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Supercritical Defatting, Composition and Digestibility of Meals from Black Soldier Fly (*Hermetia illucens*) Larvae Fed Olive Leaves, Olive Pomace or Quinoa Husk By-Products

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Simple Summary

This study examined whether feeding black soldier fly larvae (BSFL) with challenging by-products from the olive oil industry (olive leaves and olive pomace), as well as an emerging by-product from quinoa processing (quinoa husk) affects the subsequent defatting process using supercritical CO_2 (a clean extraction method), as well as the nutritional quality and digestibility of the resulting insect meal. We found that the efficiency of fat removal mainly depended on the content and solubility of extractable lipids rather than on structural differences in the larvae powder due to diets. All diets produced protein-rich meals, although most by-products slightly reduced protein levels. However, a diet containing 50% dried olive pomace produced larvae with protein levels like those fed conventional diets, with more essential amino acids and no loss in digestibility. The meals were also enriched in chitin, but without negative effects on digestibility. Thus, this study demonstrated that relevant by-products can be successfully converted into high-quality insect protein of BSFL without affecting the processing efficiency of subsequent fat removal, supporting sustainable reuse of agricultural residues for food and feed applications.

Abstract

The nutritional composition of insect-derived meals is strongly influenced by insect diet, while defatting can further modulate nutritional quality. However, some defatting methods, such as supercritical CO_2 extraction, depend on sample properties, including density and macromolecule distribution. Therefore, diet-induced changes may affect lipid extraction efficiency and kinetics, a relationship that remains unexplored. This study evaluated the impact of feeding *Hermetia illucens* larvae with by-products from olive oil industry (olive leaves, OL, at 15, 30 or 50%; dry full-fat olive pomace, OP, at 30, 50, 70, 90%) or quinoa processing (husk, QH, at 15, 30 or 50%) on supercritical CO_2 defatting performance, meal composition, amino acid profile and digestibility. Despite diet-induced variations in lipid accumulation, defatted kinetics mainly depended on the content and solubility of extractable material, while differences in packed bed microstructure had a minor effect. Protein-rich meals were obtained (25–35%), although most diets reduced protein content, except OP50. QH15 and OP30 worsened essential amino acids in meals, whereas OP50

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improved them. Chitin content increased, especially for OP-based meals. Digestibility slightly improved with OP30, OP70, QH15, and QH50. These results show the potential of olive oil and quinoa by-products to be up-cycled by *H. illucens* into high-value insect meals, without compromising the processing by supercritical CO_2 defatting, supporting sustainable insect-based food and feed production.

Keywords: edible insects; black soldier fly; by-products; digestibility; defatting; supercritical CO_2; olive oil; quinoa

1. Introduction

Among edible insects, one of the most popular is the larvae of *Hermetia illucens* (black soldier fly, BSFL). This species is allowed for animal feeding in aquaculture, pigs and poultry, Commission Regulation (EU) 2017/893 of 24 May 2017 [1] and Commission Regulation (EU) 2021/1372 of 17 August 2021 [2]. Furthermore, its form as dried defatted powder is currently under evaluation by the European Food Safety Authority as a novel food. The popularity of BSFL is due to their interesting nutritional value, containing a high level of proteins, in the range of 20–40%, as well as a noticeable lipid content of 25–45% [3–5].

It should be noted that the wide range of variability in nutritional composition in proteins and lipids seems to be particularly dependent on diverse factors, but the insect diets are remarked as one of the most important modulators of composition [6–8]. Furthermore, by subsequent technological processing of the larvae, it is possible to continue the modulation of the nutritional composition of the products. Thus, defatting processes of the dried larvae are typically performed, allowing the production of meals with a much higher protein concentration than the former full-fat larvae, as well as the generation of a valuable lipid co-product [9]. However, the consideration of the impact of the first factor, namely the insect diet, on the subsequent behavior during the second factor, namely the technological defatting process, has not been extensively considered. In this sense, one of the methods of defatting that is being intensively explored for edible insects is the supercritical fluid CO_2 technology [4,10–12]. For this method, it is known that properties of the samples, such as apparent density, particle size or macromolecules distribution and interaction, can impact the efficiency of the defatting process. Thus, considering that insect diets can modulate the protein, lipid or chitin content, but also their distribution, interaction, structure, and specific chemical composition, this may later affect not only solubility behaviour but also the mass transfer during the supercritical extraction process, thereby likely varying its efficiency and kinetics. For example, the solubility of different fatty acids in supercritical CO_2 varies significantly depending on their molecular structure, especially the length of the carbon chain and the degree of unsaturation [13,14]. Therefore, insects fed diets that promote the accumulation of unsaturated fatty acids could present greater ease and efficiency in the defatting process. For *H. illucens*, we also previously evidenced that a variable moisture content in the dried larvae had no negative effect on the oil recovery efficiency by supercritical CO_2, which was mainly determined by the initial oil content [4]. However, specific studies that evaluate the interrelation between the insect diet and the supercritical CO_2 defatting behavior have not been approached and would be of interest to contribute to the advance on the understanding of this defatting technology for edible insects.

In addition to the nutritional properties, BSFL offer another significant advantage, as the remarkable ability to act as a bioconverter of organic materials [15]. Consequently, a widely explored strategy for insect feeding involves rearing BSFL on agri-food by-products, which are allowed for this purpose [1,2]. This approach effectively transforms agri-food by-

products into valuable products, aligning with the principles of the circular economy and sustainability inherent to the emerging insect industry. One example of scarcely explored by-products for insect feeding is those derived from the olive oil industry, such as olive leaves or olive pomace, both generated in tons for each ton of processed olive oil. Although revalorization is tried into edible and non-edible products, the surplus amounts of these side streams are still a sustainable issue with the finding of alternative approaches needed to transform these materials into valuable products [16,17]. Besides these conventional by-products, emerging side streams from new expanding crops may also be considered as interesting materials for being valorized using insect-based bioconversion strategies. This can be the case of quinoa, whose production has significantly increased worldwide, motivated by the recommendation of the FAO, due to its excellent nutritional composition and resilience under undesirable growth conditions [18]. However, quinoa production generates a problem due to the large amount of the by-product quinoa husk, a low-value waste produced during grain processing [19]. Therefore, both examples of conventional and emerging by-products for insect feeding present an opportunity to valorize these problematic materials. In this sense, we recently demonstrated the efficient bioconversion of these different by-products by *H. illucens*, achieving proper growth, with simultaneous enhanced health properties [20,21].

The objective of this study was to evaluate the impact of feeding BSFL with by-products from olive oil (olive leaves or olive pomace), as well as from quinoa (quinoa husk), incorporated at different levels into the substrate of the larvae, on the behavior during the defatting processing by supercritical fluid CO_2 by evaluating the kinetic of the lipid extraction. Furthermore, the later composition of the obtained BSFL defatted meals, including amino acid profile and digestibility, as affected by the fed diets, was evaluated.

2. Materials and Methods

2.1. Raw Materials for BSFL Feeding

Dry olive leaves from the "manzanilla" variety were supplied by Natac Biotech SL (Alcorcón, Madrid, Spain) and were ground. The full-fat dry olive pomace was sourced as powder from Troil Vegas Altas SC (Valdetorres, Badajoz, Spain). Quinoa husk in powder form was provided by Naturquinoa (Madrid, Spain).

2.2. BSFL Rearing and Processing

BSFL were reared by Entomo AgroIndustrial (Murcia, Spain) on substrates based on laying hen feed (control diet, NANTA®, Madrid, Spain) or partially replaced with the experimental by-products: dry olive leaves at 15%, 30% or 50% (OL15, OL30, OL50); full-fat dry olive pomace at 30%, 50%, 70%, 90% (OP30, OP50, OP70, OP90); and quinoa husk at 15%, 30% or 50% (QH15, QH30, QH50). These values were determined based on a preliminary small-scale trial using the same diets at a 50% replacement level, which enabled the identification of potential mortality or observable negative effects on larval growth. Detailed information about the impact of the assayed diets on the growth performance of the larvae can be found in our previous study [20].

The proximate composition of the substrates is shown in Table 1. Each diet (5 kg) was mixed with water to produce 15 kg of substrate, which was divided into triplicate on plastic trays. A total of 13.000 larvae (91 g) were reared per replicate for 12 days. The experiment was conducted in a climate-controlled chamber at 26 ± 1 °C and 65 ± 5% humidity. After the feeding, larvae were sieved, rinsed in cool water, and blanched in water at 90 °C (ratio of sample to water of 1:10, w/v) for 40 s, following the method of Hurtado-Ribeira et al. [9]. The larvae were then immersed in cold water, drained and oven-dried at 55 °C for 72 h. Then, the dried larvae were powdered.

Table 1. Nutritional composition of black soldier fly substrates (g/100 g, dry matter).

	Crude Protein	Crude Lipids	Crude Fiber	Ashes	NSC [1]	GE [2]	Dry Matter [3]
Control	18.18 ± 0.44 ^{a}	2.77 ± 0.03 ^{fgh}	2.51 ± 0.09 ^{h}	22.03 ± 0.10 ^{a}	54.51	1447	88.93 ± 0.10 ^{g}
OL15	16.84 ± 0.26 ^{b}	2.65 ± 0.02 ^{g}	3.70 ± 0.18 ^{g}	19.29 ± 0.08 ^{bc}	57.52	1461	90.43 ± 0.22 ^{de}
OL30	15.14 ± 0.24 ^{cd}	2.62 ± 0.11 ^{g}	5.22 ± 0.12 ^{f}	16.87 ± 0.19 ^{d}	60.14	1464	91.23 ± 0.17 ^{b}
OL50	13.51 ± 0.19 ^{e}	2.93 ± 0.04 ^{efg}	7.77 ± 0.20 ^{d}	14.29 ± 0.16 ^{g}	61.49	1460	91.88 ± 0.09 ^{a}
OP30	16.63 ± 0.02 ^{b}	5.91 ± 0.14 ^{d}	7.15 ± 0.30 ^{e}	19.92 ± 0.16 ^{b}	50.39	1464	90.48 ± 0.00 ^{cde}
OP50	14.94 ± 0.27 ^{cd}	7.27 ± 0.13 ^{c}	11.50 ± 0.12 ^{c}	15.25 ± 0.32 ^{f}	51.04	1488	90.73 ± 0.36 ^{cd}
OP70	14.35 ± 0.21 ^{de}	9.50 ± 0.11 ^{b}	14.99 ± 0.28 ^{b}	13.50 ± 0.31 ^{h}	47.65	1504	90.90 ± 0.17 ^{bcd}
OP90	12.62 ± 0.42 ^{f}	10.82 ± 0.13 ^{a}	19.98 ± 0.23 ^{a}	10.48 ± 0.38 ^{i}	46.10	1488	90.96 ± 0.12 ^{bc}
QH15	16.37 ± 0.19 ^{b}	2.72 ± 0.08 ^{fgh}	2.35 ± 0.03 ^{h}	19.60 ± 0.11 ^{bc}	58.95	1477	89.94 ± 0.14 ^{f}
QH130	15.46 ± 0.39 ^{c}	2.97 ± 0.10 ^{ef}	2.59 ± 0.19 ^{h}	19.05 ± 0.25 ^{c}	59.93	1481	90.17 ± 0.09 ^{ef}
QH50	14.96 ± 0.33 ^{cd}	3.14 ± 0.04 ^{e}	3.20 ± 0.15 ^{g}	16.08 ± 0.07 ^{e}	62.63	1521	90.44 ± 0.09 ^{de}

[1] NSC (non-structural carbohydrates); [2] GE (gross energy) calculated using the following energy coefficients: protein 23.6 kJ/g, lipids 38.9 kJ/g and carbohydrates 16.7 kJ/g and expressed as kJ/100 g of dry matter. [3] Value for the dry diets before hydration with 1:2 water. OL, dry olive leaves; OP, full-fat dry olive pomace; QH, quinoa husk. Different letters in the same column within the substrates formulated with by-products results (a–i) indicate significant differences ($p \leq 0.05$).

2.3. Supercritical Fluid CO_2 Defatting

2.3.1. Experimental Procedure

Defatting was performed in a supercritical CO_2 extractor (Thar Technology, Pittsburgh, PA, USA, model SF20,00), which includes a cylinder extraction cell (273 cm^3) and a separator (500 cm^3) with independent control of temperature and pressure. For each extraction, the cell was filled with 125–148 g of the powdered dried larvae, depending on the substrate used for the BSFL rearing, which caused different densities of the insect powders and, hence, different masses to completely fill the volume of the cell. The extraction conditions set were 450 bars, 60 °C, and a CO_2 flow of 80 g/min for 40 min, based on our previous experience on defatting of BSFL by supercritical CO_2 [9].

The kinetic behaviour of the defatting procedure was studied by collecting the extracted material from the separator (50 bars, 60 °C) every 10 min. The defatted protein meal was stored at −20 °C until use.

Extraction yield (Y) was calculated according to the following equation:

$$Y = \frac{W_{ext}}{W} \times 100 \quad (1)$$

where W_{ext} is the weight of material extracted by and W is the weight of the dried larvae powder placed in the extraction vessel.

To estimate the initial lipid content of the insect powders, hexane extraction was used, according to Cantero-Bahillo et al. [11]. Thus, 2 g of ground sample were homogenized with hexane at a ratio of sample to solvent of 1:5 (w/v) in an Ultra-turrax (Ultra-turrax T18 basic, IKA, Staufen, Germany) (11,000 rpm) for 5 min. Then, the mixture was centrifuged at 4500 rpm for 10 min at 20 °C. The supernatant was removed, and the precipitate was defatted again following the same procedure. Hexane was removed using a vacuum rotary evaporator.

2.3.2. Defatting Kinetic Modelling

The experimental extraction curves were represented using a simple model [22] in which two different extraction periods are considered: in the first period ($t < t_c$) it is

assumed that the mass extracted depends on the equilibrium conditions and thus, the yield is related to the solute's solubility (S) according to the following equation:

$$Y = S \times q \times t \qquad 0 < t < t_c \tag{2}$$

where t is the extraction time, q is the ratio between the supercritical CO_2 flow rate and the mass of larvae (W) placed in the extraction vessel. That is, in the first period, it is assumed that the external mass transfer resistance is negligible and thus, the extraction yield vs. time is represented by a straight line and the extraction proceeds very fast.

In the second extraction period, the yield is described by:

$$Y = X_u \times [1 - C_1 \times \exp(-C_2 \times q \times t)] \qquad t > t_c \tag{3}$$

where C_1 and C_2 are parameters related to the larvae-packed cake mass transfer resistance, and X_u is the ratio between the mass of extractable substances and the mass of insoluble material in the larvae cake. In this second extraction period, the model suggests that the kinetic behavior is controlled by the mass transfer resistance and thus, the straight line of the first period becomes more curved and the extraction velocity decreases.

The model considers that in the first period, the mass extracted depends only on the solutes' solubility in supercritical CO_2. Thus, in the first period, the yield depends only on the amount of CO_2 that flows through the vessel. Since the CO_2 flow rate is constant with time, the extraction yield increases linearly with extraction time. But in the second period, the model takes into account mass transfer resistance within the larvae-packed cake, and thus, the extraction curve in this period is not as linear (extraction velocity decreases) and follows the exponential expression given by Equation (3).

Model parameters were optimized using Excel 365 software and minimizing the Mean Standard Deviation (MSD) by the method of least squares:

$$MSD = (1/4)\sqrt{\left[\left(Y_{exp} - Y_{cal}\right)/Y_{exp}\right]^2} \tag{4}$$

where Y_{exp} and Y_{cal} are the experimental and calculated extraction yields. The t_c values were calculated by equating Equations (2) and (3).

2.4. Proximate Composition of the Samples

Determination of proximate composition was carried out on both dry substrates and defatted insect meals using the official methods of analysis AOAC.2005 for dry matter (934.01), crude protein (954.01), crude fat (920.39), ash (942.05) and crude fiber (978.10). The amount of crude protein was assessed by the Kjeldahl method, with a conversion factor of 6.25 for diets and of 4.43 for fresh larvae to avoid overestimation due to non-protein nitrogen compounds such as chitin, small peptides, urea, and other compounds [23]. The analysis of the chitin content [24,25] was based on the determination of acid detergent fiber (ADF) according to AOAC method 973.18, the quantification of nitrogen in the ADF fraction using the Kjeldahl method (AOAC 955.04), and the subsequent calculation of chitin content (%) as ADF (%)—ADIP (%), where ADIP refers to the amount of protein bound to the ADF fraction. The non-structural carbohydrate content (NSC, % dry matter) was calculated as: 100 − (crude lipid + crude protein + ash + crude fiber for substrate or quitin for larvae).

2.5. Amino Acid Profile of the Insect Meals

Amino acid analysis of the defatted insect meals was based on Galafat et al. [26]. Samples were hydrolyzed (20 mg in 1 mL HCl 6 M) at 110 °C for 24 h under an inert

atmosphere (N_2). After that, 50 µL of the hydrolysate was mixed with 50 µL of 6 M NaOH. Then, 100 µL of internal standard (2.5 mM norleucine) and 800 µL sodium citrate loading buffer (pH 2.2) were added and mixed by vortex for 5 s and then filtered (0.2 µm). A sample (20 µL) of this mixture was analyzed with a Biochrom 30 amino acid analyzer (Biochrom Ltd., Cambridge, UK), according to the manufacturer's protocol.

2.6. Protein Digestibility of the Defatted Insect Meals

Protein digestibility of the samples was performed according to Varga et al. [27]. For the gastric phase digestion, 0.05 g of BSFL defatted meals were added to a 4 mL solution containing 2 g/L NaCl and 7 mL/L HCl, with a pH adjusted to 2. Right before starting the digestion process, 3.2 mg/mL of fresh pepsin (porcine pepsin 2000 U/g, Merck 7190) was added to the mixture, and the digestion was carried out for 3 h in a water bath shaker at 37 °C. Subsequently, intestinal phase digestion was performed. For this purpose, the pH of the gastric phase mixture was adjusted to 6–8 with NaOH and 2 mL of a solution containing 1.5 mg/mL of pancreatin (porcine pancreatin grade VI, Sigma, Madrid, Spain) and 24 mg/mL of bile extract were added. The incubation continued for 2.5 h under the same conditions described before.

A sample of 100 µL was withdrawn at the beginning of the process and at the end of the gastric and intestinal phase and mixed with 100 µL of 20% trichloroacetic acid solution. The mixture was centrifuged at 12,000× *g* for 15 min at 4 °C. The supernatant was collected and measured spectrophotometrically at 340 nm, according to the o-phthaldialdehyde (OPA) assay [28]. This method specifically measures free α-amino groups (–NH_2) released as a consequence of peptide bond cleavage during enzymatic digestion. Therefore, the increase in OPA-reactive amino groups reflects the extent of protein hydrolysis.

The degree of hydrolysis (DH) was estimated as the DH obtained in gastric (DH gastric) or intestinal digestion (DH intestinal) or the sum of both (DH total) as:

$$DH\% = (NH_{2\ gastric}\ \text{or}\ NH_{2\ intestinal}/NH_{2\ total}) \times 100 \quad (5)$$

where $NH_{2\ gastric}$ and $NH_{2\ intestinal}$ are the amount of NH_2 groups released at the end of the gastric or intestinal phase, respectively, and NH_2 total is the total content of NH_2 groups of the undigested meal. To determine the total NH_2 group content, a complete hydrolysis of protein was performed for each defatted insect meal by heating 0.05 g in an oven at 110 ± 1 °C for 24 h with 2.5 mL of 6 N HCl. The samples were then diluted with distilled water in a ratio of 1:1, centrifuged at 12,000× *g* and the supernatant was assayed with the OPA method for the NH_2 content.

Furthermore, the amino acid digestibility of the meals was determined. Briefly, the supernatant, which was obtained by centrifugation of the mixture of the sample, withdrawn at the end of the intestinal digestive phase, and 20% trichloroacetic acid solution, was hydrolysed with 6 N HCl at 110 ± 1 °C for 24 h. The total NH_2 group content of the supernatant hydrolysate was quantified by OPA assay ($NH_{2\ hydrolysed}$). The digestibility based on this assay method was calculated as follows:

$$\text{Digestibility}\ (\%) = (NH_2\ HCl_{\ hydrolysed}/NH_{2\ total}) \times 100 \quad (6)$$

where $NH_2\ HCl_{\ hydrolysed}$ represents the amino groups present in the supernatant from the end of the in vitro digestion after hydrolysation with 6 N HCl at 110 ± 1 °C for 24 h and $NH_{2\ total}$ is the total NH_2 group content of the undigested meal.

2.7. Statistical Analysis

The statistical analysis was performed by a one-way analysis of variance using the general linear model procedure of the SPSS 26.0 statistical package (SPSS Inc., Chicago, IL, USA). When the effect of any of the factors was significant ($p \leq 0.05$), differences between groups were analyzed by using the post-hoc Tukey test. Pearson correlation tests were conducted for additional analyses.

3. Results and Discussion

3.1. Supercritical CO_2 Defatting of the Larvae

After the feeding trial of the larvae with the experimental diets, the obtained dried larvae as powder were defatted by supercritical CO_2 and the potential impact of the treatments on the defatting behaviour was studied. The experimental extraction yields, and defatted meal yields obtained after 40 min of the supercritical defatting procedure are given in Table 2 and the kinetic behaviour of the extractions is depicted in Figure 1. In the case of the OL50 substrate, the kinetic data could not be obtained due to the low amount of dried larvae mass obtained with this substrate.

Table 2. Supercritical defatting yields and kinetic model parameters for dried BSFL larvae reared with the different experimental substrates.

Substrate	Oil Content in Dried Larvae (g/100 g)	Supercritical Extraction Yield (Y)	Defatted Meals Yield (%)	Apparent Solubility (S) (g/100 g)	Soluble/Insoluble Ratio (X_u)	t_c (min)
Control	20.7	21.5	78.8	2.71	0.375	9.9
OL15	16.4	19.2	81.8	2.30	0.345	9.7
OL30	15.2	19.2	81.9	1.85	0.312	10.9
OL50	17.1	20.6	79.6	n.a.	n.a.	n.a.
OP30	15.6	15.0	85.5	1.58	0.263	11.1
OP50	18.7	17.7	83.0	1.97	0.302	10.7
OP70	16.8	16.7	82.8	1.65	0.292	11.5
OP90	19.3	17.2	82.6	1.72	0.287	11.4
QH15	14.3	15.6	86.0	1.85	0.265	10.5
QH30	16.2	21.9	79.5	2.41	0.388	10.0
QH50	19.0	15.9	85.7	1.59	0.274	11.3

n.a., no apply.

Since the sum of the extraction yield and defatted meal yield should be 100%, it can be deduced from Table 2 that the mass balance of all experiments closed with a deviation lower than 5%. Table 2 also shows the content of lipids in the dried larvae powders, as determined by the hexane method.

Due to the non-polar character of supercritical CO_2 and considering the main macronutrients present in the larvae meals, the major substances expected to be extracted are lipids. Nevertheless, no general correlation nor tendency was found between the oil content in the dried larvae samples and the supercritical extraction yields (Y), which may be attributed to (i) differences in the lipid composition of the dried larvae packed bed, hence, potential different solubility in supercritical CO_2; and/or (ii) differences in the packed bed microstructure, which may affect in a different way the mass transfer behaviour.

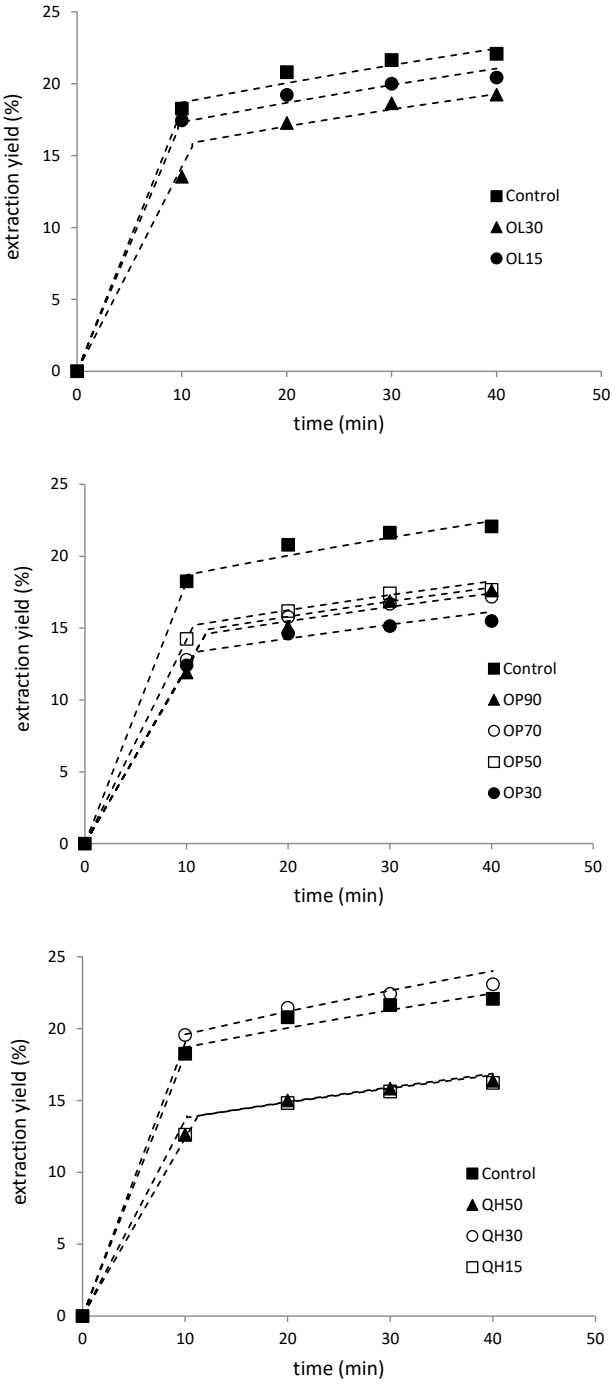


Figure 1. Kinetic behaviour of the supercritical CO_2 defatting (60 °C and 450 bar) of BSFL reared with the different substrates (OL, olive leaves; OP, olive pomace; and QH, quinoa husk) and comparison with the control diet. Dashed lines: model fitting.

To elucidate which of these two potential reasons was more relevant in the supercritical defatting procedure, the model used to represent the extraction kinetic curves (Equations (2) and (3)) was applied considering as a hypothesis that mass transfer behaviour in all dried larvae packed beds is similar (i.e., assuming equal C_1 and C_2 parameters for all kinetic curves) and fitting different apparent solubility (S) and soluble-to-insoluble material ratio (X_u) for each kinetic curve. Then, to carry out the mathematical modelling, it is assumed that differences in the microstructure of the dried larvae packed beds are not substantial to affect the supercritical extraction (similar mass transfer behaviour) and thus,

the different yields obtained strongly depend on the amount and solubility in supercritical CO_2 of the substances present in the dried larvae powders.

Table 2 shows the optimal parameters (S and X_u) and t_c values obtained for each kinetic curve (Figure 1). The mass transfer related parameters obtained were $C_1 = 0.540$ and $C_2 = 0.011$.

Defatted meal yields exhibited good correlation with both S ($R^2 = 0.696$) and X_u ($R^2 = 0.873$) as can be observed in Figure 2, supporting the hypothesis adopted in the modelling procedure. That is, defatted meal yield mainly depends on the content and solubility of extractable substances in the dried larvae packed bed, while differences in porosity, apparent density, particle size or macromolecule distribution in the packed bed have a minor effect. Therefore, the greater the apparent solubility, the greater the ratio between soluble and insoluble material, and the lower the defatted meal yield. Furthermore, similar t_c values were obtained for all kinetic curves (Table 2), indicating similar length of the first extraction period (c.a. 10 min), in which the kinetic behaviour is controlled by the solubility of the extractable substances ($R^2 = 0.809$ for the correlation between t_c and S). The second extraction period, in which the kinetic behaviour is controlled by mass transfer resistance, was satisfactorily represented by the model using the same C_1 and C_2 parameters for all kinetic curves, also supporting the hypothesis adopted in the modelling procedure.

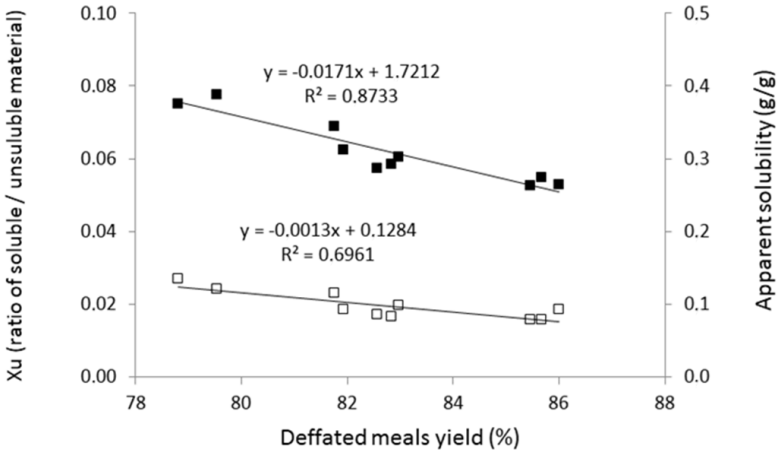


Figure 2. Correlation between model parameters Xu (■) and S (□) and defatted meal yields obtained in the supercritical defatting procedure (60 °C, 450 bar) applied for all dried larvae powders.

Therefore, in general, it seems that the experimental larvae fed with the different diets, despite different initial lipid content, do not demonstrate relevant differences in the packed bed microstructure, following a similar supercritical kinetic behaviour in the defatting process, regardless of the fed diets. The different defatting yields of the batches may be attributed to the different content and composition of the extractable substances present in the larvae samples, which caused a variable residual lipid content that remained in the meals after the defatting process, as will be elucidated in Section 3.2. Then, the defatted meal yield varied in the range of 79% for control larvae, 80–82% for OL larvae, 83–86% for OP larvae and 80–86% for QH larvae (Table 2).

3.2. Composition of Defatted Insect Meals

The proximate composition of the different defatted meals obtained is shown in Table 3. As expected, a rich-protein product was achieved, with values in the range of 25–35%. However, in general, except for OP50, all the experimental diets caused significantly lower protein content with respect to the control larvae. This trend agreed with the same trend observed for the composition of the diets (Table 1).

Table 3. Proximate composition (g/100 g) of the defatted BSFL meals.

Substrate	Crude Protein	Crude Lipid	Chitin	ADF	ADIP	Ashes	Moisture	NSC
Control	34.84 ± 0.12 ^{a}	0.36 ± 0.02 ^{g}	5.56 ± 0.39 ^{g}	8.50 ± 0.93 ^{e}	34.44 ± 2.53 ^{a}	27.14 ± 0.16 ^{ab}	6.60 ± 0.08 ^{c}	25.50
OL15	32.91 ± 0.10 ^{c}	0.55 ± 0.06 ^{g}	7.18 ± 0.26 ^{f}	10.89 ± 0.54 ^{d}	34.01 ± 1.11 ^{ab}	27.56 ± 0.10 ^{a}	7.01 ± 0.22 ^{b}	24.79
OL30	28.08 ± 0.17 ^{f}	11.38 ± 0.29 ^{a}	9.84 ± 0.20 ^{d}	15.18 ± 0.64 ^{c}	35.11 ± 1.39 ^{a}	24.47 ± 0.03 ^{d}	5.70 ± 0.06 ^{f}	20.53
OL50	26.99 ± 0.10 ^{g}	0.43 ± 0.19 ^{g}	11.14 ± 0.17 ^{c}	15.99 ± 0.14 ^{c}	30.34 ± 0.63 ^{bcd}	25.33 ± 0.20 ^{c}	6.19 ± 0.18 ^{de}	29.92
OP30	32.45 ± 0.07 ^{d}	9.43 ± 0.24 ^{b}	8.78 ± 0.30 ^{e}	12.05 ± 0.38 ^{d}	27.15 ± 0.57 ^{d}	22.58 ± 0.39 ^{f}	4.85 ± 0.09 ^{g}	21.91
OP50	35.22 ± 0.15 ^{a}	1.24 ± 0.25 ^{f}	11.33 ± 0.77 ^{c}	16.10 ± 1.24 ^{c}	29.60 ± 0.70 ^{cd}	23.30 ± 0.10 ^{e}	5.77 ± 0.11 ^{f}	23.14
OP70	29.55 ± 0.09 ^{e}	2.33 ± 0.01 ^{e}	19.14 ± 0.22 ^{b}	22.96 ± 0.26 ^{b}	16.65 ± 1.59 ^{e}	19.54 ± 0.14 ^{h}	5.94 ± 0.17 ^{ef}	23.50
OP90	25.40 ± 0.27 ^{h}	1.61 ± 0.21 ^{f}	23.22 ± 0.54 ^{a}	28.41 ± 1.60 ^{a}	15.77 ± 2.06 ^{e}	18.76 ± 0.21 ^{i}	5.76 ± 0.10 ^{f}	25.25
QH15	32.78 ± 0.18 ^{cd}	5.56 ± 0.30 ^{c}	7.22 ± 0.22 ^{f}	10.71 ± 0.48 ^{de}	32.54 ± 1.00 ^{abc}	25.39 ± 0.06 ^{c}	4.95 ± 0.11 ^{g}	24.10
QH30	33.68 ± 0.07 ^{b}	0.60 ± 0.08 ^{g}	6.97 ± 0.06 ^{f}	10.37 ± 0.09 ^{de}	32.77 ± 0.09 ^{abc}	26.77 ± 0.17 ^{b}	8.17 ± 0.05 ^{a}	23.81
QH50	34.12 ± 0.10 ^{b}	4.65 ± 0.08 ^{d}	7.75 ± 0.28 ^{ef}	11.05 ± 0.45 ^{d}	29.88 ± 0.34 ^{cd}	21.75 ± 0.20 ^{g}	6.47 ± 0.19 ^{cd}	25.26

ADF, acid detergent fiber; ADIP, protein bound to the ADF Fraction; NSC, Non-structural carbohydrate content; OL, dry olive leaves; OP, full-fat dry olive pomace; QH, quinoa husk. Different letters in the same column mean significant differences ($p \leq 0.05$).

Ashes were the second major component for all the meals. These values were high, even in the case of control larvae (27%), but in agreement with the high ash content of the used control diet (Table 1). In any case, the ash content tended to decrease with the inclusion of the by-products, reflecting the same trend as the diet compositions (Table 1).

One remarkable result was that the experimental diets impacted the chitin content of the obtained insect meals (Table 3). Thus, a significant increase was observed with respect to the control. Additionally, this increase was dose-dependent with the level of olive leaves and olive pomace. Thus, the chitin content of the control (close to 6%) was almost duplicated in the case of OL50 (11%) and quadruplicated in the case of OP90 (23%). In general, it is known that different factors may modulate the chitin content of BSFL, such as the development stage and diet [29]. When exploring the relationship with the used substrates, significant positive correlations were obtained for the chitin content of the insect meals and the fiber and lipid contents of substrates (r = 0.967, $p < 0.001$ and r = 0.903, $p < 0.001$, respectively). This strong linear relationship was observed regardless of the specific by-product used, suggesting that the total fiber content of the substrate, rather than the specific included by-product, may determine the increase in the chitin content of the larvae. However, in the case of the effect of the lipid content of substrates on the chitin content of meals, the linear relationship was especially evident for the olive pomace diets (r = 0.963). On the contrary, the lower the protein and ashes contents of the substrates the higher the chitin of the BSFL meals (protein: r = −0.782, $p = 0.004$, and ashes: r = −0.884, $p < 0.001$). All these dietary factors, as expected, modulated in the same way the value of ADF for the insect meals and caused the contrary effect for the ADIP value. Thus, the higher the dietary protein and ashes, the higher the protein content bound to the ADF fraction of the insect meals. Therefore, it can be concluded that all nutrients of the BSFL substrates seem to modulate the final reached chitin content of the meals.

It should be remarked that the obtained results on chitin content could be considered negative or positive depending on the last tentative use of the products. Thus, from the food and feed point of view, the beneficial effect of chitin on gastrointestinal health has been described [30–33], but excessive levels of chitin are not always desirable [34]. In this case, the experimental diets based on quinoa husk would be more desirable than olive leaf or olive pomace, since quinoa husk did not extensively impact the chitin content of the BSFL meals. On the contrary, in case the last purpose is obtaining chitin, or chitosan from chitin, having a starting material with an initial enriched chitin concentration may be proposed, as an innovative mode to increase the yield and efficiency of chitosan production. This may be of great interest since the production of chitosan, due to bioactive properties as well as technological, technical and industrial uses, is being established as one of the major

industries of valorisation of chitin from natural sources, including insects [33,35]. Thus, in this case, the BSFL feeding with olive leaves at 50% or full-fat dry olive pomace at ≥50% would be of great interest.

Finally, and as expected, the moisture and lipids were minor components in general for most samples. Furthermore, the variability in the defatting efficiency obtained for some treatments, as previously commented, was reflected in a variable remaining minor lipid content in the defatted meals (Table 3).

Therefore, nutrients of the BSFL substrates seem to modulate the final nutritional composition of the defatted meals. However, it is important to note that the subsequent technological processing of the larvae, such as drying and defatting, also contributes to the final meal composition. Thus, despite the impact of substrates on the nutritional composition of fresh larvae, as previously described in detail [20], additional compositional differences in the defatted meals emerge due to the concentration effects associated with drying and lipid removal.

3.3. Amino Acid Profile of the Defatted Insect Meals

The detailed amino acid profile of the BSFL meals as affected by the diets is shown in Figure 3. The specific numerical data and statistical differences are detailed as Supplementary Materials (Table S1). It should be noted that the total protein content of the samples determined by the Kjeldahl method (Table 3) did not completely coincide with the total amino acid content. This discrepancy may be due to the limitations of each method and the nature of the sample analysed.

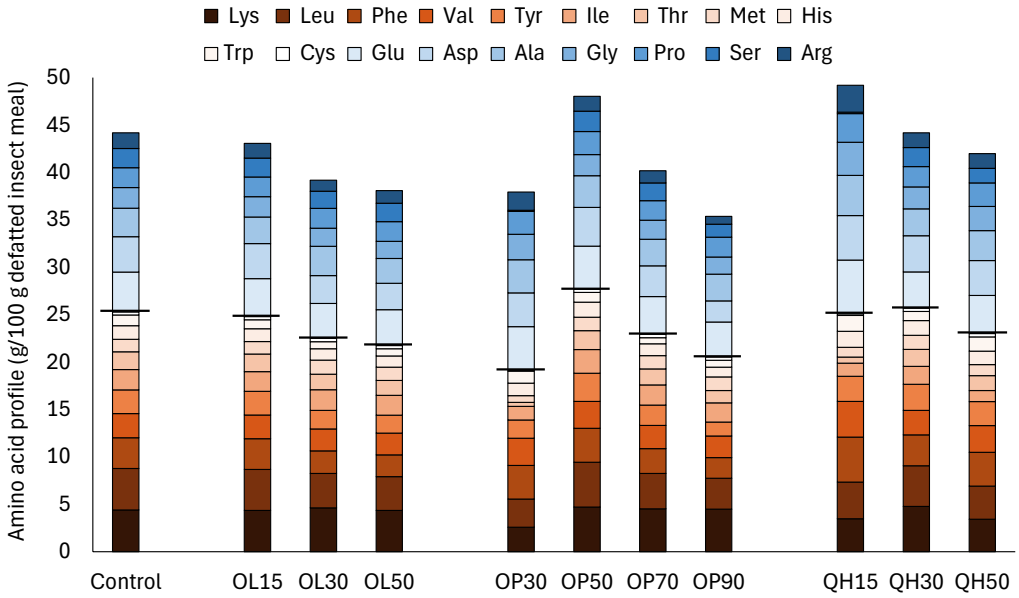


Figure 3. Amino acid profile of defatted BSFL meals as affected by the experimental diets (OL, dry olive leaves; OP, full-fat dry olive pomace, QH, quinoa husk). Black line denotes the limit between essential amino acids (below the line) and non-essential amino acids (above the line).

According to Figure 3, the control larvae showed a major content of essential amino acids, being 57% of total amino acids. The major essential amino acids were lysine and leucine, followed by phenylalanine, valine and tyrosine. Among non-essential amino acids, glutamic acid and aspartic acid, plus asparagine, were the most abundant. In general, this profile was in agreement with the description in previous studies for BSFL [36,37].

In general, the qualitative amino acid profile remained almost the same as that of control larvae for OL-based diets, although with generally lower amino acid contents as the level of olive leaves increased in the diets. Therefore, it seems that olive leaves

mainly impacted the total protein (Table 3) and amino acid content of larvae but without qualitatively modifying the amino acid profile (Figure 3). Similarly, in the case of QH-based diets, a pattern of lower amino acids was observed with the level of replacement. Additionally, QH15 also caused a significant decrease in the ratio of essential amino acids with respect to non-essential (50% of total amino acids), despite QH15 larvae containing the highest total amino acids. Concerning the effect of olive pomace, a clear pattern due to the level of replacement in the diets was not observed. On one hand, OP30 simultaneously caused a lower total amino acid content and the most remarkable decrease in essential amino acid content and ratio. On the contrary, OP50 larvae were especially remarkable, as these simultaneously contained higher total amino acids than control (48 g/100 g and 44 g/100 g, respectively), in agreement with total protein content in Table 3, as well as higher content of essential amino acids than control (28 g/100 g of meal and 25 g/100 g of meal, respectively).

In general, previous studies on BSFL have concluded that different diets do not seem to impact the amino acid profile of the larvae extremely [38–40]. Nevertheless, more extensive studies are still needed, including a wider diversity of diets, to reach a robust conclusion. As a contribution, the present study suggests that specific ingredients at specific inclusion in the BSFL diet, although slightly, can modify the amino acids of BSFL, either quantitatively or qualitatively. Further studies are needed to clarify the specific metabolic pathways behind such effects, as well as whether such modifications would be enough to impact the global nutritive value of these meals, as well as the potential bioactive peptides of these meals.

3.4. Digestibility of the Defatted Insect Meals

To evaluate the impact of the different diets on the digestibility of the defatted insect meals, the degree of gastrointestinal hydrolysis was initially tested (Figure 4a). Lack of significant differences was found, either for gastric, intestinal or total gastrointestinal hydrolysis due to the diets. Thus, in general, the degree of hydrolysis was in the range of 9–15% for gastric digestion, 7–12% for intestinal digestion and 18–23% for total gastrointestinal digestion. These values were similar to the ones obtained by Rodríguez-Rodríguez [41], who found values in the range of 20–28% total gastrointestinal digestion for a full-fat *H. illucens* meal using the same method of in vitro digestion and hydrolysis estimation. It should be remarked that the method used for hydrolysis, based on measuring the free amino groups, did not include peptidases to break down dipeptides or oligopeptides. Therefore, the maximum expected theoretical hydrolysis would be 50%, since by generating dipeptides, one free amino group is obtained for every two amino acids instead of one per amino acid [42]. Considering this, it could be estimated that the total gastrointestinal digestion of the assayed samples would be in the range of 36–46% of the maximum theoretical hydrolysis, which could be considered moderate.

To have wider information about digestibility, the soluble fraction of hydrolysed proteins after digestion was estimated, as it is typically measured for protein digestibility studies [43,44]. Thus, as shown in Figure 4b, digestibility was in the range of 63–75%. In general, these values agree with the previously reported ones by Rodríguez-Rodríguez [41]. Furthermore, in the present study, it was evidenced that the impact of experimental diets was remarkable, since no diet negatively impacted digestibility, but some of them positively did. Thus, OP30 and OP70 diets produced meals with slightly higher digestibility than control diets (70%, 74% and 66%, respectively). Similarly, QH15 and QH50 meals also showed higher digestibility (75% and 69%, respectively). Therefore, despite a clear pattern with the level of these by-products in the diets not being found, it is evident that specific

levels of full-fat dry olive pomace and quinoa husk slightly improved the digestibility of the insect meals.

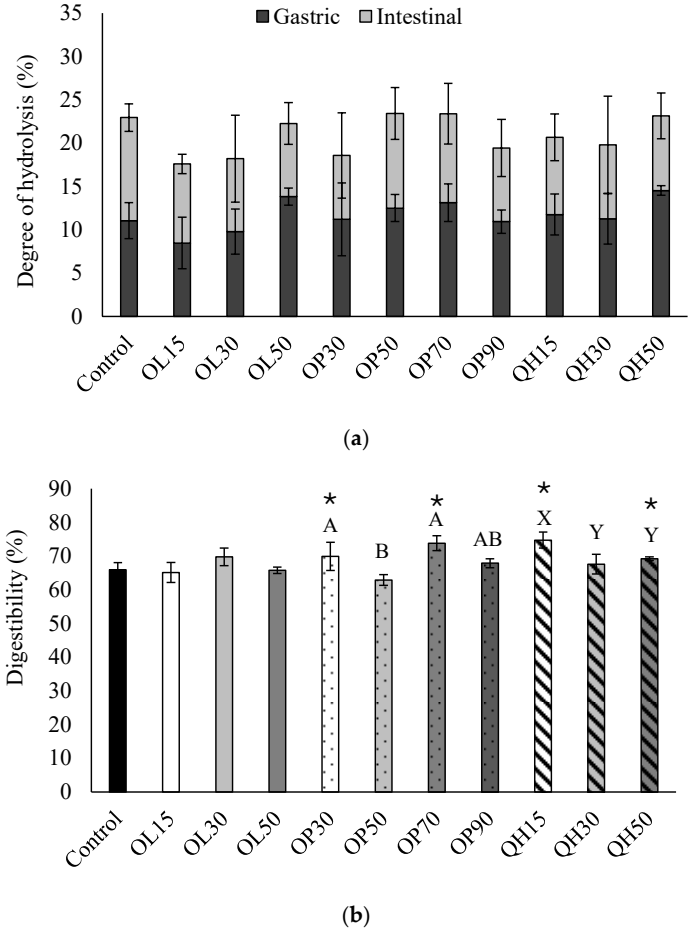


Figure 4. Degree of hydrolysis (**a**) and digestibility (**b**) of defatted BSFL meals as affected by the experimental diets (OL, dry olive leaves; OP, full-fat dry olive pomace, QH, quinoa husk). Different letters represent significant differences (A-B, among OP-based meals, X-Y, among QH-based diets). * Represents significant differences with respect to the control. Bars without letters or asterisks indicate no significant differences.

According to previous studies, and despite some of them having concluded that the insect proteins have a high digestibility, this cannot be generalized because there are different factors that can affect these results, such as the insect species or the composition of the meals. In this sense, the chitin co-existence of insect meals has been described to interfere with the digestibility of proteins [25], although this has not been globally generalized. In the present study, it is interesting to remark that, despite the significant higher chitin content of the meals obtained due to the level of replacement with the experimental by-products, as well as the variable values of ADF and ADIP (Table 3), which may affect the digestibility [25], such effects did not impact the global hydrolysis of the proteins of the meals (Figure 4a), which should be considered a noticeable result. At least, this was not evidenced by the used digestibility method, which did not include the total breakdown of dipeptides or oligopeptides during digestion. Therefore, further studies, in vivo as possible, would be needed to validate the observed results.

4. Conclusions

Feeding BSFL with by-products from olive or quinoa production, despite causing different lipid content in the larvae, does not impact the supercritical CO_2 defatting. Thus,

the behaviour of the supercritical CO_2 process primarily depends on the content and solubility of extractable material in the dried larvae, while differences in the packed bed microstructure have a minor effect.

Furthermore, the feeding assay with these by-products, after following the supercritical defatting process, allows us to produce protein-rich meals, although with lower protein content due to most of the used by-products. Nevertheless, the feeding with full-fat dry olive pomace at 50% in the larvae diets can be concluded as a remarkable substrate, since this allows the production of larvae with the same protein as conventional-fed larvae, a higher ratio of essential amino acids, and no impact on the digestibility of the meal, while maintaining defatting process performance. Additionally, all the experimental BSFL defatted meals present a notable enrichment in chitin that can be considered of interest depending on the final intended use of the meal and that does not compromise the digestibility of the meals.

Therefore, this study demonstrates the potential of incorporating agro-industrial by-products from olive oil and quinoa production into BSFL diets as an effective strategy for the bioconversion of these low-value residues into high-value insect-derived meals, enabling the modulation of nutritional properties and digestibility of the insect meals, and without compromising the efficiency of the supercritical CO_2 defatting process of the larvae.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/insects17020161/s1, Table S1: Amino acid profile (g/100 g) of the defatted BSFL meals.

Author Contributions: Conceptualization, T.F. and D.M.; methodology, E.R.-G., D.M.-H., M.D.H., A.T.V., D.F., T.F., M.R.G.-R. and D.M.; formal analysis, E.R.-G., D.M.-H., M.D.H., A.T.V., D.F., M.R.G.-R. and D.M.; investigation, E.R.-G., D.M.-H., M.D.H., A.T.V. and D.F.; resources, M.D.H. and D.M.; data curation, E.R.-G., D.M.-H., M.D.H., A.T.V. and D.F.; writing—original draft preparation, E.R.-G., M.D.H. and D.M.; writing—review and editing, T.F. and D.M.; visualization, E.R.-G., T.F. and D.M.; supervision, M.R.G.-R. and D.M.; project administration, D.M.; funding acquisition, M.D.H. and D.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

BSFL	Black soldier fly larvae
FAO	Food and Agriculture Organization of the United Nations
OL	Olive leaves
OP	Olive pomace
QH	Quinoa husk

AOAC	Association of Official Analytical Chemists
ADF	Acid detergent fiber
ADIP	Amount of protein bound to the ADF fraction
OPA	O-phthaldialdehyde
DH	Degree hydrolysis
NSC	Non-structural carbohydrate content

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