 ^{ab}	1030.40 ± 120.77 ^b	1298.64 ± 156.57 ^a	662.28 ± 93.22 ^c	1226.66 ± 237.68 ^{ab}	1181.95 ± 139.87 ^{ab}	1247.37 ± 143.03 ^{ab}
ΣMUFA	209.21 ± 12.49 ^b	289.31 ± 15.49 ^{ab}	257.84 ± 30.72 ^b	329.81 ± 46.68 ^a	285.08 ± 42.71 ^{ab}	242.18 ± 62.24 ^b	240.98 ± 42.36 ^b
ΣPUFA	48.82 ± 2.05 ^{abc}	94.88 ± 55.73 ^{ab}	53.93 ± 7.36 ^c	95.98 ± 24.38 ^a	50.62 ± 10.81 ^c	54.93 ± 3.77 ^c	58.26 ± 18.75 ^{bc}

Results are reported as the mean ± standard deviation. The different letters in the same row indicate significant differences ($p < 0.05$). nd= not detected.

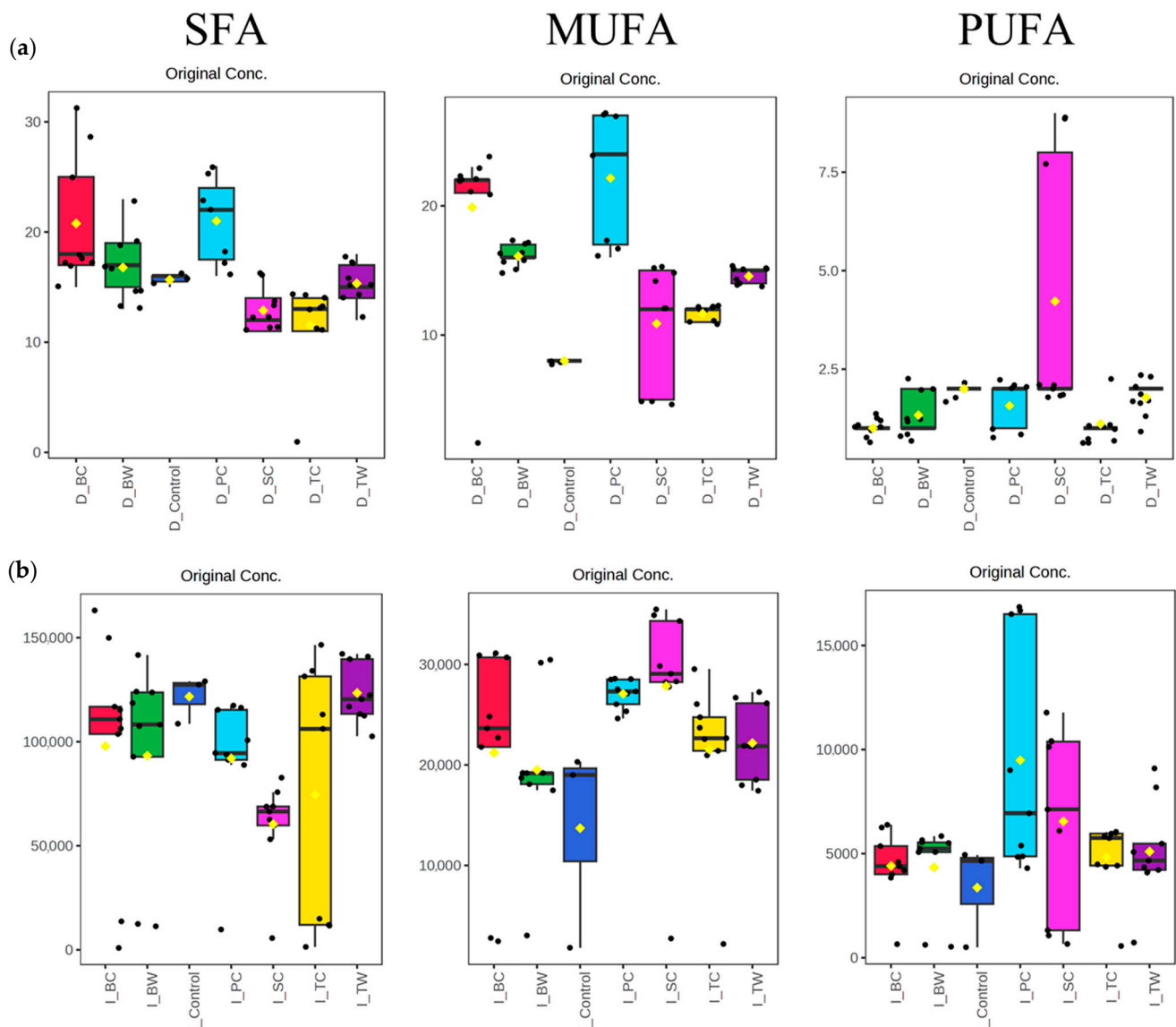


Figure 3. Box plots showing variations in fatty acid groups analysed by ANOVA ($p < 0.05$) for diets (a) and their corresponding larvae (b). Each box represents the interquartile range (25th–75th percentile), the horizontal line indicates the median, whiskers denote the range, and dots represent outliers. Yellow dots mark the mean values. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 4. Correlation coefficients (r) among the fatty acid concentrations in BSFL and their corresponding diets.

Saturated FAs	C12:0	C14:0	C16:0	C17:0	C18:0	C20:0	Σ_{SFA}
(r)	−0.05	0.56	0.93	0.66	0.62	0.40	0.35
Unsaturated FAs	C18:1 n-9c	C18:1 n-9t	C18:2	C20:3	C20:1	Σ_{MUFA}	Σ_{PUFA}
(r)	−0.14	−0.03	−0.11	0.83	−0.29	−0.13	0.80

3.3. Amino Acids Profile

The total AA content varied across both diets and BSFL samples, but no statistically significant differences were detected, as seen in Figure 4 and Table 5. Among the diets, D_TC showed the highest AA content (6.40 ± 0.93 mg/100 g dw), whereas D_CTRL was lowest (4.34 ± 1.05 mg/100 g). Across all diet treatments, values spanned 4.34–6.40 mg/100 g, indicating moderate dispersion. In contrast, BSFL samples consistently exhibited higher totals than the diets, averaging approximately 1.8-fold greater overall. I_TC had the highest AA concentration (9.94 ± 0.77 mg/100 g), while I_BW was lowest (8.72 ± 1.63 mg/100 g), with a narrower range across insect treatments (8.72–9.94 mg/100 g), suggesting that dietary formulation produced limited carry-over effects on whole-larval AA accumulation. In the BSFL, lysine was the most abundant essential AA, and glutamic acid was the most abundant non-essential AA.

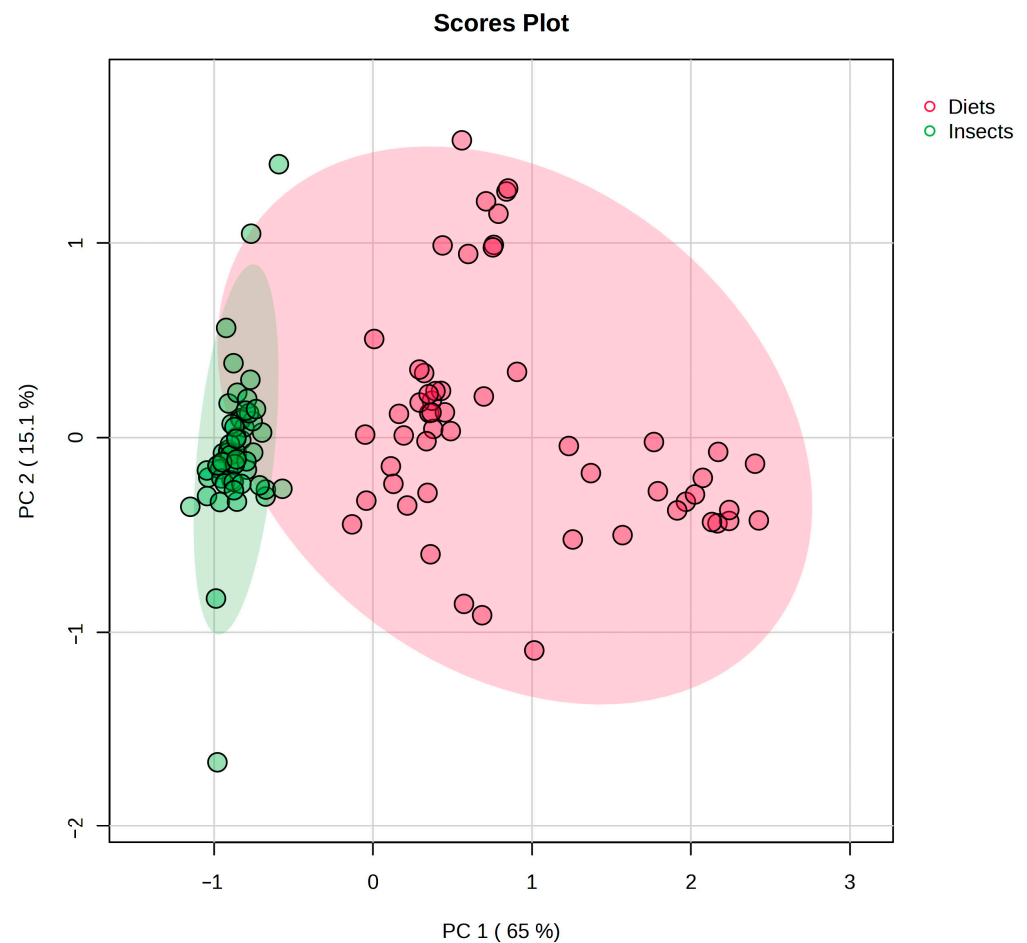
**Figure 4.** Comparison between diets and BSFL (insects) based only on AAs. Data were normalized by median and mean-centered.

Table 5. Total amino acid in diets and BSFL.

Samples (Diets)	Amino Acid (mg/100 g dw)	Samples (BSFL)	Amino Acid (mg/100 g dw)
D_CTRL	4.34 ± 1.05	I_CTRL	9.31 ± 1.26
D_BC	5.10 ± 0.41	I_BC	9.36 ± 0.87
D_BW	4.68 ± 0.95	I_BW	8.72 ± 1.63
D_PC	5.23 ± 0.73	I_PC	8.90 ± 0.76
D_SC	5.40 ± 0.96	I_SC	9.18 ± 1.03
D_TC	6.40 ± 0.93	I_TC	9.94 ± 0.77
D_TW	4.45 ± 0.78	I_TW	9.76 ± 1.21

Results are reported as the mean ± standard deviation.

3.4. Carotenoids and Tocopherols

Figure 5 presents the distribution of tocopherols and carotenoids across the experimental diets (a) and the corresponding BSFL samples (b). Each box plot illustrates the variability among replicates within each treatment group, showing median values (horizontal lines), interquartile ranges (boxes), and outliers (dots). These plots visualize the relative abundance and dispersion of antioxidant compounds, highlighting how different feed formulations influenced the accumulation of these metabolites in both the diets and larvae. Consistent with previous observations, the diets exerting the largest effects on both the feed matrices and the corresponding BSFL were D_SC, D_TC, and D_TW (Tables 6 and 7). In contrast, D_PC, D_BW, and D_BC contained comparatively low concentrations of tocopherols (particularly α -tocopherol) and carotenoids (predominantly lutein and zeaxanthin) and therefore contributed minimally to the antioxidant potential of BSFL; these treatments were not considered further. Tomato-based diets showed elevated tocopherols (Figure 5): α -tocopherol was 1.68 mg/kg dw in D_TC and 1.85 mg/kg dw in D_TW, while γ -tocopherol was 15.06 mg/kg dw in D_TC and 3.35 mg/kg dw in D_TW. The corresponding insect samples contained α -tocopherol at 13.98 mg/kg dw (I_SC), 5.31 mg/kg dw (I_TC), and 22.54 mg/kg dw (I_TW), the latter being the highest measured. Lutein was detected in all diet formulations. The spinach-containing diet (D_SC) exhibited the greatest xanthophyll content, with lutein, neoxanthin, and violaxanthin at 31.63, 87.05, and 162.01 mg/kg dw, respectively.

Table 6. Bioactive compounds content in the diets expressed as mg/kg dry weight.

	D_CTRL	D_BC	D_BW	D_PC	D_SC	D_TC	D_TW
α -tocopherol	nd	nd	0.25 ± 0.4 ^b	nd	0.80 ± 1.2 ^b	1.68 ± 0.5 ^{ab}	1.85 ± 2.3 ^a
β -tocopherol	8.55 ± 0.3 ^a	1.67 ± 3.3 ^b	7.64 ± 0.2 ^a	2.31 ± 3.5 ^b	nd	6.43 ± 2.4 ^a	6.63 ± 0.3 ^a
γ -tocopherol	nd	nd	1.73 ± 0.7 ^{bc}	0.68 ± 1.1 ^c	2.12 ± 1.7 ^{bc}	15.06 ± 2.6 ^a	3.35 ± 0.4 ^b
α -tocotrienol	1.87 ± 0.8 ^{ab}	0.80 ± 1.2 ^b	2.28 ± 0.2 ^{ab}	1.89 ± 2.3 ^{ab}	0.79 ± 0.8 ^b	1.16 ± 0.5 ^{ab}	2.60 ± 0.4 ^a
γ -tocotrienol	4.43 ± 0.2 ^a	nd	1.22 ± 0.1 ^b	nd	nd	nd	0.43 ± 0.6 ^c
Neoxanthin	nd	nd	nd	nd	87.05 ± 58.1 ^a	nd	nd
Violaxanthin	nd	nd	nd	nd	162.01 ± 146.8 ^a	nd	nd
Zeaxanthin	9.54 ± 0.1 ^{ab}	nd	nd	nd	nd	9.94 ± 0.4 ^a	6.06 ± 4.5 ^b
Lutein	9.81 ± 0.1 ^b	9.44 ± 0.3 ^b	14.16 ± 3.3 ^b	11.33 ± 2.1 ^b	31.63 ± 19.9 ^a	10.80 ± 0.7 ^b	10.40 ± 0.5 ^b
δ -carotene	nd	nd	nd	nd	nd	41.58 ± 7.4 ^a	50.77 ± 14.8 ^a
β -cryptoxanthin	nd	nd	nd	nd	nd	52.44 ± 11.5 ^a	98.45 ± 38.1 ^b
Lycopene	nd	nd	nd	nd	nd	824 ± 378.3 ^a	2318.69 ± 1230.7 ^b
β -carotene	nd	nd	nd	nd	nd	116.09 ± 33.7 ^a	nd

Results are reported as the mean ± standard deviation. The different letters in the same row indicate significant differences ($p < 0.05$). nd = not detected.

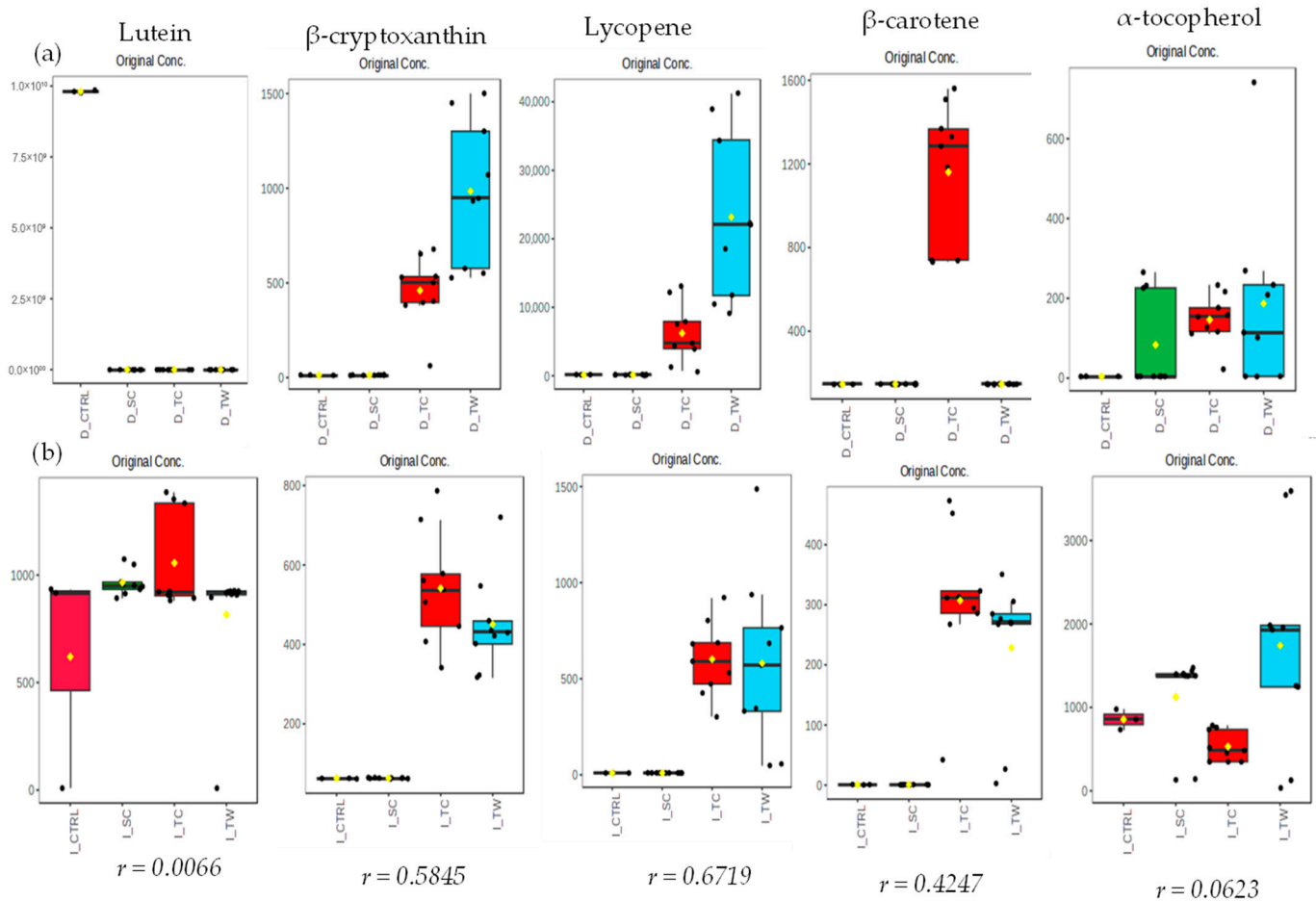


Figure 5. Boxplots showing the distribution of tocopherols and carotenoids in (a) experimental diets and (b) corresponding BSFL samples. Variations among treatments were analysed by ANOVA. The yellow dots in each box represent the mean values, while the black dots indicate the individual data points (outliers) plotted over the boxplot distribution.

Table 7. Bioactive compounds content in BSFL samples expressed as mg/kg dry weight.

	I_CTRL	I_BC	I_BW	I_PC	I_SC	I_TC	I_TW
α-tocopherol	8.56 ± 1 ^{bc}	nd	2.99 ± 0.8 ^c	7.22 ± 2.6 ^c	13.98 ± 0.4 ^b	5.31 ± 1.8 ^c	22.54 ± 10.2 ^a
α-tocotrienol	16.76 ± 0.5 ^{ab}	nd	15.15 ± 2.8 ^{ab}	11.34 ± 8.5 ^b	15.81 ± 2.6 ^{ab}	19.79 ± 1.2 ^a	nd
Zeaxanthin	3.86 ± 0.2 ^a	nd	3.06 ± 0.2 ^b	2.66 ± 0.1 ^b	2.99 ± 0.3 ^b	3.62 ± 0.8 ^a	2.93 ± 0.1 ^b
Lutein	9.17 ± 0.1 ^{ab}	nd	9.52 ± 0.3 ^{ab}	9.06 ± 0.2 ^b	9.65 ± 0.6 ^{ab}	10.56 ± 2.2 ^a	9.15 ± 0.1 ^b
β-cryptoxanthin	nd	nd	3.69 ± 0.4 ^b	nd	nd	5.41 ± 1.4 ^a	4.51 ± 1.2 ^{ab}
Lycopene	nd	nd	nd	nd	nd	6.02 ± 1.9 ^a	6.87 ± 3.6 ^a
β-carotene	nd	nd	nd	nd	nd	3.49 ± 0.7 ^a	2.89 ± 0.3 ^b

Results are reported as the mean ± standard deviation. The different letters in the same row indicate significant differences ($p < 0.05$). nd= not detected.

4. Discussion

Comprehensive chemical profiling and PCA analyses reinforce the view that BSFL act as selective bioconverters rather than passive reflectors of their diet. In our study, the experimental diets diverged markedly in carotenoid and tocopherol composition, particularly the tomato- and spinach-based diets, but the larvae clustered into just two distinct compositional regimes, indicating constraints on assimilation or retention. Among the discriminating metabolites, α-tocopherol, lutein, lycopene, and saturated fatty acids (especially lauric acid, C12:0, and palmitic acid, C16:0) stood out. For instance, C12:0 accounted for 35–57% of the total fatty acids in our study, consistent with recent reports showing

approximately 32–48% under diverse rearing conditions [43–46]. Leong et al. [47] further reported that this proportion can exceed 70% when larvae are reared on a certain fruit-waste diet. This metabolic trait underpins the increasing interest in BSFL oil as a sustainable lipid source in aquafeeds, where lauric acid contributes both nutritional value and antimicrobial properties [48].

The distinct clustering of larvae reared on the spinach–chickpea substrate suggests a non-linear metabolic response triggered by a specific combination of dietary carotenoids and tocopherols. Spinach is particularly rich in lutein and α -tocopherol, while chickpea contributes complementary lipids and sterols; together, these may have exceeded a physiological threshold that altered larval assimilation or allocation strategies. Such threshold effects have been noted in other enrichment studies, where only high dietary loads of fat-soluble bioactives are translated into measurable larval accumulation [49]. Importantly, recent work confirms that BSFL are capable of bioaccumulating carotenoids and tocopherols, although the efficiency of uptake is variable and compound-specific [50]. The identification of α -tocopherol and carotenoids as key discriminants in our study aligns with these observations and emphasizes the potential of substrate engineering for enhancing the micronutrient profile of insect biomass.

From a nutritional perspective, the enrichment of tocopherols and carotenoids in BSFL biomass is of particular relevance. These compounds not only enhance the antioxidative stability of insect oils but also carry functional benefits when insects are used in feed or food applications [45,51]. However, the convergence of most diet treatments into a single larval compositional cluster suggests that metabolic constraints limit the degree of achievable enrichment [52]. This underscores the need for further targeted studies, including tracer experiments to quantify assimilation pathways, dose–response designs to assess threshold effects, and post-harvest analyses to evaluate stability and bioavailability of retained micronutrients [45,53]. Together, such efforts will refine strategies to tailor BSFL nutritional profiles and further establish their role as sustainable bio-converters of plant-derived bioactive compounds.

4.1. Fatty Acid Profile

Our findings confirm that substrate composition can modulate BSFL lipid profiles, particularly MUFAs, while the overall class hierarchy (SFA > MUFA > PUFA) remains conserved. The prominence of medium-chain and long-chain SFAs, especially lauric acid, agrees with a broad literature base reporting SFA-rich BSFL lipids, with lauric acid being frequently dominant and functionally relevant (antimicrobial, fast energy) in feed applications [54,55]. Importantly, BSFLs naturally exhibit an imbalanced SFA/PUFA ratio, yet their fatty acid profile can be partially shaped by diet composition [25]. In our study, this was evident in the significant correlations between dietary and larval PUFA levels, which mirror earlier reports of dietary reflection [35,37,56].

The strong diet–larva correlations for C16:0 and total PUFAs indicate notable dietary control over larval lipids, although this response occurs within the inherent metabolic constraints typical of BSFL. Oonincx and Finke [57] observed that larvae reared on low-fat diets accumulate shorter-chain SFAs, consistent with our finding of substrate-driven modulation of medium-chain FA. Similarly, Danieli et al. [58] documented moderate positive correlations between dietary and larval unsaturated FAs, supporting our observation that total PUFA levels in larvae followed dietary supply ($r = 0.80$). Barroso et al. [59] demonstrated that BSFL can even be enriched with omega-3 FAs through diet, and Truzzi et al. [60], as well as Ratti et al. [25], suggested supplementation with *Schizochytrium* sp. or spirulina alongside plant by-products as viable strategies to enhance omega-3 PUFA in BSFL biomass.

Interestingly, in our experiment, the spinach–chickpea and pea–chickpea treatments produced the highest larval PUFA levels, pointing to the potential of plant-based blends to improve unsaturated FA retention. This aligns with recent studies showing that targeted substrates, whether legumes, leafy vegetables, or microalgae, can elevate larval PUFA fractions, though gains often plateau due to metabolic constraints [60,61]. Together, these findings reinforce the view that BSFL FA profiles are steerable but not fully re-programmable by diet. From a sustainability perspective, the capacity to nudge BSFL lipids toward higher MUFA/PUFA proportions has practical value for circular feed systems. BSFL fat has been investigated as a partial substitute for conventional lipids across species, and studies highlight both nutritional and environmental benefits when insect fats replace palm, coconut, or fish oils [62–64]. The higher MUFA/PUFA ratio in insect-derived fats contributes to improved oxidative stability in feed, reducing the need for synthetic antioxidants [65]. Furthermore, this substitution could alleviate the pressure on land and marine resources associated with traditional feed fats, thus aligning with the sustainability goals of circular feed systems [66,67].

4.2. Amino Acid Composition

BSFL samples showed lysine as the most abundant essential amino acid and glutamic acid as the most abundant non-essential amino acid. This pattern is consistent with findings from Fuso et al. [68], who reported that when BSFLs are reared on vegetable by-products, lysine and leucine predominate among essential amino acids while glutamic acid is the most frequent non-essential amino acid. Similarly, Cappellozza et al. [69] observed that BSFL biomass reared on diets composed of fruits and vegetables is particularly rich in glutamic acid, reinforcing the tendency for glutamate to dominate the non-essential class in insect protein. The relatively high lysine content may partly be derived from inclusion of legumes in the rearing substrate, as legume proteins tend to be lysine-rich [70,71].

Although many studies have explored how substrate composition affects the amino acid profile of BSFL, their results are somewhat inconsistent. Differences across studies may arise due to variation in substrates (e.g., agricultural by-products, food waste, manure), insect strain, rearing conditions, developmental stage, and processing approaches [37,68,72,73]. Processing steps such as fat removal (defatting) can concentrate the protein (and amino acids) in the residual biomass: for example, Schiavone et al. [74] showed that defatting larvae increases AA content in the dried meal. More broadly, the interplay of substrate and processing yields intra- and interstudy variability in AA profiles [75].

In our investigation, despite the differences among diet treatments, the whole-larvae AA profiles across insect groups were quite stable, with relatively little spread in total AA concentrations and dominant AA identities, suggesting biological regulation. Essential AAs like lysine, leucine, and valine in our BSFL are comparable to values reported by Lu et al. [76], which supports their nutritional adequacy. According to several authors, BSFL AA profiles meet the needs for substitution in key feed sectors, such as fishmeal in aquaculture [77], swine diets [78], and poultry diets [79].

From a feed-industry perspective, the observed AA profile lends support for using BSFL as a partial or full protein source replacement, particularly when essential AAs like lysine are limited in many plant-derived feeds. The high glutamic acid content may also contribute to palatability or metabolic functions in target animals, although non-essential AAs tend to be less limiting. The nutritional value does not depend purely on amino acid presence but also on digestibility, bioavailability, and the balance (ratio) of AAs, metrics which can vary with insect processing and feed formulation [75]. Nevertheless, our results and the wider literature suggest caution. While some studies find strong substrate effects

on AA profiles, others find limited change, and it is possible that beyond a threshold of dietary variation, larvae regulate AA composition more tightly [80]. Furthermore, recent research is progressively exploring targeted supplementation or adjustment of substrates (e.g., amino acid precursors) to influence insect AA profiles [81]. This could open avenues for tailoring BSFL protein for specific feed needs.

4.3. Carotenoid and Tocopherol Profile

This study demonstrates that dietary enrichment strategies strongly influence the antioxidant profile of BSFL via modulation of tocopherol and carotenoid contents in the rearing substrates (i.e., diets). Among the tested formulations, D_SC, D_TC, and D_TW exerted the most pronounced effects, whereas D_PC, D_BW, and D_BC contributed only marginally. These findings align with previous work showing that substrate composition is a key determinant of insect biochemical composition and can be used strategically to enhance nutritional quality [82].

Tomato-based diets showed particularly high concentrations of α -tocopherol and γ -tocopherol, confirming the potential of tomato by-products as substrates for functional insect rearing. In the larvae, α -tocopherol reached up to 22.54 mg/kg dw in I_TW, surpassing levels in I_SC (13.98 mg/kg) and I_TC (5.31 mg/kg). This amplification indicates efficient uptake or conversion by the insects, in agreement with previous observations on tocopherol assimilation in insect systems [83]. Since tocopherols act as potent lipid antioxidants, their presence in larvae may improve oil stability, prolong shelf life, and add functional value to insect-derived products. Such dietary strategies reduce the need for synthetic antioxidants post-harvest, aligning with sustainable processing and clean-label approaches.

The abundance of xanthophylls in green leafy vegetables such as spinach is well established [9], and accordingly, the major source of lutein in the D_SC diet can be attributed to spinach. This diet yielded particularly high amounts of lutein, neoxanthin, and violaxanthin, accounting for 31.63, 87.05, and 162.01 mg/kg dw, respectively, highlighting the potential of leafy by-products to enrich insect biomass with nutritionally relevant carotenoids.

Tomato-based diets, in combination with wheat or chickpeas, displayed a different carotenoid profile dominated by lycopene, followed by β -carotene, β -cryptoxanthin, and δ -carotene. Indeed, tomato by-products typically contain lycopene, β -carotene, α -carotene, lutein, β -cryptoxanthin, and zeaxanthin, although concentrations may vary depending on cultivar, cultivation system, climatic conditions, and ripening stage [10]. In the present study, lycopene accounted for 824 mg/kg dw in D_TC and 2318.69 mg/kg dw in D_TW. The variability between diets can be explained by the natural heterogeneity of secondary metabolites in tomato residues, as samples were deliberately collected at different periods of the production line to mimic industrial conditions.

Importantly, the carotenoid profiles of the larvae closely matched those of their diets, consistent with recent findings on tomato- and carrot-based diets [31]. Correlation analysis in our study revealed positive associations between dietary and larval levels of lycopene ($r = 0.6719$), β -cryptoxanthin ($r = 0.5845$), and β -carotene ($r = 0.4247$). However, lutein and α -tocopherol in larvae were not correlated to diet (Figure 5), suggesting selective assimilation or metabolism of these compounds. Similarly, Leni et al. [31] reported strong correlations between total carotenoids and xanthophylls ($r = 0.882$) when BSFL were fed bean- and bran-based by-products, highlighting that substrate type influences the degree of nutrient transfer.

The observed enrichment of BSFL with dietary carotenoids, particularly provitamin A types (β -carotene, β -cryptoxanthin), has important nutritional implications. Previous research has shown that larvae raised on provitamin A-enriched substrates can contribute

to dietary vitamin A restoration in the food chain [30,84]. Given the global burden of vitamin A deficiency, integrating agricultural by-products rich in carotenoids into insect farming could represent a novel, sustainable strategy to improve micronutrient intake.

From a sustainability standpoint, these findings reinforce the role of insects as bioconverters that can upcycle heterogeneous agri-food residues into nutrient-rich biomass [85,86]. This dual function, waste valorization and nutritional enhancement, positions insect farming as a promising component of circular food systems [45,86–89]. The ability to tailor antioxidant content through diet further extends their potential, providing insect oils and meals with greater stability and functional value for feed and food applications.

5. Conclusions

This study confirms that BSFLs act as selective bioconverters rather than passive reflectors of dietary inputs, with distinct constraints on the assimilation and retention of bioactive compounds. While tomato- and spinach-based diets enhanced larval profiles of α -tocopherol, lycopene, and lutein, most treatments converged into a limited range of compositional outcomes, highlighting the constrained flexibility of BSFL metabolism. The prominence of lauric acid across treatments reinforces its status as a metabolic characteristic of BSFL lipids and a functionally relevant trait for feed applications. Enrichment of larvae with tocopherols and carotenoids not only improves the antioxidative stability of insect-derived oils but also offers a sustainable route to restore essential micronutrients, including provitamin A carotenoids, in the food chain. These findings highlight the dual role of BSFL in waste valorization and nutritional enhancement, while also revealing metabolic thresholds that may limit the degree of achievable enrichment.

While the study demonstrates the nutritional enrichment potential of BSFL reared on mixed vegetable by-products, several limitations should be acknowledged. The experiments were conducted under controlled laboratory conditions using selected substrate blends, which may not fully represent the variability and operational complexity of industrial-scale systems. From an industry and policy perspective, these findings support the integration of insect-based bioconversion into sustainable feed and waste management frameworks, aligning with circular economic objectives and EU directives on resource efficiency and waste reduction. Despite the promising results, further research is needed to evaluate industrial feasibility, process optimization, and the stability of bioactive compounds during feed processing to ensure the scalability of these benefits.

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