Fatty acids profile of black soldier fly (*Hermetia illucens*): influence of feeding substrate based on coffee-waste silverskin enriched with microalgae

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Highlights

Hermetia illucens (HI) enriched with PUFA through the revalorization of organic waste

Reuse of coffe silverskin (CS) enriched with *Schizochytrium* sp or *Isochrysis* sp.

Hermetia illucens prepupae: first study of fatty acid composition coupling GC-MS and FTIR.

Ability of HI prepupae to accumulate significative amounts of polyunsaturated fatty acids

HI prepupae reared on CS enriched with *Schizochytrium* sp are beneficial to health

CS substrate enriched with a 10% of *Schizochytrium* sp is the most covenient one

ABSTRACT

The aim of this work was to find alternative low-cost and environmentally friendly rearing substrates for the growth of *Hermetia illucens* (HI) (Diptera, Stratiomydae), used as feed. At this purpose, insect feeding substrates based on the re-use of coffee silverskin, the main waste product of the coffee-roasting industry, enriched with various percentages of microalgae (i.e., *Schizochytrium* sp. or *Isochrysis* sp.), were tested. The fatty acid profile, as well as the relative amount of lipids, proteins and carbohydrates (these latter calculated as ratio to the total biomass of the sample) of ingredients, insect feeding substrates and HI prepupae, were determined for the first-time coupling Gas Chromatography-Mass Spectrometry and Fourier Transform Infrared Spectroscopy. A multivariate statistical analysis (Principal Component Analysis) was performed to better read into results. In general, the inclusion of microalgae caused in both feeding substrates and in HI prepupae an increase in the relative amount of lipids and proteins, improving their nutritional value. Higher amounts of unsaturated fatty acids, particularly of omega-3, and good nutritional indices were detected in HI prepupae reared on substrates enriched with 10%, 20% or 25% of *Schizochytrium* sp. with respect to HI prepupae fed with coffee silverskin enriched with *Isochrysis* sp., suggesting them as new nutraceutical ingredients for future functional feed and food. In addition, the substrate enriched with a 10% inclusion level of *Schizochytrium* sp. has to be considered the most convenient one since a greater inclusion of microalgae did not promote additional benefits in terms of nutritional value of HI prepupae.

KEYWORD: *Hermetia illucens, coffee silverskin, microalgae, FA profile, relative macromolecular composition, Principal Component Analysis*

Abbreviations. CARBO, carbohydrates; CS, coffee silverskin; DHA, docosahexaenoic fatty acid; DM, dry matter; EPA, eicosapentaenoic fatty acid; FA, fatty acid; FAMEs, fatty acid methyl esters;

FTIR, Fourier Transform InfraRed; GC-MS, gas-chromatography-mass spectrometry; HI, Hermetia illucens; I, *Isochrysis* sp.; IR, InfraRed; LIP, lipids; MUFAs, monounsaturated fatty acids; NIST, National Institute of Standard & Technology; PCA, Principal Component Analysis; PUFAs, polyunsaturated fatty acids; PRT, proteins; S, *Schizochytrium* sp.; UNSAT, unsaturated fatty acids.

1. Introduction

Due to the rapid increase in world population, the production of enough feed for farmed-animals and food for humans represents a serious challenge for the future. Moreover, the increase in food-demand along with not-sustainable food production practices will generate a rise in waste and by-product production (Van Huis, 2013). Therefore, the revalorization of by-products for feed and food production is strongly supported by several research proposals and studies (Diener et al., 2011; Li et al., 2011; Salomone et al., 2017). Insects may represent a valuable alternative ingredient for feed and food production in a new interesting approach of sustainable circular economy, since they show high reproductive rate and nutritional value and can grow on organic by-products (Henry et al., 2015; Gasco et al., 2016; Barragan-Fonseca et al., 2017; Liu et al., 2017; Vargas et al., 2018). Recently, the EFSA Scientific Committee (2015) proposed a list of insect species with the greatest potential as food and feed ingredients in the EU, including *Hermetia illucens* (HI, Diptera, Stratiomydae). Due to its rapid development (Hall and Gerhardt, 2002), reduced environmental footprint (Sheppard et al., 1994), and preference for organic waste as growth substrate (Van Huis et al., 2013; Nguyen et al., 2015; Meneguz et al., 2018), HI is one of the most promising insect species to respond to the joint problems of the future lack of conventional feed and food ingredients and the excessive production of agro-food waste (Cutrignelli et al., 2018; Zarantoniello et al., 2019). In general, HI shows high lipid content (up to 500 g/kg) (Makkar et al., 2014; Barragan-Fonseca et al., 2017), but its fatty acid (FA) composition is not always optimal for animal and human nutrition and health (Nordøy et al., 2001; Gómez-Candela et al., 2011), because characterized by low amounts of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids and high amounts of saturated ones (SFA) (St-Hilaire et al., 2007; Ushakova et al., 2016; Barragan-Fonseca et al., 2017; Caligiani et al., 2018; Zarantoniello et al., 2018; Cardinaletti et al., 2019). HI nutritional composition are deeply influenced by the rearing substrates (Tomberlin et al., 2002; Nguyen et al., 2013), and it has been demonstrated that rearing HI larvae on a substrate based on organic waste containing desirable omega-3 fatty acids could be a suitable way to enrich the final insect biomass (St-Hilaire et al., 2007; Barroso et al., 2017).

Coffee silverskin (a coffee roasting by-product, CS) is an industrial waste rich in bioactive compounds and characterized by antioxidant and potential prebiotic activities (Narita and Inouye, 2014; Costa et al., 2018; Iriondo-DeHond et al., 2019), suggesting it as ingredient for functional food. Large amounts of CS are produced worldwide every year (Galanakis, 2017), representing a discharge, and thus a cost, for coffee companies. In the concept of circular economy, a general effort to valorize this waste is of great interest.

Marine microalgae are characterized by the presence of essential amino-acids and high contents of omega-3 and -6 PUFAs (da Silva Vaz et al., 2016). *Schizochytrium* sp. are heterotrophic marine traustochytrids of which 35 g/100g of their total fatty acids consists out of DHA (Zhu et al., 2007; Barclay et al., 2010), while *Isochrysis* sp. are microalgae of the genus haptophytes characterized by a high content of PUFAs such as DHA, stearidonic acid and alpha-linolenic acid (Aussant et al., 2018).

The aim of this work was to find environmentally friendly rearing substrates for the growth of PUFA-enriched *Hermetia illucens*, to be used as a perspective feed ingredient. At this purpose, CS was chosen as main growth substrate for HI, while *Schizochytrium* sp. and *Isochrysis* sp. (at various inclusion percentages) were added as PUFA source. The fatty acid profile, as well as the relative amount of lipids, proteins and carbohydrates (calculated as ratio to the total biomass of the sample) of ingredients, insect feeding substrates and HI prepupae, were determined for the first-time coupling Gas Chromatography-Mass Spectrometry and Fourier Transform Infrared Spectroscopy. This latter is a label free analytical technique, successfully applied in recent years to characterize the macromolecular features of biological samples at vibrational level (Giorgini et al., 2018; Zarantoniello et al., 2019).

2 Materials and methods

2.1. *Rearing and harvesting*

2.1.1. *Insect feeding substrate preparation*

Nine different insect feeding substrates (from here below indicated as "substrates") were tested during the experiment. The basal substrate consisted of by-products obtained from roasting coffee (a mixture of Arabica and Robusta varieties) process (coffee silverskin, CS) [provided by Saccaria Caffè S.R.L., Marina di Montemarciano (AN), Italy]. CS (moisture 440 g/kg) was collected in plastic bags, frozen at -20°C, and ground in an Ariete 1769 food processor (De' Longhi Appliances Srl, Italy) to a particle size of 2±0.4 mm before the feeding substrate preparation. *Schizochytrium* sp. and *Isochrysis* sp. were freeze-dried provided by AlghItaly Società Agricola S.R.L. (Sommacampagna (VR), Italy) and stored at 4°C. Feeding substrates were formulated as follow (Table 1): substrate E, 100% coffe silverskin (CS); substrates As, Bs, Cs and Ds: CS added with 5%, 10%, 20% and 25% of *Schyzochytrium* sp., respectively; substrates Ai, Bi, Ci and Di: CS added with 5%, 10%, 20% and 25% *Isochrysis sp*., respectively. All substrates were added with water to reach an optimal moisture (as suggested by literature) close to 700 g/kg (Table 1) (Makkar et al., 2014). Both separate ingredients (CS and microalgae) and feeding substrates (a mixture of CS and microalgae) samples were stored at -80°C for further analyses.

2.1.2. *Rearing of Hermetia illucens larvae*

HI rearing was carried out at the D3A experimental facility (Polytechnic University of Marche) starting from 6 days old larvae purchased from Smart Bugs s.s. [Ponzano Veneto (TV), Italy]. Larvae were divided in the following groups (five replicates, each containing 150 larvae) (Van Broekhoven et al., 2015): HI E, prepupae reared on substrate E (100% CS); HI As, HI Bs, HI Cs, HI Ds: prepupae reared on substrate CS enriched with 5%, 10%, 20% and 25% of *Schyzochytrium* sp, respectively; HI Ai, HI Bi, HI Ci, HI Di: prepupae reared on substrate CS enriched with 5%, 10%, 20% and 25% of *Isochrysis* sp., respectively. Each group contained 750 larvae (6 days old, hand counted). Larvae were reared at a density of 0.3 ind./cm^2 (Barragan-Fonseca et al., 2018), in a climatic chamber at a 27±1°C temperature, 650±50 g/kg relative humidity (Spranghers et al., 2017), in continuous darkness.

Each larva was provided with a feeding rate of 100 mg/day (Diener et al., 2009) within plastic boxes (28 x 19 x 14 cm). Boxes were screened with fine-mesh cotton gauze and covered with a lid provided with a single ventilation hole (Spranghers et al., 2017). Substrates were completely replaced once a week (larvae were gently transferred into another box containing the new feed). Larvae were visually inspected every day and when prepupae were identified by the change in tegument colour from white to black (May, 1961), they were manually collected using forceps and brushes and sampled and stored at -80°C for further analyses.

Experiments were performed in compliance with the Italian laws and institutional guidelines. No specific authorization is requested to conduct experiments on invertebrates such as insects.

2.2. Analytical methods

2.2.1. Lipid extraction and fatty acids analysis

Moisture of samples was determined in an oven at 105°C for 24h (index no. 934.01) (Association of Official Analytical Chemists, 2002). To determine the total lipid content and the overall FA profile of the single ingredients, substrates, and HI prepupae, samples were thawed, and homogenized. Aliquots of 200 mg of each replicate were added with 100 µl of Internal Standard (methyl ester of nonadecanoic acid, 99.6%, Dr. Ehrenstorfer GmbH, Germany), and extracted overnight with the method of Folch et al. (1957). HI prepupae larvae were washed to eliminate substrate particles, and finely ground before lipid extraction. Lipid extracts were evaporated under laminar flow inert gas (N_2) until constant weight. After drying, the mass of extracted lipids was determined gravimetrically (as g/kg DM). GC-MS analysis was carried out on three aliquots *per* replicate (three GC-MS runs for aliquot).

The extracted lipids were resuspended in n-epthane to transesterify fatty acids. Fatty acid methyl esters (FAMEs) were prepared using sodium methylate, according to Canonico et al. (2016). FAMEs were determined on an Agilent-6890 GC equipped with a split-splitless injector and coupled to an Agilent-5973N quadrupole Mass Selective Detector. A CPS ANALITICA CC-wax-MS (30 m × 0.25 mm ID, 0.25 μm film thickness) glass capillary column coated with polyethylene glycol was used. Instrumental conditions were as reported in Truzzi et al. (2017, 2018): sample injections of 1 μL were made in a split mode ratio 1:5 using a glass cup liner (Agilent Liner, splitless, double taper 5583-4705). The inlet temperature was set at 250°C. Helium carrier gas (99.9999%, Air Liquide, Italy) (8.0 psi) was used at a flow rate of 1 mL/min. The oven temperature started at 100°C for 1 min, and it was subsequently increased to 150°C at the rate of 25°C min^{-1}, to 200°C at the rate of 5°C min^{-1} and to 230°C at the rate of 1°C min^{-1}, for a total run time of 43 min. The ion source and the quadrupole temperatures were set at 230°C and 280°C, respectively. The electron energy was 70 eV. A mass range from 50 to 400 m/z was scanned at a rate of 3.15 scan/s. Data collection, identification, and quantification of FAs were as reported in Truzzi et al. (2017). Retention times and mass spectra of 37-component FAME Mix standard (≥ 99%, Supelco, Bellefonte, PA, USA) were used to confirm the NIST (National Institute of Standard & Technology) identification of FAs in the sample. For each aliquot, at least three runs were performed on the GC-MS. The method performances were as those obtained for the determination of FAMEs in insects and experimental diets of the experiment performed in Vargas et al. (2018): the linearity was checked up to 320 mg mL^{-1}, and the limit of detection and limit of quantification, calculated as reported by Truzzi et al. (2014), ranged from 4 mg mL^{-1} to 22 mg mL^{-1} and from 13 mg mL^{-1} to 66 mg mL^{-1}, respectively. Moreover, the method showed a good accuracy and precision. For ingredients, substrates, and prepupae, the intraday and interday precision were, for major FAs with an amount greater than 1g/100 g FAs, <3% and <8%, respectively, indicating a good repeatability of the analyses. For FAs with an amount minor than 1 g/100 g FAs, intraday and interday precision ranged from 6% to 15%, and from 7% to 20%, respectively.

2.2.2. Fourier Transform InfraRed spectroscopy analysis

FTIR (Fourier Transform InfraRed) spectroscopy was exploited to define the relative amount of lipids, proteins and carbohydrates of all ingredients, substrates and HI prepupae groups. For each experimental group, one 5 mg aliquot for each replicate (five) were analyzed (5 spectra for each aliquot).

InfraRed (IR) measurements were performed by using a Spectrum GX1 Spectrometer (Perkin Elmer, Waltham, Massachusetts, USA) equipped with a Attenuated Total Reflectance accessory for measurements in reflectance. IR spectra were acquired in the medium IR region from 4000 to 800 cm^{-1} (spectral resolution 4 cm^{-1}). Each spectrum was the result of 64 scans. Before each sample acquisition, a background spectrum was collected. Raw IR spectra were converted in absorbance, two-points baseline linear fitted in the 4000-800 cm^{-1} spectral range and vector normalized in the same interval (OPUS-IR™ 7.1, 2016).

On pre-processed IR spectra, specific bands with biological meaning were detected and analyzed in terms of position and integrated areas (Integration routine, OPUS 7.1 software). In particular, the following bands were investigated: ~3013 cm^{-1} (spectral range of integration 3035-2996 cm^{-1}, named unsaturated FA, UNSAT); ~2925 and ~2855 cm^{-1} (spectral range of integration 2996-2804 cm^{-1}, named lipids, LIP); ~1744 cm^{-1} (spectral range of integration 1786-1709 cm^{-1}, named fatty acids, FA); ~1647 and ~1542 cm^{-1} (spectral range of integration 1709-1480 cm^{-1}, named proteins, PRT), and ~1144 cm^{-1} (spectral range of integration 1187-1123 cm^{-1}, named carbohydrates, CARBO). The above defined integrated areas were used to calculate the following band area ratios: LIP/TBM (relative amount of total lipids with respect to total sample biomass), UNSAT/TBM (unsaturated groups in lipid alkyl chains with respect to total sample biomass), FA/TBM (relative amount of fatty acids with respect to total sample biomass), PRT/TBM (relative amount of total proteins with respect to total sample biomass), and CARBO/TBM (relative amount of total carbohydrates with respect to total sample biomass). TBM, defined as total sample biomass, was the sum of the integrated areas at 3035-2996 cm^{-1}, 2996-2804 cm^{-1} and 1807-811 cm^{-1}.

2.3. Health benefits: fatty acid index calculation

From the fatty acid profile (as g of each fatty acid/100 g of total fatty acids), three nutritional indices were calculated. These indices provide different importance to each fatty acid depending on the different contribution of this to the promotion or prevention of cardiovascular disorders: atherogenicity (AI) and thrombogenicity (TI) indices (Ulbricht and Southgate, 1991), and the Hypocholesterolemic to Hypercholesterolemic fatty acid ratio (HH) (Santos-Silva et al., 2002):

$$AI = [12{:}0 + (14{:}0 \times 4) + 16{:}0] / (\Sigma MUFAs + \Sigma PUFA\text{-}n6 + \Sigma PUFA\text{-}n3)$$

$$TI = \Sigma\,(14{:}0 + 16{:}0 + 18{:}0) / [(0.5 \times \Sigma MUFAs + 0.5 \times \Sigma(n6) + 3 \times \Sigma(n3) + (n3/n6)]$$

where: MUFAs are monounsaturated Fatty Acids, PUFAs are polyunsaturated Fatty Acids, distinguished in PUFA-n6 (sum of omega-6 PUFAs) and PUFA-n3 (sum of omega-3 PUFAs).

$$HH = (18{:}1n9 + 18{:}2n6 + 20{:}4n6 + 18{:}3n3 + 20{:}5n3 + 22{:}5n3 + 22{:}6n3)/(14{:}0 + 16{:}0)$$

Also, other nutritional indices such as n-3/n-6, PUFAs/SFAs, were calculated from the fatty acid profile.

2.4. Statistical analysis

IR band area ratios (presented as mean±S.D), lipid content and fatty acid data were analyzed by one-way-ANOVA test, followed by the Multiple Range Test (Daniel and Cross, 2013), after testing the homogeneity of variance with Levene's test. Significant differences were evaluated at the 95% confidence level. When the ANOVA test gave a P-value equal to 0.0000, in the text it was indicated as $P<0.001$. Principal Component Analysis (PCA) was carried out on standardized data; significant components were obtained through the Wold cross-validation procedure (Wold, 1978). ANOVA test, Multiple range test and PCA were performed using STATGRAPHICS Centurion 18 software (Manugistics Inc., 2018).

3. Results

The fatty acids (FA) profile as well as the relative amount of lipids, proteins and carbohydrates of single ingredients (CS and microalgae *Schizochytrium* sp. and *Isocrysis* sp.), substrates (E, As, Bs, Cs, Ds, Ai, Bi, Ci, Di), and HI prepupae fed on the different substrates were analyzed by GC-MS and FTIR techniques.

3.1. Single ingredients

3.1.1. Fatty acid profile

The total lipid content extracted from CS with the Folch's method (96 ± 6 g/kg DM) was consistent with the few data available (Borrelli et al., 2004; Esquivel and Jiménez, 2012; Pourfarzad et al., 2013; Toschi et al., 2014). The content of total lipids in *Schizochytriums* sp (78±1 g/kg DM) and *Isochrysis* sp (70±5 g/kg DM) was lower than literature data (Zhu et al., 2007; Ren et al., 2009; Shah et al., 2014; Vidyashankar et al., 2015). The content of fatty acids (calculated as in Truzzi et al., 2017) was 1.5 g/kg in rehydrated coffee, 63 g/kg in *Schizochytrium* sp. and 1.2 g/kg in *Isochrysis* sp.

The FA profiles of CS and microalgae *Schizochytrium* sp. and *Isochrysis* sp. are reported in Table 2. In CS, the most represented FA was linoleic acid (18:2n6, ~26 g/100 g FAs), followed by palmitic (16:0, ~22 g/100 g FAs), stearic (18:0, ~15 g/100 g FAs), arachic (20:0, ~11 g/100 g FAs) and bebenic (22:0, ~9 g/100 g FAs) acids. In general, CS was mainly composed of SFA (~62 g/100 g FAs), followed by PUFA (~29 g/100 g FAs) and MUFA (~9 g/100 g FAs). The FA profile of *Schizochytrium* sp. was dominated by 22:6n3, (docosahexaenoic acid DHA, ~79 g/100 g FAs), and 16:0 (~13 g/100 g FAs). This microalga was then rich in PUFA (~82 g/100 g FAs), whereas SFA and MUFA represented only ~16 g/100 g FAs and ~1 g/100 g FAs, respectively. Moreover, the very high content of 22:6n3 resulted in a high n-3/n-6 ratio (~47). The FA composition of *Isochrysis* sp. was characterized by a high content of 22:6n3 (~32 g/100 g FAs), myristic acid (14:0, ~17 g/100 g FAs), α-linoleic acid (18:3n3, ~13 g/100 g FAs), and oleic acid (18:1n9, ~11 g/100 g FAs). PUFA was the main class (~55 g/100 g FAs), followed by SFA (~27 g/100 g FAs) and MUFA (~18 g/100 g FAs). n-3/n-6 ratio (~5) was about 9-fold lower than that of *Schizochytrium* sp.

3.1.2. Relative macromolecular composition

The absorbance IR spectra of lyophilized samples of coffee silverskin (CS) and microalgae *Schizochytrium* sp. (S) and *Isocrysis* sp. (I) were reported in Fig. 1. In all the spectra, the bands related to lipids (2925 and ~2855 cm^{-1}, asymmetric and symmetric stretching vibrations of CH_2 groups in lipid alkyl chains, ν_{asym} CH_2 and ν_{sym} CH_2) (Kiefer et al., 2010), proteins (~1647 cm^{-1}, mainly ν C=O, Amide I band of proteins) (Mayers et al., 2013), carbohydrates and polysaccharides (~1032 cm^{-1}, stretching vibration of C-O moieties in carbohydrates, ν C-O) (Mayers et al., 2013) were detected. In *Schizochytrium* sp., additional bands associated to unsaturated lipids (3013 cm^{-1}, stretching vibration of =CH groups in lipid alkyl chains, ν =C-H) (Kiefer et al., 2010), fatty acids (1744 cm^{-1}, stretching vibration of carbonyl moiety in fatty acids, ν C=O) (Mayers et al., 2013), proteins (1542 cm^{-1}, ν C-N and δ N-H, Amide II band of proteins) (Mayers et al., 2013), and carbohydrates and polysaccharides (1144 cm^{-1}, stretching vibration of C-O-C bonds in carbohydrates and polysaccharides, ν C-O-C) (Duygu et al., 2012), were also found. In *Isochrysis* sp., only the bands at 3013 cm^{-1} and 1542 cm^{-1} were detected, the former showing a lower absorbance value with respect to *Schizochytrium* sp.

3.2. Insect feeding substrates

3.2.1. Fatty acid profile

The extraction of total lipids from substrates with Folch method (Folch, 1957) showed that the inclusion of microalgae in the substrates caused a statistically significant increase of total lipids with respect to the CS substrate E (Fig. 2), positively related to microalgae inclusion levels in the substrate. In particular, the inclusion of *Schizochytrium* sp. (at all tested percentages) and of *Isochrysis* sp. (exclusively at 20% (Ci) and 25% (Di)) (Fig. 2b), caused a statistically significant increase of lipid content in the substrate compared to CS substrate E ($P>0.001$, $P=0.002$, respectively).

Table 3 shows the FA composition of substrates. The FA profile of substrates enriched with *Schizochytrium* sp. was dominated by docosahexaenoic acid (DHA) 22:6n3, which increased with the increase of the microalga inclusion, from ~61 to ~71 g/100 g FAs (Ds). The second most represented fatty acid was 16:0 (from ~16 in As to ~13 g/100 g FAs in Cs), followed by 18:0 (from 4.5 in As to 2.7 g/100 g FAs in Ds), 18:2n6 (from 6.1 in As to 2.5 g/100 g FAs in Ds), 20:0 (from 3.0 in As to 1.7 g/100 g FAs in Ds), and 22:0 (from 2.0 in As to 1.3 g/100 g FAs in Ds). The FA profile of substrates enriched with *Isochrysis* sp. was dominated by 16:0, that decreased with the increase of the microalga inclusion, from ~23 (Ai) to ~19 g/100 g FAs (Di). Other well represented fatty acids were 18:0 (from ~16in Ai to ~7 g/100 g FAs in Di), and 18:2n6 (from ~16 in Ai to ~14 g/100 g FAs in Di), followed by 18:3n3 (from ~9 in Ai to ~17 g/100 g FAs in Di), 20:0 (from ~10 in Ai to ~6 g/100 g FAs in Di) and 22:0 (from ~10 in Ai to ~6 g/100 g FAs in Di). DHA varied from 1.5 in Ai to 5.7 g/100 g FAs in Di, whereas the content of 20:5n3 were below the detection limit.

Fig. 3 compares the amounts of FA classes of substrates containing different microalgae. In general, an increasing inclusion of *Schizochytrium* sp. in substrates determined a statistically significant decrease of SFA ($P<0.001$), MUFA ($P<0.001$), n-6 ($P<0.001$), n-9 ($P<0.001$) amounts, and a statistically significant increase of PUFA ($P<0.001$) and n-3 ($P<0.001$) amounts, and of n-3/n-6 ratio ($P<0.001$). An increasing inclusion of *Isochrysis* sp. in substrates determined in general a statistically significant decrease of SFA ($P<0.001$) and n-6 ($P=0.005$) amounts, and a statistically significant increase of MUFA ($P<0.001$), PUFA ($P<0.001$), n-3 ($P<0.001$), n-9 ($P<0.001$) amounts, and of n-3/n-6 ratio ($P=0.008$). Substrates enriched with *Schizochytrium* sp. showed statistically significant lower amounts of SFA ($P<0.001$), MUFA ($P<0.001$), n-6 ($P<0.001$) and n-9 ($P<0.001$), and statistically significant higher amounts of PUFA ($P<0.001$) and n-3 ($P<0.001$) compared to those enriched with *Isochrysis* sp. Consequently, substrates containing *Schizochytrium* sp. showed a n-3/n-6 ratio significantly higher than substrates enriched with *Isochrysis* sp. ($P<0.001$).

3.2.2. Relative macromolecular composition

The absorbance IR spectra of tested feeding substrates were reported in Fig. 4. For a better comparison, IR spectra of microalgae *Schizochytrium* sp. (S) and *Isochrysis* sp. (I) were also shown. It is interesting to notice that, in all substrates enriched with increasing amounts of *Schizochytrium* sp. (As, Bs, Cs and Ds), a corresponding and well evident increase of the absorbance of the peaks already detected in the microalga was detected: ~2925 cm^{-1} and ~2855 cm^{-1} (asymmetric and symmetric stretching vibrations of CH_2 groups in lipid alkyl chains, ν_{asym} CH_2 and ν_{sym} CH_2); ~3013 cm^{-1} and ~1744 cm^{-1} (respectively stretching vibration of =CH groups in lipid alkyl chains, ν =C-H, and stretching vibration of carbonyl moiety in fatty acids, ν C=O); ~1647 cm^{-1} and ~1542 cm^{-1} (Amide I and II bands of proteins), and ~1144 cm^{-1} (stretching vibration of C-O-C bonds in carbohydrates and polysaccharides, ν C-O-C) (Fig. 2a). Conversely, except a tiny increase of the band at ~1647 cm^{-1} and ~1542 cm^{-1} (Amide I and II bands of proteins), no meaningful differences were observed by comparing the spectral profiles of the CS substrate (E) with those of substrates enriched with increasing percentages of *Isochrysis* sp. (Ai, Bi, C, and Di) (Fig. 2b).

The statistical analysis of specific band area ratios (Fig. 5a), confirmed that the inclusion of *Schizochytrium* sp. caused in all substrates (As, Bs, Cs and Ds), a statistically significant increase of the relative amount of total lipids (LIP/TBM, $P<0.001$), unsaturated lipid alkyl chains (UNSAT/TBM, $P<0.001$), fatty acids (FA/TBM, $P<0.001$) and carbohydrates (CARBO/TBM, $P<0.001$) with respect to E. Conversely, a statistically significant increase of relative amounts of proteins (PRT/TBM) was detected only in Bs, Cs and Ds substrates ($P=0.035$), while no significant differences were observed between E and As ($P=0.494$). In substrates enriched with *Isochrysis* sp., due to the absence of meaningful bands attributable to this microalga, only the band area ratios LIP/TBM and PRT/TBM were analyzed (Fig. 5b). With respect to E, a statistically significant increase of the relative amount of total lipids (LIP/TBM) was observed only in Ci e Di substrates ($P=0.025$), while no changes were detected in Ai and Bi ($P=0.852$); conversely, the relative amount of proteins (PRT/TBM) significantly increased in all the substrates enriched with *Isochrysis* sp. ($P<0.001$).

3.3 Hermetia illucens prepupae

The dry matter (DM) content of fresh prepupae reared on the different experimental substrates was 320±20 g/kg, and no statistically significant differences between groups were evidenced.

3.3.1. Fatty acid profile

The analysis of total lipids extracted through Folch method evidenced that, in general, an increase of total lipid content in the substrate corresponded to an increase in total lipid content of prepupae (Fig. 2). In fact, the lipid content of HI prepupae reared on substrates enriched with *Schizochytrium* sp. showed a statistically significant linear correlation (r=0.905, P=0.035) with lipid content of substrates. Moreover, HI prepupae showed a statistically higher lipid content (from ~140 in HI As to ~210g/kg DM in HI Ds) than that of prepupae reared on substrate E (~8 g/kg DM), and significant differences between groups were also evidenced (P<0.001), a part between HI Cs and HI Ds. About HI prepupae reared on substrates enriched with *Isochrysis* sp., a statistically higher lipid content (from ~120 in HI Ai to ~140g/kg DM in HI Di) was observed with respect to HI prepupae reared on substrate E (P=0.015). In this case, no statistically significant correlation between lipid content of prepupae and of substrates was evidenced.

Table 4 and Fig. 6 show FA composition and the amount of FA classes of HI prepupae reared on tested substrates, respectively. The FA profile of prepupae reared on CS substrate E was characterized by high quantities of saturated fatty acids (i.e. 74±2 g/100 g FAs, Fig. 6), such as 12:0, 16:0, 18:0, 20:0, and 22:0, followed by 18:1n9, 18:2n6 and 16:1n7. This profile reflected the FA composition typical for CS, with a higher prevalence of SFA (Table 2).

The inclusion of *Schizochytrium* sp. in substrates induced, in prepupae, a statistically significant increase in the amount of 22:6n3 (DHA) and 20:5n3 (EPA), and a statistically significant general decrease in saturated fatty acids with respect to prepupae HI E. It should be noted that lauric acid (12:0) increased in prepupae HI Cs and HI Ds if compared with prepupae Hi E. Moreover, HI prepupae Bs, HI Cs, and HI Ds showed a similar FA composition, especially in relation to unsaturated FAs, and they showed amounts of 22:6n3 and 20:5n3 statistically higher than prepupae HI As. The inclusion of *Schizochytrium* sp. allowed to obtain a DHA/EPA ratio of 1.3-1.6. This behavior modified the quantities of FA classes (Fig. 6a): HI Bs, HI Cs, HI Ds showed a significantly lower SFA amount (P<0.001), and significantly higher amounts of PUFA (P<0.001), n-3 (P<0.001), n-6 (P<0.001), n-9 (P<0.001), and n-3/n-6 ratio (P<0.001), than prepupae HI As and HI E. No significant differences were evidenced in FA classes of prepupae reared on substrates including 10%, 20% and 25% of microalgae. The inclusion of *Isochrysis* sp. in substrates caused the following changes in FA profile of HI prepupae with respect to HI E (Table 4): (i) a statistically significant marked increase (2 fold-higher) of lauric acid (12:0); ii) a statistically significant increase of 14:0, 18:0, and 18:1n9; (iii) a statistically significant decrease of 20:0 and 22:0; (iv) a statistically significant increase of EPA and DHA only for prepupae HI Ci and HI Di. Concerning FA classes (Fig. 6b), prepupae HI Ci and Di showed a statistically lower amount of SFA (P<0.001), and a statistically higher quantities of MUFA (P=0.0005), PUFA (P<0.001), n-3 (P<0.001), n-9 (P<0.001), than prepupae HI Ai and Bi and those reared on CS substrate (HI E). The n-3/n-6 ratio significantly increased with the increasing inclusion percentage of *Isochrysis* sp. in the substrate (P<0.001).

HI Bs, HI Cs and HI Ds prepupae showed a statistically lower amount of SFA (~45 g/100 g FAs) with respect to HI prepupae reared on substrates enriched with *Isochrysis* sp. (more than 60 g/100 g FAs) (P<0.001), and significantly higher quantities of PUFA (~37 g/100 g FAs (P<0.001)), and n-3 (~28 g/100 g FAs) (P<0.001), than prepupae reared on substrates enriched with *Isochrysis* sp. (PUFA < 20 g/100 g FAs, n-3 < 10 g/100 g FAs). Consequently, the n-3/n-6 ratio is significantly higher in prepupae reared on *Schizochytrium* sp. than in those reared on *Isochrysis* sp (P<0.001). Moreover, comparing prepupae reared on substrates enriched with the same inclusion level of *Schizochytrium* sp. or *Isochrysis* sp., the amounts of EPA and DHA were significantly higher (about 10-folds) in prepupae reared on *Schizochytrium* sp. with respect to prepupae reared on *Isochrisis* sp. (P<0.001 for both EPA and DHA).

3.3.2. Relative macromolecular composition

For the first time, HI prepupae were analyzed by FTIR spectroscopy. In all IR spectra (Fig. 7), the bands attributable to lipids (~2922 cm^{-1} and ~2850 cm^{-1}), proteins (~1648 cm^{-1} and ~1540 cm^{-1}), carbohydrates and polysaccharides (~1040 cm^{-1}) were detected. In addition, the absorbance IR spectra of HI reared on substrates enriched with *Schizochytrium* sp., showed both the increase of the bands at ~1575 cm^{-1} (stretching vibration of carboxylate groups, ν COO^{-}) (Aryee et al., 2009) and ~1540 cm^{-1} (Amide II band of proteins), and the occurrence of additional bands at ~3013 cm^{-1} (attributable to unsaturated fatty acids) and ~1742 cm^{-1} (associated to fatty acids) (Fig. 7a). Less marked differences were observed by comparing the IR spectra of HI prepupae reared on substrates enriched with *Isochrysis* sp. with CS substrate E. In this latter case, only the increase of the bands at ~1575 cm^{-1} and ~1540 cm^{-1} was observed (Fig. 7b). In addition, in all HI prepupae fed on substrates enriched with microalgae, no significant differences were observed in the spectral range 1200-900 cm^{-1}, related to carbohydrates vibrational modes.

Specific band area ratios were analyzed (Fig. 8) suggesting that HI reared on substrates enriched with increasing amounts of *Schizochytrium* sp. (HI As, HI Bs, HI Cs and HI Ds) showed a corresponding statistically significant increase of total lipids (LIP/TBM, $P<0.001$) (as pinpointed by lipid extraction with Folch method), fatty acids (FA/TBM, $P<0.001$), and unsaturated lipid alkyl chains (UNSAT/TBM, $P<0.001$). Moreover, a statistically significant increase of proteins (PRT/TBM) was also observed in HI Bs, HI Cs and HI Ds ($P<0.001$), while no changes were detected in HI As ($P=0.527$) (Fig. 8a). Considering prepupae reared on substrates enriched with *Isochysis* sp., a statistically significant increase of total lipids (LIP/TBM) was detected only in insects reared on substrates enriched with higher inclusions of microalga (HI Ci and HI Di) ($P<0.001$), confirming in general the results obtained with Folch method. Statistically significant higher amounts of proteins (PRT/TBM, $P<0.001$) were observed in all insect groups with respect to HI E (Fig. 8b). Due to the absence of the band at 1144 cm^{-1} in all the analyzed insect samples (reared on substrates enriched with microalgae), it was not possible to evaluate the band area ratio CARBO/TBM.

3.4. Principal Component Analysis

To better understand the relationships between type and percentage of microalgae included in the substrates and relative amount of lipids and proteins and FAs composition of prepupae, a multivariate analysis (Principal Component Analysis, PCA) on HI prepupae data was performed to reduce the dimensionality of the data set to few components that summarize the information contained in the overall data set. The amount of total lipids in g/kg DM (TL), FAs greater than 1 g/100 g FAs, and band area ratios LIP/TBM and PRT/TBM, were included in the data matrix (band area ratio UNSAT/TBM and FA/TBM were not included because of lacking data for prepupae reared on substrates enriched with *Isochrysis* sp.). By applying PCA to the data set (9 observations, 18 variables), it was possible to extract three significant, cross-validated principal components (PC), that accounted for ~92% of the variability in the original data (Table 5). On examining the loading matrix (Table 5) and the graphical distribution of analyzed groups on the reported biplot (showing *loadings* and *scores* plots simultaneously) of PC1 *vs* PC2 (Fig. 9), specimens were divided based on their FAs composition, lipid content and protein relative amount. PC1, that explained ~47% of the variance, was associated to the prevalence of saturated or polyunsaturated fatty acids: prepupae HI E, and HI As, HI Ai and HI Bi (positive scores) were characterized by higher content of SFA such as 16:0, 18:0, 20:0, and 22:0 (positive loadings on PC1), than other groups, whereas prepupae reared on substrates enriched with 10%, 20% and 25% of *Schizochytrium* sp. (HI Bs, HI Cs, and HI Ds, respectively), were characterized by a higher amount of lipids (LIP/TBM and TL negative loadings on PC1), and by a FA composition with higher content of PUFA, in particular omega-3 (20:5n3, 22:6n3), and omega-6 (20:4n6, 18:3n6) (negative loadings on PC1), than other groups. PC2 (~34% of explained variance) was dominated by the type of microalga added to the substrate: HI prepupae reared on substrates enriched with *Isochrysis* sp. showed positive scores, whereas HI prepupae reared on substrates enriched with *Schizochytrium* sp. showed negative scores on PC2. Prepupae reared on *Isochrysis* sp., showed higher protein relative amount, and higher content of precursors of n-3 and n-6 FAs (18:3n3 and 18:2n6, respectively), and of short-chain fatty acids (12:0 and 14:0) than prepupae reared on *Schizochytrium* sp. (negative scores), which showed instead higher amounts of n-3 and n-6 FAs, and of SFA from 16 to 22 carbons (medium- and long-chain fatty acids). PC3 (Table 5) was mainly dominated by the contrast between 18:0 and its metabolite 18:1n9; HI Ci and HI Di (positive scores on PC3) showed higher content of 18:1n9, 18:2n6 and 18:3n3, than HI Ai and HI Bi prepupae (negative scores on PC3). In any case, the variance explained by PC3 was only ~10%, then it did not provide further information about FA composition differences between studied groups.

3.5. Health benefits indices

Table 6 shows Atherogenic (AI), thrombogenic (TI), and Hypo/Hyper-cholesterolemic (HH) indices of HI prepupae. Low values of AI (≤ 0.51) and TI (≤ 0.30) are beneficial to health (Ulbricht and Southgate, 1991). AI values of all groups of *H. illucens* are higher than the suggested value, but HI reared on substrates enriched with *Schizochytrium* sp. showed statistically lower values than prepupae reared on substrates enriched with *Isochrysis* sp. ($P<0.05$). HI reared on substrates including from 10% to 25% of *Schizochytrium* sp. showed a TI value ≤ 0.30, whereas the TI value of prepupae reared on substrate enriched with *Isochrysis* sp. was far above this limit. Finally, the HH index (high values correspond to hypocholesterolemic effects (Santos-Silva et al., 2002) was significantly higher, from ~2.6 to ~3.3, in HI prepupae reared on substrate enriched with 10%, 20% or 25% of *Schizochytrium* sp. than other groups. The recommended PUFAs/SFAs ratio is > 0.45, i.e. the minimum recommended value to avoid the potential to raise blood cholesterol level (Department of Health and Social Security (DHSS), 1984). Only prepupae of HI reared on substrates including 10%, 20% and 25% of *Schizochytrium* sp. showed a PUFA/SFA ratio > 0.45.

4. Discussion

In agreement with the concept of circular economy, in the present study, rearing substrates for HI larvae were based on the re-use of the organic by-product coffee silverskin while *Schizochytrium* sp. and *Isochrysis* sp. were tested as PUFA-rich ingredients in order to improve the nutritional quality of the final produced insect biomass. Microalgae can be considered environmental-friendly ingredients for the improvement of insect feeding substrates (Vidyashankar et al., 2015). The fatty acid profile and the relative macromolecular composition of ingredients, substrates and prepupae were investigated by conventional GC-MS and innovative FTIR techniques.

The analysis of coffee silverskin confirmed lower amounts of proteins and lipids with respect to microalgae (Vargas et al., 2018); FAs were mainly represented by SFA, and, to a lesser extent, by PUFA and MUFA (data consistent with those reported by Costa et al (2018)). Conversely, the infrared analysis of the microalgae *Schizochytrium* sp. suggested higher relative amounts of proteins, carbohydrates, and unsaturated lipids with respect to those detected in coffee silverskin. In addition, the FA profile of this microalga was rich in PUFA, mainly in docosahexaenoic acid DHA, and 16:0, as already reported in literature, even if with different relative quantities (Zhu et al., 2007; Wang and Wang, 2012). Higher relative amounts of proteins and lipids were also detected in *Isochrysis* sp. with respect to those detected in coffee silverskin, and its FA profile was rich in PUFA. The n-3/n-6 ratio (~5) of *Isocrysis* sp. was about 9-fold lower than that of *Schizochytrium* sp. (~47), suggesting that this latter microalga contains a major amount of PUFA with respect to both *Isochrysis* sp. and CS. These results, in agreement with data reported by Poisson and Ergan (2001), but quite different from those found by Aussant et al. (2018) for *Isochrysis galbana*, and by Vidyashankar et al. (2015) for *Isochrysis* sp., suggested a different FA composition of the two microalgae. Moreover, it is known that the FA composition of microalgae is influenced by both the nutritional composition of culture media and the growing environmental conditions as well as by the specific species and strains (Robertson et al., 2013).

The study of the FA composition of substrates showed that CS substrate E was poor in unsaturated FA, and particularly in the omega-3 DHA and EPA. *Schizochytrium* sp. inclusion lead to a statistically significant increase of lipids and unsaturated fatty acids (such as n-3 and n-6), and to a detriment of saturated ones. A similar trend was also pinpointed for substrates enriched with *Isochrysis* sp., even if the content of unsaturated fatty acids was lower than substrates enriched with *Schizochytrium* sp. The infrared analysis of the substrates pinpointed that the microalgae inclusion determined an increase in the relative amount of proteins and lipids with respect to the CS substrate E, thus improving the nutritional value of the substrate at all levels. In coffee silverskin enriched with increasing percentages of *Schizochytrium* sp., a well evident increase of the bands related to proteins, carbohydrates, lipids and, mainly, to unsaturated fatty acids was observed. Conversely, substrates enriched with increasing percentages of *Isochrysis* sp. showed only a higher relative amount of proteins, and, to a lesser extent, of lipids, this latter statistically significant only in substrates enriched with 20% and 25% of microalga.

The dry matter (DM) content of fresh prepupae reared on the different experimental substrates was 320±20 g/kg, in accordance with data obtained in HI prepupae reared on various organic substrates (Barragan-Fonseca et al., 2017; Caligiani et al., 2018), and no statistically significant differences between groups were evidenced. The inclusion of microalgae in the substrate influenced the lipid content of HI prepupae; this result agreed with previous studies that demonstrated a strong influence of the lipid composition of the diet on lipid content in insects (Tomberlin et al., 2002; Nguyen et al., 2013; Barroso et al. 2017; Spranghers et al., 2017). The lipid content of HI prepupae reared on CS-based substrates enriched with microalgae *Schizochytrium* sp. or *Isochrysis* sp. was in general consistent with the lipid content (from 70 to 390 g/kg DM) found in HI larvae reared on animal waste, such as chicken manure, swine manure, or liver (Barragan-Fonseca et al., 2017), but the nutritional quality of prepupae analyzed in this study was higher. In fact, whereas HI prepupae reared on CS substrate E showed a FA profile consistent with that of HI larvae reared on animal waste (Barragan-Fonseca et al., 2017), HI prepupae reared on CS enriched with microalgae showed a FA profile with a high content of omega-3 fatty acids, such as DHA and EPA, which are in general absent in the FA profile of HI larvae reared on animal or vegetables waste (St-Hilaire et al., 2007; Ushakova et al., 2016; Barragan-Fonseca et al., 2017; Caligiani et al., 2018).

HI prepupae reared on feeding substrates containing microalgae showed high percentages of lauric acid 12:0. This fatty acid is synthetized by HI larvae when there are sufficient amounts of carbohydrates in their substrates (Spranghers et al., 2017). Fatty acids from the substrate are also being transformed into 12:0 by the larvae. Lauric acid has been shown to demonstrate an intestinal anti-inflammatory role in fish, promoting gut’s welfare by mitigating inflammatory conditions such as inflammation caused by insect-chitin (Aleström et al., 2006; Dahm and Geisler, 2006; De-Santis and Jerry, 2007; Zarantoniello et al., 2019). The levels of 12:0 in the larvae reared on *Isochrysis* sp. enriched substrates are in compliance with literature, but their fat content is still low (120-130 g/kg DM). The fact that HI E larvae do not contain much 12:0 means that CS solely is not a good growth substrate. This is also reflected in the very low-fat content of these larvae (only 10%). *Schyzochytrium* sp. looks to be a very good enrichment substrate given that not only the percentage of n-3 in the larvae was increased, but also the total lipid content and thus 12:0.

Moreover, prepupae reared on substrates enriched with *Schizochytrium* sp. showed a higher relative content of proteins, lipids and unsaturated fatty acids, with respect to the total biomass, than insect reared on CS substrate E. In the case of HI reared on substrates enriched with *Isocrhysis* sp., the inclusion of this microalga caused a consistent increment of the relative amount of proteins compared to HI reared on CS substrate E, while only a tiny increase of lipids was observed.

PCA analysis highlighted that the type of microalga included in the substrate strongly influenced the FA composition and hence the nutritional composition of HI prepupae. In particular, prepupae reared on *Schizochytrium* sp. enriched substrates, showed a better FA profile, with significantly lower amounts of saturated fatty acids and significantly higher quantities of unsaturated ones and of n-3/n-6 ratio than prepupae reared on *Isocrhysis* sp. enriched substrates. Moreover, from PCA analysis no relevant differences were observed in the overall nutritional quality of prepupae reared on substrates enriched with 10%, 20% or 25% of *Schizochytrium* sp. (HI Bs, HI Cs, and HI Ds, respectively). Noteworthy, the inclusion of *Schizochytrium* sp. over 10% did not bring a significant improvement in the FA profile in terms of saturated and unsaturated fatty acids, particularly omega-3.

Health benefits indices recorded for HI prepupae were in general consistent with those of different species of microalgae (Aussant et al., 2018) and demonstrated that a regular inclusion of HI prepupae as feed/food ingredient reared on substrate enriched with 10%, 20% or 25% of *Schizochytrium* sp. could be beneficial to health and produce hypocholesterolemic effects (Santos-Silva et al., 2002). On the light of the overall results and considering that the heterotrophic production of this microalga is much cheaper than the autotrophic *Isochrisis* sp. production (Ren et al., 2009; Perez-Garcia et al., 2011; Vidyashankar et al., 2015), *Schizochytrium* sp. seems to be the best microalga to be added to the CS substrate.

5. Conclusions

This work demonstrated an easy and efficient way to produce high nutritional quality *Hermetia illucens* prepupae through the revalorization of organic industrial waste (coffee silverskin), and its polyunsaturated fatty acids-enrichement with environmentally friendly microalgae, promoting the circular economy concept.

Schyzochytrium sp. looks to be a very good source of polyunsaturated fatty acids, given that not only the percentage of n-3 in the larvae was increased, but also the total lipid content. Moreover, the inclusion of *Schizochytrium* sp. supported a *Hermetia illucens* prepupae production characterized by higher nutritional values than those reared on *Isochrysis* sp. diets. No differences in the fatty acid profile and nutritional indices were evidenced among *Hermetia illucens* prepupae reared on substrates enriched with 10%, 20% or 25% of *Schizochytrium* sp. Therefore, the substrate enriched with a 10% inclusion level of *Schizochytrium* sp. should be considered the most convenient one since a greater inclusion of microalgae did not promote additional benefits in terms of nutritional value of *Hermetia illucens* prepupae. Finally, another advantage related to the use of *Schyzichytrium* sp. is that the heterotrophic production of this microalga is much cheaper than the autotrophic *Isochrisis* sp. production.

Thanks to fat quality, these *Hermetia illucens* prepupae enriched with polyunsaturated fatty acids deserve a special attention both as feed ingredient in the present, as well as food ingredient in the future.

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Figure Captions

Fig. 1 InfraRed (IR) absorbance spectra of coffee silverskin (CS), *Isocrysis* sp. (I) and *Schizochytrium* sp. (S) in the 4000-800 cm^{-1} spectral range. Spectra are shifted along y-axis for better reading.

Fig. 2 Total lipid content (g/kg DM, dry matter) of coffee silverskin substrate (E) and substrates and *Hermetia illucens* (HI) prepupae reared on corresponding substrates enriched with: (a) 5% (As), 10% (Bs), 20% (Cs) and 25% (Ds) of *Schizochytrium* sp.; (b) 5% (Ai), 10% (Bi), 20% (Ci) and 25% (Di) of *Isochrysis* sp. White bar, substrate; grey bar, Insect. Values are presented as mean ± SD (mean represents 5 replicates). Different letters indicate statistically significant differences among experimental groups compared within the same matrix ($P<0.05$).

Fig. 3 Comparison of fatty acid (FA) classes (g/100 g FAs) between substrates enriched with 5% (A), 10% (B), 20% (C), 25% (D) of *Schyzochytrium* sp. or *Isochrysis* sp. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-3, omega-3 polyunsaturated fatty acids; n-6, omega-6 polyunsaturated fatty acids, n-9, omega-9 polyunsaturated fatty acids; n-3/n-6, omega-3/omega-6 ratio. Different letters indicate statistically significant differences among rearing substrates containing the same microalga ($P<0.05$). Values are presented as mean ± SD (mean represents 5 replicates). The coffee silverskin substrate (E) was not reported in the figure, because the FA profile is the same of the ingredient silverskin, with the only difference between them being the content of water.

Fig. 4 InfraRed (IR) absorbance spectra of coffee silverskin substrate (E) and of substrates enriched with: (a) 5% (As), 10% (Bs), 20% (Cs) and 25% (Ds) of *Schizochytrium* sp.; (b) 5% (Ai), 10% (Bi), 20% (Ci) and 25% (Di) of *Isochrysis* sp. For a better comparison, the spectra of *Schizochytrium* sp. (S) and *Isochrysis* sp. (I) are also reported. Spectra are showed in absorbance mode in the 4000-800 cm^{-1} spectral range and shifted along y-axis.

Fig 5 Statistical analysis of band area ratios of substrates enriched with *Schizochytrium* sp. (a) and *Isochrysis* sp. (b): LIP/TBM (lipids/total sample biomass, representative of total lipids), UNSAT/TBM (unsaturated lipids/total sample biomass, representative of unsaturated lipid alkyl chains), FA/TBM (fatty acids/total sample biomass, representative of total fatty acids), PRT/TBM (proteins/total sample biomass, representative of total proteins), and CARBO/TBM (carbohydrates/total sample biomass, representative of total carbohydrates). Coffe silverskin substrate (E); substrates enriched with 5% (As), 10% (Bs), 20% (Cs) and 25% (Ds) of *Schizochytrium* sp. (S); substrates enriched with 5% (Ai), 10% (Bi), 20% (Ci) and 25% (Di) of *Isochrysis* sp. (I); microalgae *Schizochytrium* sp. (S) and *Isochrysis* sp. (I). Values are presented as mean±SD (mean represents 5 replicates). Different letters denote significant differences among experimental groups ($P<0.05$).

Fig. 6 Fatty acid (FA) classes (g/100 g FAs) of *Hermetia illucens* (HI) prepupae reared on coffe silverskin substrate (HI E) and on substrates enriched with: (a) 5% (HI As), 10% (HI Bs), 20% (HI Cs) and 25% (HI Ds) of *Schizochytrium* sp.; (b) 5% (HI Ai), 10% (HI Bi), 20% (HI Ci) and 25% (HI Di) of *Isochrysis* sp.. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-3, omega-3 polyunsaturated fatty acids; n-6, omega-6 polyunsaturated fatty acids, n-9, omega-9 polyunsaturated fatty acids; n-3/n-6, omega-3/omega-6 ratio. Values are presented as mean ± SD (mean represents 5 replicates).

Fig. 7 InfraRed (IR) absorbance spectra of *Hermetia illucens* (HI) prepupae reared on coffe silverskin substrate (HI E), and on substrates enriched with: (a) 5% (HI As), 10% (HI Bs), 20% (HI Cs) and 25% (HI Ds) of *Schizochytrium* sp.; (b) 5% (HI Ai), 10% (HI Bi), 20% (HI Ci) and 25% (HI Di) of *Isochrysis* sp. Spectra are showed in absorbance mode in the 4000-800 cm^{-1} spectral range and shifted along y-axis.

Fig. 8 Statistical analysis of band area ratios of HI prepupae reared on CS substrate (HI E) and on substrates enriched with: (a) 5% (HI As), 10% (HI Bs), 20% (HI Cs) and 25% (HI Ds) of *Schizochytrium* sp.; (b) 5% (HI Ai), 10% (HI Bi), 20% (HI Ci) and 25% (HI Di) of *Isochrysis* sp. LIP/TBM (lipids/total sample biomass, representative of total lipids), FA/TBM (fatty acids/total sample biomass, representative of total fatty acids), UNSAT/TBM (unsaturated lipids/total sample biomass, representative of unsaturated lipid alkyl chains) and PRT/TBM (proteins/total sample biomass, representative of total proteins). Values are presented as mean±SD (mean represents 5 replicates). Different letters denote significant differences among experimental groups ($P<0.05$).

Fig. 9 Principal Component Analysis: 2D Biplot of PC1 (first Principal Component) versus PC2 (second Principal Component). HI E: prepupae reared on substrate E (100% coffee silverskin, CS); HI As, HI Bs, HI Cs, HI Ds: prepupae reared on substrate CS enriched with 5%, 10%, 20%, and 25% of *Schizochytrium* sp., respectively; HI Ai, HI Bi, HI Ci, HI Di: HI prepupae reared on substrates CS enriched with 5%, 10%, 20%, and 25% of *Isochrysis* sp., respectively. TL: total lipids, g/kg DM (dry matter); LIP/TBM: amount of lipids relative to total sample biomass; PRT/TBM: amount of proteins relative to total sample biomass.

Table 1 Percentage of ingredients in tested feeding substrates, and relative water content.

Substrate	CS (%)	*Schizochytrium* sp. (%)	*Isochrysis* sp. (%)	Moisture, g/kg (n=3)
As	95	5	-	695±3
Bs	90	10	-	693±1
Cs	80	20	-	692±2
Ds	75	25	-	693±4
E	100	0	0	686±5
Ai	95	-	5	688±3
Bi	90	-	10	700±3
Ci	80	-	20	683±2
Di	75	-	25	694±6

Substrates As, Bs, Cs and Ds: coffe silverskin (CS) enriched with 5%, 10%, 20% and 25% of *Schyzochytrium* sp., respectively; substrate E: 100% CS; substrates Ai, Bi, Ci and Di: CS enriched with 5%, 10%, 20% and 25% *Isochrysis sp*., respectively.

Table 2 Fatty acid (FA) profile (as g/100 g FAs) of the ingredients coffee silverskin (CS), *Schizochytrium* sp. and *Isochrysis* sp.

Fatty acids	CS (Saccaria)	*Schizochytrium* sp.	*Isochrysis* sp.
10:0	nd	0.05±0.004	nd
12:0	0.20±0.06	0.13±0.01	0.06±0.01
14:0	2.4±0.1	0.9±0.01	16.7±0.3
15:0	0.61±0.04	0.12±0.01	0.31±0.02
16:0	22.1±0.6	12.9±0.7	9.2±0.4
16:1n9	0.42±0.02	0.59±0.01	4.6±0.2
16:2n7	0.09±0.04	nd	2.0±0.1
17:0	0.38±0.03	0.12±0.01	nd
18:0	15.5±0.1	1.9±0.3	1.0±0.1
18:1n9	7.2±0.5	0.2±0.01	11.5±0.2
18:1n7	0.85±0.03	0.61±0.01	1.3±0.1
18:2n6	26.2±0.9	0.4±0.1	7.5±0.1
18:3n6	nd	0.21±0.01	1.2±0.1
18:3n3	2.9±0.1	0.5±0.1	12.8±0.3
20:0	11.4±1.1	0.3±0.1	nd
20:1n9	0.54±0.02	nd	nd
20:4n6	nd	1.1±0.1	nd
20:5n3	nd	1.2±0.1	nd
22:0	8.7±0.5	nd	nd
23:0	0.38±0.03	nd	nd
24:0	0.17±0.09	nd	nd
22:6n3	nd	78.8±0.5	31.7±0.7
Total SFAs	61.8±1.1	16.3±0.7	27.3±0.5
Total MUFAs	9.0±0.3	1.4±0.1	17.5±0.2
Total PUFAs	29.2±0.4	82.2±0.9	55.2±0.8
n-3	2.9±0.3	80.5±0.9	44.6±0.8
n-6	26.2±0.3	1.7±0.0	8.7±0.1
n-9	8.14±0.3	0.8±0.0	16.1±0.2
n-3/n-6	0.11±0.01	46.8±1.4	5.1±0.1

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids, PUFAs, polyunsaturated fatty acids, n-3, omega-3 polyunsaturated fatty acids, n-6 omega-6 polyunsaturated fatty acids, n-9 omega-9 polyunsaturated fatty acids, n-3/n-6, omega-3/omega-6 ratio. Data represent mean ± standard deviation (n. aliquots per sample = 3, replicates for each aliquot = 3).

nd, not detected

Table 3 Fatty acid (FA) profile (as g/100 g FAs) of control substrate (E), and substrates enriched with *Schyzochytrium* sp. or *Isochrysis* sp.

FA	E	As (5%)	Bs (10%)	Cs (20%)	Ds (25%)	P-value	Ai (5%)	Bi (10%)	Ci (20%)	Di (25%)	P-value
10:0	nd	0.03±0.002	0.04±0.007	0.04±0.001	0.04±0.001		nd	nd	nd	nd	
12:0	0.15±0.01	0.15±0.06	0.16±0.03	0.13±0.01	0.12±0.002		0.11±0.01	0.13±0.01	0.10±0.01	0.11±0.01	
14:0	2.5±0.2	1.2±0.1^{a}	1.1±0.1^{a}	1.0±0.01^{a}	1.0±0.01^{a}	0.054	2.6±0.1^{a}	3.3±0.1^{b}	4.4±0.1^{c}	4.4±0.2^{c}	0.001
15:0	0.63±0.05	0.23±0.01	0.21±0.03	0.19±0.01	0.18±0.01		0.61±0.01	0.57±0.01	0.60±0.01	0.56±0.01	
16:0	22.7±0.4	16.4±1.4^{b}	15.4±1.1^{a,b}	13.1±0.6^{a}	14.3±0.9^{a}	<0.001	22.5±0.3^{c}	21.5±0.1^{b,c}	21.3±0.3^{b}	19.3±0.4^{a}	0.0023
16:1n9	0.43±0.04	0.47±0.02	0.53±0.06	0.55±0.01	0.55±0.01		2.4±0.3^{a}	2.7±0.3^{a}	3.8±0.1^{b}	5.1±0.1^{c}	0.0005
16:2n7	0.11±0.01	0.02±0.01	0.03±0.01	0.03±0.01	0.03±0.01		2.3±0.1^{b}	1.7±0.1^{a}	2.9±0.1^{c}	5.0±0.1^{d}	<0.001
17:0	0.38±0.02	0.17±0.01	0.16±0.01	0.15±0.01	0.14±0.01		0.38±0.01	0.36±0.01	0.36±0.01	0.33±0.01	
18:0	16.9±0.5	4.5±0.4^{d}	3.5±0.1^{c}	3.2±0.3^{b}	2.7±0.1^{a}	<0.001	15.8±0.1^{d}	14.1±0.1^{c}	9.4±0.1^{b}	6.6±0.1^{a}	<0.001
18:1n9	6.3±0.4	1.7±0.2	1.1±0.1	0.9±0.1	0.7±0.05		5.8±0.1^{a}	6.0±0.1^{a}	7.3±0.2^{b}	7.5±0.1^{b}	0.0005
18:1n7	0.83±0.02	0.56±0.01	0.58±0.04	0.60±0.01	0.57±0.004		1.4±0.1	1.3±0.01	1.6±0.01	1.9±0.03	
18:2n6	24.0±1.4	6.1±0.3^{d}	4.2±0.3^{c}	2.8±0.2^{b}	2.5±0.2^{a}	<0.001	15.8±0.1^{b}	15.4±0.2^{b}	15.7±0.1^{b}	13.8±0.1^{a}	0.0002
18:3n6	nd	0.14±0.01	0.17±0.02	0.17±0.01	0.17±0.01		0.15±0.01	0.18±0.01	0.24±0.01	0.30±0.02	
18:3n3	2.7±0.1	1.0±0.1	0.9±0.1	0.8±0.1	0.7±0.02		8.7±0.1^{b}	7.3±0.1^{a}	11.5±0.1^{c}	16.8±0.1^{d}	<0.001
20:0	11.9±0.6	3.0±0.2^{c}	2.1±0.1^{b}	1.9±0.1^{a}	1.7±0.1^{a}	<0.001	9.9±0.2^{c}	10.3±0.2^{c}	7.8±0.3^{b}	5.9±0.2^{a}	<0.001
20:1n9	0.48±0.02	0.13±0.01	0.10±0.01	0.07±0.01	0.06±0.01		0.35±0.04	0.35±0.01	0.37±0.06	0.29±0.01	
20:4n6	nd	0.9±0.1	0.9±0.1	1.0±0.1	1.0±0.02		nd	nd	nd	nd	
20:5n3	nd	0.8±0.1	0.9±0.1	1.0±0.1	1.0±0.03		nd	nd	nd	nd	
22:0	9.4±1.2	2.0±0.3^{b}	1.3±0.2^{a}	1.2±0.1^{a}	1.3±0.07^{a}	<0.001	9.6±0.2^{c}	10.6±0.2^{d}	7.3±0.2^{b}	6.2±0.1^{a}	<0.001
23:0	0.35±0.04	0.09±0.01	0.06±0.01	0.05±0.01	0.05±0.01		0.29±0.02	0.38±0.03	0.24±0.04	0.22±0.01	
24:0	0.21±0.04	nd	nd	nd	nd		nd	nd	nd	nd	
22:6n3	nd	60.6±1.9^{a}	66.4±1.5^{b}	71.1±0.4^{c}	71.3±1.4^{c}	<0.001	1.5±0.1^{a}	3.9±0.3^{b}	5.1±0.1^{c}	5.7±0.1^{d}	<0.001

Substrate E: 100% coffe silverskin (CS); substrates As, Bs, Cs and Ds: CS enriched with 5%, 10%, 20% and 25% of *Schyzochytrium* sp., respectively; substrates Ai, Bi, Ci and Di: CS enriched with 5%, 10%, 20% and 25% *Isochrysis sp*., respectively.

Data represent mean ± standard deviation (replicates for each group = 5; n. aliquots per replicate = 3).

Means within rows of rearing substrates containing the same microalga bearing different letters are significantly different (P<0.05). FAs <1g/100 g FAs were excluded from any statistical analyses because their concentrations were close to the limit of detection.

Table 4 Fatty acid (FA) profile (as g/100 g FAs) of *Hermetia illucens* (HI) prepupae reared on tested feeding substrates.

FA	HI E	HI As (5%)	HI Bs (10%)	HI Cs (20%)	HI Ds (25%)	HI Ai (5%)	HI Bi (10%)	HI Ci (20%)	HI Di (25%)	P-value
10:0	0.25±0.04	0.38±0.08	0.52±0.14	0.85±0.02	0.55±0.03	0.87±0.01	1.03±0.01	0.94±0.02	0.85±0.02	
12:0	14.1±1.5^{c}	9.4±0.4^{b}	8.0±0.2^{a}	19.5±0.3^{d}	19.9±1.9^{d}	28.3±0.3^{e}	32.5±1.5^{f}	30.2±1.4^{f}	28.2±0.6^{e}	<0.001
14:0	3.2±0.7^{b,c}	2.7±0.1^{b}	2.0±0.2^{a}	5.9±0.9^{d}	4.0±0.4^{c}	5.7±0.1^{d}	6.7±0.1^{e}	6.8±0.2^{e}	6.9±0.2^{e}	<0.001
15:0	0.46±0.02	0.32±0.02	0.22±0.05	0.16±0.02	0.12±0.01	0.24±0.01	0.27±0.01	0.25±0.01	0.20±0.01	
16:0	18.1±2.0^{e}	16.6±0.6^{d,e}	15.9±1.2^{d}	12.1±0.6^{b}	10.8±1.0^{a}	14.4±0.5^{c}	14.3±0.6^{c}	12.7±0.5^{b}	12.0±0.6^{b}	<0.001
16:1n7	4.7±0.5^{b,c}	4.1±0.4^{a,b}	5.2±0.1^{c}	4.3±0.5^{a,b}	5.0±0.3^{c}	3.8±0.1^{a}	4.6±0.1^{b}	3.9±0.2^{a}	3.6±0.2^{a}	0.0051
16:2n7	nd	nd	nd	nd	nd	nd	nd	nd	nd	
17:0	0.51±0.01	0.42±0.06	0.35±0.09	0.21±0.02	0.16±0.02	0.27±0.01	0.20±0.01	0.20±0.01	0.24±0.02	
18:0	10.8±0.7^{d}	17.1±0.5^{f}	12.6±0.5^{e}	4.7±0.6^{a}	5.8±0.6^{b}	21.4±0.9^{g}	12.9±0.7^{e}	11.0±0.6^{d}	9.0±0.6^{c}	<0.001
18:1n9	9.0±0.9 ^{b,c}	8.2±0.3^{b}	11.3±0.3^{d}	11.7±0.8^{d,e}	12.9±0.7^{e,f}	7.4±0.2^{a}	10.2±0.7^{c}	12.4±0.7^{e}	13.9±0.8^{f}	<0.001
18:1n7	2.5±0.3^{f}	1.5±0.1^{d,e}	1.4±0.1^{d}	0.9±0.2^{b}	0.5±0.1^{a}	1.6±0.1^{e}	1.2±0.1^{c}	1.2±0.1^{c}	1.1±0.1^{b,c}	<0.001
18:2n6	6.2±0.6^{c,d}	4.6±0.2^{b}	4.9±0.6^{b}	3.7±0.2^{a}	3.9±0.4^{a}	4.0±0.3^{a}	4.8±0.3^{b}	5.9±0.4^{c}	6.8±0.5^{d}	<0.001
18:3n6	0.4±0.1	0.9±0.1	1.6±0.2	1.6±0.4	1.9±0.6	0.1±0.1	0.1±0.1	0.2±0.1	0.2±0.1	
18:3n3	1.0±0.2^{a}	0.9±0.1^{a}	1.1±0.2^{a}	1.0±0.1^{a}	1.1±0.2^{a}	1.2±0.3^{a}	2.3±0.3^{b}	3.8±0.4^{c}	5.3±0.4^{d}	<0.001
20:0	11.0±0.3^{e}	6.9±0.7^{d}	2.7±0.7^{b}	1.2±0.1^{a}	1.3±0.2^{a}	4.2±0.5^{c}	2.9±0.4^{b}	2.6±0.5^{b}	2.4±0.4^{b}	<0.001
20:1n9	0.02±0.01	0.15±0.05	0.12±0.05	0.01±0.01	0.05±0.04	0.04±0.01	0.06±0.01	0.04±0.01	0.07±0.01	
20:4n6	0.1±0.3^{a}	2.2±0.4^{d}	3.2±0.4^{e}	3.6±0.3^{e}	3.9±0.7^{e}	0.4±0.3^{a,b}	0.7±0.2^{b}	1.4±0.1^{c}	2.0±0.1^{d}	<0.001
20:5n3	0.8±0.2^{a}	6.5±0.3^{e}	10.6±0.2^{f}	10.6±0.2^{f}	11.7±0.1^{g}	0.6±0.1^{a}	1.2±0.1^{b}	2.2±0.1^{c}	3.0±0.1^{d}	<0.001
22:0	16.0±0.4^{e}	8.9±0.7^{d}	2.8±0.3^{b}	1.3±0.2^{a}	1.1±0.3^{a}	5.0±0.4^{c}	3.3±0.3^{b}	3.0±0.2^{b}	2.8±0.3^{b}	<0.001
23:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	
24:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	
22:6n3	0.7±0.2^{a}	8.3±0.6^{c}	15.6±0.8^{d,e}	16.7±0.3^{e}	15.2±0.2^{d}	0.5±0.1^{a}	0.6±0.1^{a}	1.2±0.2^{b}	1.4±0.2^{b}	<0.001

HI E: prepupae reared on substrate E (100% coffe silverskin, CS); HI As, HI Bs, HI Cs, HI Ds: prepupae reared on substrate CS enriched with 5%, 10%, 20% and 25% of *Schyzochytrium* sp, respectively; HI Ai, HI Bi, HI Ci, HI Di: prepupae reared on substrate CS enriched with 5%, 10%, 20% and 25% of *Isochrysis* sp., respectively.
Data represent mean ± standard deviation (replicates for each group = 5; n. aliquots per replicate = 3).
Means within rows bearing different letters are significantly different (P<0.05). FAs <1g/100 g FAs were excluded from any statistical analyses because their concentrations were close to the limit of detection.

Table 5 Principal Component Analysis. Eigenvalues, explained and cumulative variance, loadings of the variables for the first three Principal components.

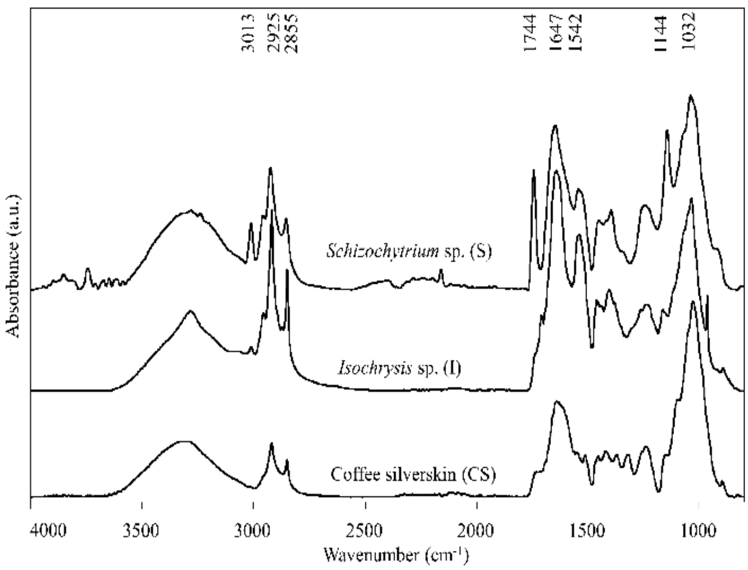
	Principal Component		
	1	2	3
Variance explained			
Eigenvalues	8.508	6.209	1.812
% of variance	47.26	34.50	10.07
Cumulative %	47.26	81.76	91.83
Factor loadings			
12:0	0.061	0.364	-0.175
14:0	0.019	0.373	-0.113
16:0	0.236	-0.271	0.064
16:1n7	-0.114	-0.264	0.100
18:0	0.230	-0.054	-0.422
18:1n9	-0.216	0.206	0.419
18:1n7	0.291	-0.174	0.145
18:2n6	0.167	0.133	0.585
18:3n6	-0.292	-0.207	0.034
18:3n3	0.036	0.347	0.312
20:0	0.258	-0.220	0.184
20:4n6	-0.330	-0.062	0.075
20:5n3	-0.311	-0.160	0.025
22:0	0.252	-0.215	0.217
22:6n3	-0.298	-0.189	-0.022
TL	-0.328	-0.064	-0.082
LIP/TBM	-0.299	0.161	0.126
PRT/TBM	0.077	0.372	-0.128

TL: total lipids, g/kg DM (dry matter); LIP/TBM: amount of lipids relative to total sample biomass; PRT/TBM: amount of proteins relative to total sample biomass.

Table 6 Atherogenic index (AI), thrombogenic index (TI), Hypo/Hyper-cholesterolemic index (HH), and PUFA/SFA ratio of HI prepupae reared on coffee silverskin (E) and on coffee silverskin enriched with different percentages of *Shizochytrium* sp. or *Isochrysis* sp.

Nutritional indices	HI E	HI As (5%)	HI Bs (10%)	HI Cs (20%)	HI Ds (25%)	HI Ai (5%)	HI Bi (10%)	HI Ci (20%)	HI Di (25%)	P-value
HI	1.83±0.08^{d}	0.99±0.05^{c}	0.58±0.06^{a}	1.02±0.08^{c}	0.84±0.05^{b}	3.34±0.07^{g}	2.86±0.06^{f}	2.18±0.07^{e}	1.81±0.03^{d}	<0.001
TI	1.68±0.23^{f}	0.61±0.04^{c}	0.31±0.02^{b}	0.22±0.01^{a}	0.20±0.02^{a}	0.20±0.02^{a}	1.42±0.02^{e}	0.87±0.01^{d}	0.63±0.01^{c}	<0.001
HH	0.85±0.06^{a}	1.58±0.09^{b,c}	2.62±0.27^{d}	2.63±0.22^{d}	3.30±0.23^{e}	0.70±0.02^{a}	0.94±0.02^{a}	1.38±0.09^{b}	1.72±0.06^{c}	<0.001
PUFA/SFA	0.12±0.02^{a}	0.37±0.02^{c}	0.82±0.07^{d}	0.81±0.03^{d}	0.86±0.04^{e}	0.08±0.01^{a}	0.13±0.01^{a}	0.27±0.01^{b}	0.30±0.01^{b}	<0.001

HI E: prepupae reared on substrate E (100% coffe silverskin, CS); HI As, HI Bs, HI Cs, HI Ds: prepupae reared on substrate CS enriched with 5%, 10%, 20% and 25% of *Schyzochytrium* sp, respectively; HI Ai, HI Bi, HI Ci, HI Di: prepupae reared on substrate CS enriched with 5%, 10%, 20% and 25% of *Isochrysis* sp., respectively.
PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
Data represent mean ± standard deviation.
Means within rows bearing different letters are significantly different ($P<0.05$)



3013
2925
2855
1744
1647
1542
1144
1032
Absorbance (a.u.)
Schizochytrium sp. (S)
Isochrysis sp. (I)
Coffee silverskin (CS)
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

Fig. 1

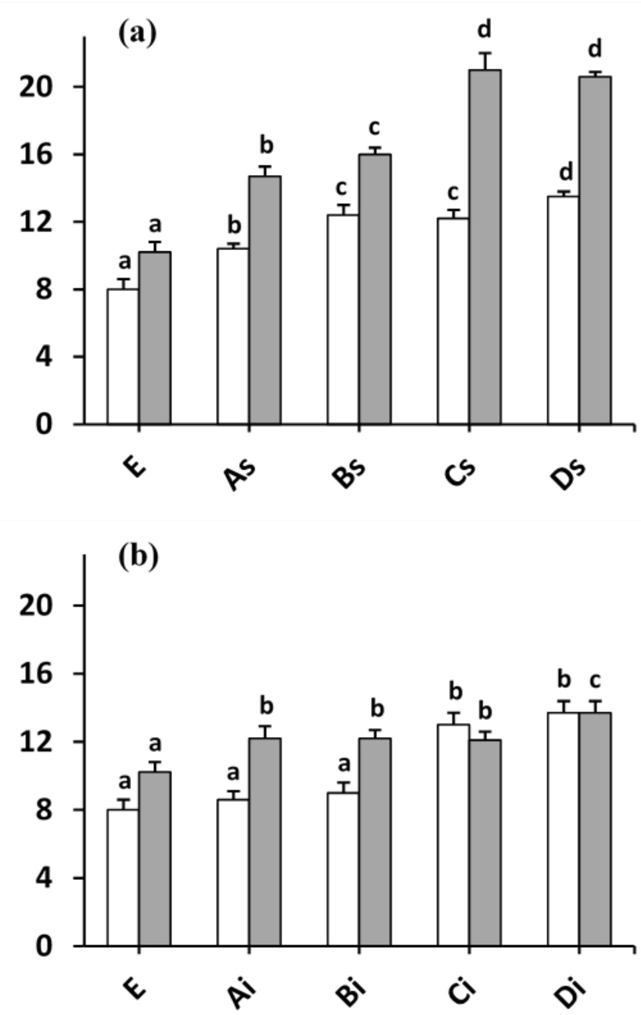

(a)
0
4
8
12
16
20
a
a
b
b
c
c
c
d
d
d
E
As
Bs
Cs
Ds
(b)
0
4
8
12
16
20
a
a
a
b
a
b
b
b
b
c
E
Ai
Bi
Ci
Di

Fig. 2

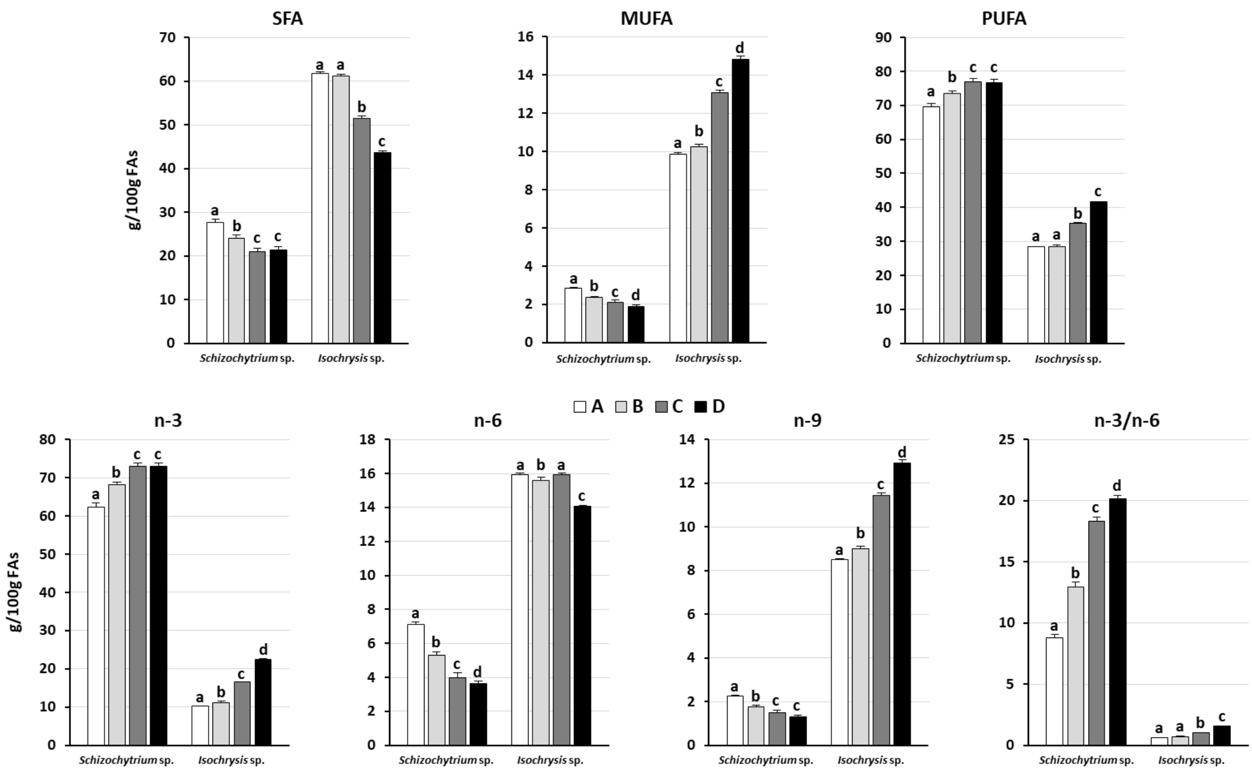
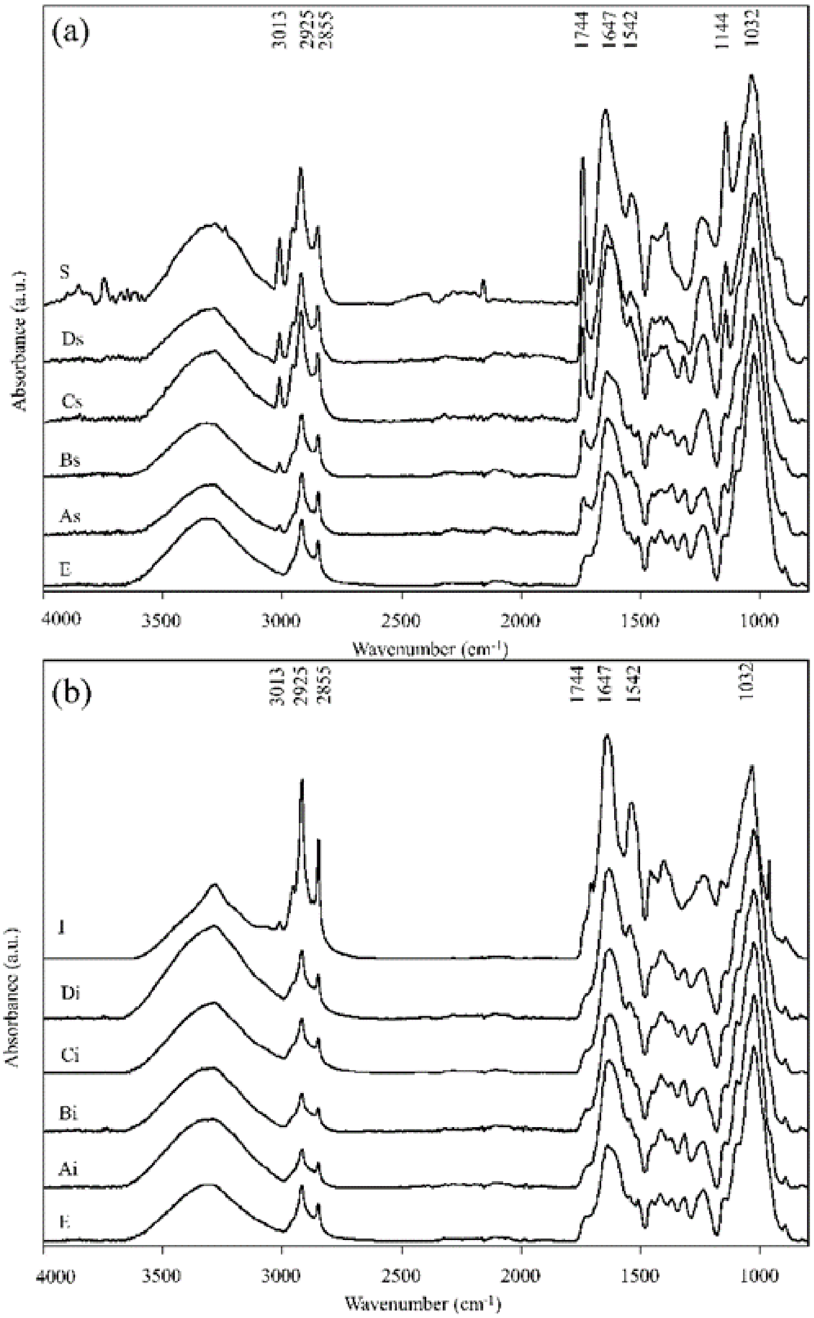

SFA
MUFA
PUFA
n-3
n-6
n-9
n-3/n-6
g/100g FAs
Schizochytrium sp.
Isochrysis sp.
A B C D

Fig. 3



(a)
3013
2925
2855
1744
1647
1542
1144
1032
S
Ds
Cs
Bs
As
E
Absorbance (a.u.)
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)
(b)
3013
2925
2855
1744
1647
1542
1032
I
Di
Ci
Bi
Ai
E
Absorbance (a.u.)
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

Fig. 4

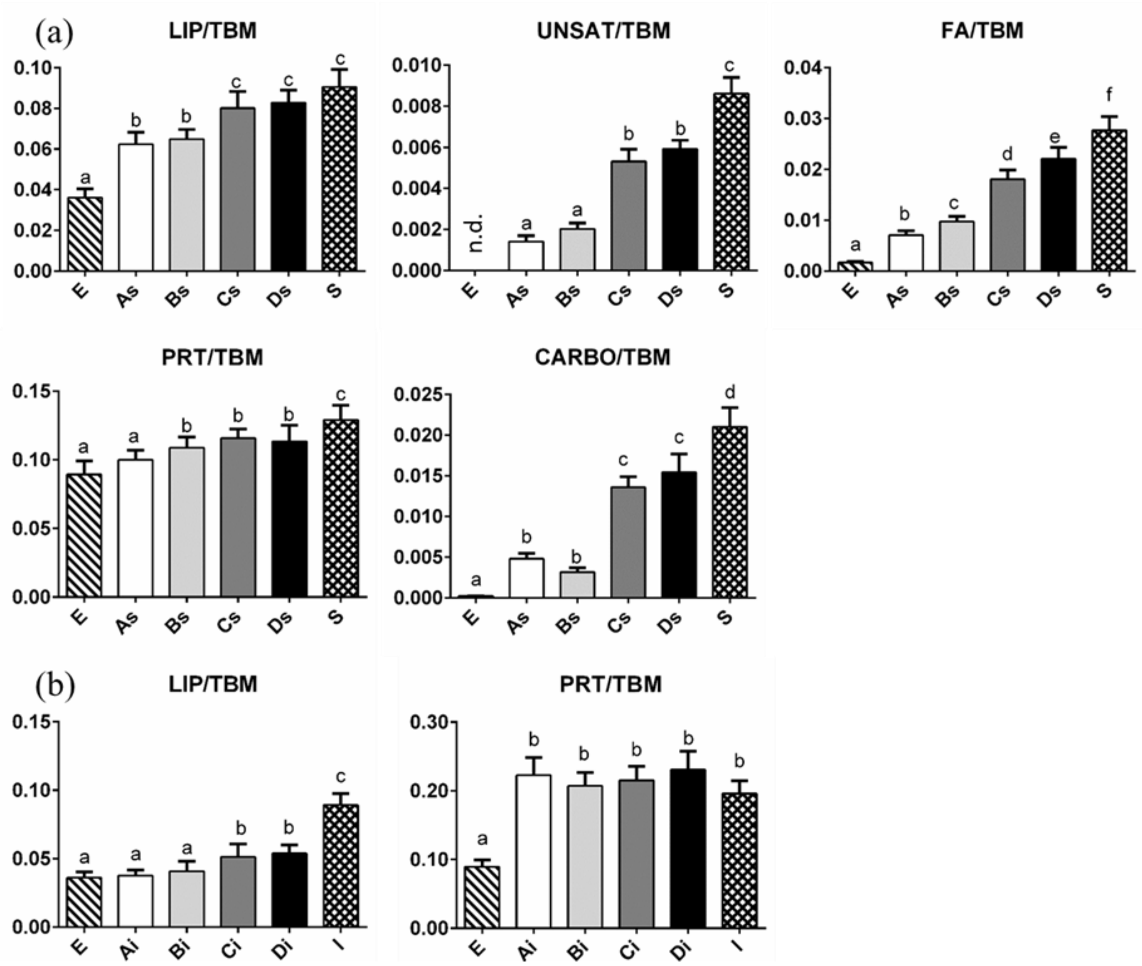

(a)
LIP/TBM
UNSAT/TBM
FA/TBM
PRT/TBM
CARBO/TBM
n.d.
E
As
Bs
Cs
Ds
S
(b)
LIP/TBM
PRT/TBM
E
Ai
Bi
Ci
Di
I

Fig. 5

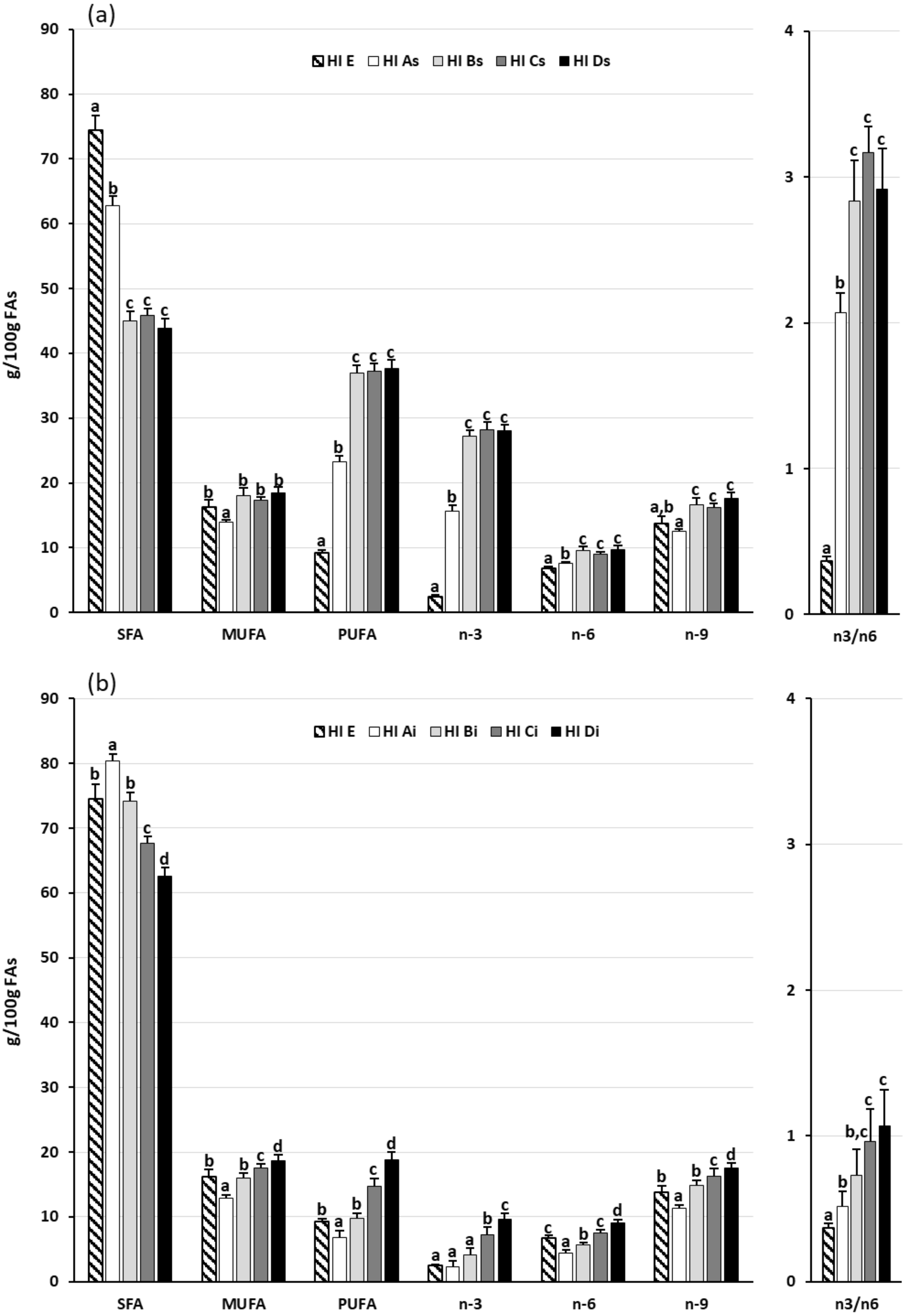

(a)
HI E
HI As
HI Bs
HI Cs
HI Ds
g/100g FAs
SFA
MUFA
PUFA
n-3
n-6
n-9
n3/n6
(b)
HI E
HI Ai
HI Bi
HI Ci
HI Di
g/100g FAs
SFA
MUFA
PUFA
n-3
n-6
n-9
n3/n6

Fig. 6