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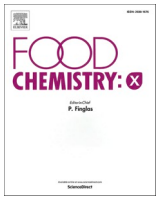

Study of the effect of feeding *Tenebrio molitor* larvae during their rearing on their growth, nutritional profile, value and safety of the produced flour

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ABSTRACT

Science and food industry must strive to ensure and improve edible insect's benefits, and especially their safety and nutritional value. This study investigated how various food substrates used in the rearing of *Tenebrio molitor* larvae influence their growth, the safety of the larvae, and the nutritional quality of the resulting flour. The main findings indicate that all samples showed significant differences in their nutritional profile, larval characteristics, and heavy metal content. Regarding the content of protein, fat and fiber it ranges from 44.1 to 51.8 %, 28.6–34.8 % and 10.5–14.9 %, respectively. These results suggest that insect diet is a very crucial parameter that can affect all that factors and must be taken into account, especially when they are intended as raw materials to be used for food production.

1. Introduction

The necessity for developing new protein sources has become increasingly urgent in the face of growing global population (nearly 10 billion by 2050) and the environmental challenges of traditional livestock production (Stull & Patz, 2020). Livestock, particularly beef, contributes up to 26 % of anthropogenic greenhouse gas emissions, 64 % of global ammonia emissions and requires up to 43,000 L of water per kg, with 70 % of global freshwater already used for agriculture (FAO, 2006; FAO, 2021). The utilization of edible insects as a sustainable protein source has garnered significant attention in recent years due to their environmental benefits and nutritional value (Can Karaca et al., 2023). In contrast to animal protein, edible insects require significantly less feed (2.1 kg/kg of live weight), land (18 m^2/kg of protein), and water (23 L/g of protein) and emit only 14–19 kg CO_2-equivalent per kg of protein compared to beef's 77–175 kg CO_2-equivalent (FAO, 2021). Moreover, insects present a protein-rich alternative to conventional livestock, boasting an average protein content ranging from 35 % to 65 %, with specific species reaching up to 77 % (Can Karaca et al., 2023; Papastavropoulou et al., 2021). Their abundance in essential amino acids and micronutrients underscores their potential as a sustainable and nutritious food source (Can Karaca et al., 2023; da Silva Lucas et al., 2020; Papastavropoulou et al., 2021; Rumpold & Schlüter, 2013). Thus, insects present a more sustainable and efficient alternative, crucial for future food security and environmental preservation (van Huis et al., 2013). Among edible insects, *Tenebrio molitor* is one of the most studied insects in the scientific world of entomophagy. The yellow mealworm (*T. molitor* larvae) is a novel food according to Regulation (EU) 2015/2283. Moreover, according to the assessments carried out by EFSA, heat-dried or freeze-dried larvae, can be consumed whole or in powder form by all population groups (EFSA Panel on Nutrition et al., 2021; Moruzzo et al., 2021). In the feed sector, currently EU Regulations 2017/893 and 2021/1372, amending Regulation 999/2001, allow the use of proteins derived from insects to feed fish, domestic animals, poultry, and pigs,

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but not for ruminants (Errico et al., 2022). In the European Union, it is included in the list of insects with the highest potential as food and animal feed (Errico et al., 2022; Gkinali et al., 2022). *T. molitor* larvae boast a protein content ranging from 37 % to 68 % of dry weight, comparable to conventional sources like meat and soybeans (Ghaly & Alkoaik, 2009; Papastavropoulou et al., 2021; Stull et al., 2019). Furthermore, their amino acid profile includes all essential amino acids crucial for human health, while their fatty acid composition is rich in beneficial unsaturated fats such as oleic acid and linoleic acid (Papastavropoulou et al., 2021; Stull et al., 2019). These insects primarily consume decaying seeds and grains but can also feed on non-spoiled items like flour, cereals, and meat scraps (Ghaly & Alkoaik, 2009). They can be obtained through wild harvesting, semi-domestication, or cultivation, with insect farming offering a more sustainable option (Yen, 2015). Currently, wild harvesting dominates, but there is growing interest in insect farming, with 241 companies worldwide producing edible insects as of March 2019 (van Huis, 2020). Mealworms, cockroaches, and some beetles are fully domesticated and ideal for cultivation (Baiano, 2020). Adherence to production and hygiene regulations is crucial for ensuring the safety and quality of insect products (van Huis et al., 2013), considering factors like microbial safety, toxicity, allergenicity, and nutritional value.

According to several studies that have been done, both the growth and the nutritional composition of the *T. molitor* larvae are qualitatively and quantitatively affected by the diet of the insects during their rearing (Bawa et al., 2020; Bordiean et al., 2022; Dreassi et al., 2017; Hong et al., 2020; Kröncke & Benning, 2023). It has been observed that cereal-based rearing does not vary the basic nutritional composition (e.g. crude protein, crude fat and moisture) of *T. molitor* larvae significantly (Alves et al., 2016; Ramos-Elorduy et al., 2002; van Broekhoven et al., 2015). While it has been shown that, the fatty acid profile is affected by a diet enriched in unsaturated fatty acids (Alves et al., 2016). Also, higher protein content and lower fat content were observed when vegetable waste was used as feed, compared to a cereal-based diet (Li et al., 2013). Furthermore, better reproductive performance and larvae growth have been observed with administration of starchy substrates (wheat bran, flour) (Rumbos et al., 2020). In addition, insects may contain hazardous chemicals from the breeding process, such as heavy metals (Hong et al., 2020; Papastavropoulou et al., 2023). Heavy metals, which can be found in insects in most cases, are lead (Pb), mercury (Hg), arsenic (As), and cadmium (Cd) (Mlček et al., 2017; Papastavropoulou et al., 2023). In a study by Mlček et al. (2017), it was reported that the content of cadmium in dry larvae of *T. molitor* was 147–230 mg/kg. Moreover, *T. molitor* larvae showed concentrations of various heavy metals within the following ranges (mg/kg wet weight): Cd 0.008–0.016, Pb 0.063–0.079, Ni 0.03–0.63, As 0.021–0.023, Hg 0.12×10^{-3}–0.49×10^{-3} (Truzzi et al., 2019). Studies have investigated the bioaccumulation of heavy metals in *T. molitor* larvae, in relation to their rearing conditions and food substrate (Mlček et al., 2017; Truzzi et al., 2019; van der Fels-Klerx et al., 2016). According to these studies the bioaccumulation factor for As ranged from 1.1 to 2.6, for Cd 0.8–2.5 and 1.5–6.2 for Hg. In fact, Truzzi et al. (2019) found that Pb bioaccumulation factor was 34 when larvae were fed with 100 % organic wheat flour compared to a mixture of 75 % organic wheat flour and 25 % organic olive-pomace, where the bioaccumulation factor was reduced to 6.1. Although the findings on the accumulation of heavy metals in edible insects are limited, however, it has been found to depend on several factors, such as the insect species, its developmental stage, rearing conditions and food substrate (Hong et al., 2020; Papastavropoulou et al., 2023). Therefore, there is a possibility of finding hazardous heavy metals in edible insects and one of the most critical parameters is the food substrate during insect rearing (Mlček et al., 2017). Interest in *T. molitor* larvae as food and feed has increased. Therefore, there is a need to develop an improved rearing substrate of *T. molitor* to ensure a better nutritional composition of yellow mealworm and make their production more efficient and safer. The promotion and use of edible insects in food systems must be improved and they should not be reared with incorrect industrial practices and cheap and often unsuitable food substrates. This study investigates the impact of diverse food substrates on the growth, safety, and nutritional profile of *T. molitor* larvae, emphasizing the crucial role of diet selection for enhancing mealworm suitability as a food source. By comprehensively analyzing many nutritional parameters, assessing heavy metal contamination, and exploring unconventional food substrates, the research offers novel insights into the optimization of edible insect production for improved food safety and sustainability.

The purpose of our study is to investigate the effect of the insect diet during its rearing, on the nutritional profile and safety of its produced flour and the growth of the *T. molitor* larvae. For this purpose, nine mealworm samples were tested, feeding by different food substrates, in order to determine if there are differences in their growth (larvae weight), the nutritional profile of produced flour and the heavy metals contamination. By analyzing various parameters, we tried to understand the implications of dietary variations on larval development and flour composition. The findings will contribute to our understanding of insect rearing practices and inform strategies for improving the safety and nutritional value of edible insects, with the aim to promoting them as a sustainable and nutritious food source for human consumption, while also addressing concerns related to heavy metal contamination and dietary composition. Because the most important factors where need to pay a lot of attention are the safety and the nutritional value of insects intended for food or feed. For these reasons, through this research we wanted to examine the effect of the food substrate, on the safety and nutritional value of these insects, with the main goal of making mealworms a safer and more nutritious food for humans.

2. Materials and methods

2.1. Samples - equipment - reagents

The samples analyzed were the mealworms (*T. molitor* larvae), reared on 9 different food substrates. Moisture, ash, protein, amino acid profile, total fat, fatty acid profile, total fiber, minerals and heavy metals were determined in these samples. Larval weight gain was also recorded for all samples. The insect samples and the food substrates which *T. molitor* larvae were grown are shown in Table 1. It is worth noting that the standard diet of *T. molitor* larvae is that of sample 1 (wheat bran), while the other diets are treatments.

In order to obtain the larvae used in the experiments, 15–20 adult *T. molitor* insects (1:1 female:male) were introduced in each of 3 boxes with wheat bran (standard diet) and allowed to oviposit. After 15 days, the adults were removed, and their larvae were collected using a sterile standard No. 10 sieve (2 mm openings). A group of 300 randomly collected larvae 0.8–1 mm in length were introduced together with 300 g of food substrate in a box. In all cases, larvae received carrot and potato cubes provided as a water source at 4-day intervals. Two boxes per treatment were used. Boxes were kept under controlled conditions (25 ± 1 °C, 65 ± 5 % RH and 16:8 L:D photoperiod) for 5 weeks. During this period, the larvae in each box were inspected and no dead

Table 1
Diets of the 9 different samples of the *Tenebrio molitor* larvae.

Mealworms	Food substrate
Sample 1	Wheat bran
Sample 2	Oats
Sample 3	Sunflower seeds-wheat bran (1:4)
Sample 4	Wheat bran +25 % flaxseed
Sample 5	Wheat bran +25 % carob flour
Sample 6	Wheat Bran +25 % seaweed (*Posidonia oceanica*)
Sample 7	Wheat bran +12.5 % flaxseed
Sample 8	Wheat bran +12.5 % carob flour
Sample 9	Wheat bran +12.5 % seaweed (*Posidonia oceanica*)

individuals were recorded. At the end of the 5-week period, the *T. molitor* larvae of each box were separated from the food substrate using a sterile standard No. 10 sieve (2 mm openings) and all the larvae weighed in groups of 10 (i.e. 30 groups (replicates) per box). Afterwards, they were kept without food for at least 24 h, in order to allow the normal excretion of the non-nutritive elements of their diet and to complete their digestion. They were then weighed into Petri dishes, which were placed in the refrigerator to inactivate and avoid stressing the insects. Then, they were placed in a deep freezer (−80 °C). Finally, they were placed for lyophilization, and the dry larvae were homogenized to obtain them in the form of flour.

Reagents used were as follows: NaH_2PO_4 and KOH (Honeywell, Fluka), Na_2HPO_4 99 %, H_3PO_4, Methanesulfonic acid (MSA), Formic acid 98 % and TDF-100 A Kit (α-amylase, protease, amyloglucosidase, celite) (Sigma Aldrich), HNO_3 65 % (Macron Fine Chemicals), HCl 37 %, $H_2SO_4 \geq 95$ %, Ammonium formate, Methanol and Diethyl ether ≥99.5 % (Fisher Chemical), NaOH, H_3BO_3 and Na_2SO_4 (Lachner), $CuSO_4$*5H2O 99 % (Alfa Aesar), K_2SO_4 and Ethanol 99.8 % (Acros Organics), Petroleum ether, Hydrogen peroxide 30 % (H_2O_2), Hexane (Carlo Erba Reagents), Acetonitrile (HPLC), Acetonitrile (LC-MS), Stock solution of Hg (1000 mg/L) (Merck), Multi-element standard solution 100 mg/L of 9 elements (Al, As, Cd, Co, Cr, Mo, Ni, Pb, Se) in 5 % HNO_3 (CPAchem Ltd) Trizma base ≥99.9 %.

The scientific equipment used was: Calibrated analytical balance with four decimal places (AB204-S/FACT, METTLER TOLEDO), Furnace (GALLENKAMP FURNACE), Rotary evaporator (EV 311H, LAB TECH), Water bath (WB12, ARGOLAB), Ultrasonic bath (D-78224, S15H ELMA), Centrifuge (NEYA8, NEYA), Kjeldahl digester (DK6 Heating Digester, VELP SCIENTIFICA), Kjeldahl distillation apparatus (UDK 139, VELP SCIENTIFICA), GC-FID Gas Chromatograph (SHIMADZU), Microwave Plasma Atomic Emission Spectrometer MP-AES (4210 MP-AES, AGILENT TECHNOLOGIES), NIR analyzer (DA 7250, PERKIN ELMER), Lyophilizer (TELSTAR), Thermoreactor (WTW CR 3200), Microwave digestion system (Mars X-PRESS, CEM), Spectrophotometer (DR12010, HACH), Hydrophilic interaction chromatograph with tandem mass spectrometry, HILIC-MS/MS (TSQ Quantum Access, THERMO FISHER SCIENTIFIC), Inductively Coupled Plasma Mass Spectrometry ICP-MS (Agilent 7900 ICP-MS, Agilent Technologies).

2.2. Methods of analysis

To determine these parameters, the following analysis methods were used. Moisture was determined by lyophilization, and ash based on AOAC 942.05. Total fat was determined according to the ISO 1443:1973 method. The fatty acid profile was determined by an in-house flame ionization detector gas chromatography method based on ISO-12966 2. Total fiber was determined based on AOAC 993.21 by a combination of enzymatic and gravimetric methods. Protein determination was done by the Kjeldahl method. Amino acids were determined by an in-house HILIC-MS/MS method. The minerals Fe, Cu, Mn, Zn, Ca, Mg, Na and K were measured using an in-house MPAES method, while phosphorus was determined according to REGULATION (EC) 152/2009. All the above analysis carried out are described in more detail in our previous publication (Papastavropoulou et al., 2021). In the case of amino acid analysis, the determination of all analytes, apart from tryptophan, was performed according to Papastavropoulou et al. (2021) with a minor modification in vial composition with acetonitrile:water 90:10 (*v/v*). In the case of tryptophan, the only step that was different was the use of 4 M NaOH instead of MSA based on Yust et al. (2004). Finally, the protein, total fat, fiber and ash content of the samples were also measured with a NIR (Near-infrared) analyzer.

For the determination of heavy metals and trace elements by ICP-MS, the calibration curves, prepared by appropriate dilution of stock solutions, ranged between 0.50 and 20.0 μg/L for all elements except for Hg for which the concentrations ranged between 0.25 and 8.0 μg/L. Table 2 presents information about the ICP-MS method.

Table 2
Analyte Parameters during ICP-MS analysis.

Element	Mass	Cell mode	Internal standard
Al	27	No Gas	Sc^{45}
Cr	52	He	Sc^{45}
Co	59	He	Ge^{72}
Ni	60	He	Ge^{72}
As	75	He	Ge^{72}
Se	78	H_2	Ge^{72}
Mo	95	He	In^{115}
Cd	111	He	In^{115}
Hg	202	He	Lu^{175}
Pb	206 + 207 + 208	He	Lu^{175}

2.3. Statistical analysis

All statistical analysis were conducted using SPSS software (IBM SPSS Statistics Version 29). The results were reported as mean ± standard deviation (SD) ($n = 3$). Tukey's multiple range test was used to compare the means. Variance (ANOVA) was used to analyze the significant differences between the samples with different diets. The threshold for significance for all tests was set at $p < 0.05$. Results followed by the same letter are not significantly different. The weight data of *T. molitor* larvae were log-transformed to meet the assumptions of ANOVA. The means were separated using the Tukey – Kramer HSD test. Analyses were conducted using the statistical package JMP (SAS Institute, 2016).

3. Results and discussion

3.1. Nutritional value and moisture content

The results of nutritional value of the insect flour samples and moisture content of fresh insects are presented in the Table 3. Through these results we can observe the variations in the analyzed parameters. Thus, we can draw conclusions about how the food substrate affects *T. molitor* larvae and their macronutrients. According to the results the composition of the larvae of *T. molitor* is affected by the breeding diet. This can be perceived both from the above results and from the results of another research (Bordiean et al., 2022; Dreassi et al., 2017; Rumbos et al., 2020). More specifically, we observe differences in the moisture content of the larvae, the highest content (65.2 %) was found in sample 9 and the lowest (51.2 %) in sample 2. The most important macronutrients are protein and fat. According to the results shown in Table 3, the highest protein percentage is contained in sample 3 (51.8 %) and the three lowest protein content values are in samples 2 (45.2 %), 4 (45.1 %) and 5 (44.1 %). Samples 6 (49.1 %), 7 and 9 (48 %) also had quite high protein percentages. Also, through the results we see variations in the percentages of the total fat of the samples as well as the fiber, with the presence of notable significant differences. For total fat the contents are from 28.6 to 34.8 %, and for fibers they are from 10.5 to 14.9 %. Finally, the ash contained in the insect flour shows significant differences in all samples, and the values range from 3.17 to 4.46 %. Such results have also been reported for mealworm and crickets in other studies (Kröncke & Benning, 2023; Oonincx et al., 2019; Rumbos et al., 2020).

3.2. Fatty acid profile

The fatty acid profile of mealworms excels in unsaturated fatty acids (UFA) compared to saturated fatty acids (SFA). The most abundant of the SFA are palmitic acid (16:0) (3.401–5.426 %), myristic (14:0) (0.624–1.428 %) and stearic acid (18:0) (0.842–1.632 %), of the MUFA are palmitoleic (C16:1n7 cis) (0.427–1.245 %) and oleic acid (18:1n-9 cis) (10.570–16.663 %) and of the PUFAs are α-linoleic acid (18:2n-6) (5.954–9.505 %), α-linolenic acid (18:3n-3) (0.061–3.000 %) and γ-linolenic acid (18:3n6) (0.617–1.669 %). These results are also in

Table 3
Nutritional Value of the insect flour samples and moisture content of fresh insects (± SD).

ANALYSIS PARAMETER	(g/100 g of insect flour ± SD)								
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Ashes	$3.17^{a} \pm 0.01$	$3.97^{b} \pm 0.03$	$4.46^{c} \pm 0.03$	$3.28^{d} \pm 0.01$	$3.39^{e} \pm 0.02$	$3.58^{f} \pm 0.02$	$4.34^{g} \pm 0.02$	$3.51^{h} \pm 0.01$	$3.31^{d} \pm 0.02$
Proteins	$47.8^{ae} \pm 0.9$	$45.2^{bd} \pm 0.4$	$51.8^{c} \pm 0.3$	$45.1^{bd} \pm 1.6$	$44.1^{d} \pm 0.6$	$49.1^{a} \pm 0.1$	$48.0^{ae} \pm 0.3$	$46.4^{be} \pm 0.2$	$48.0^{ae} \pm 0.2$
Total Fat	$31.2^{ab} \pm 0.4$	$34.8^{c} \pm 0.8$	$31.1^{ab} \pm 1.8$	$29.2^{ab} \pm 0.9$	$30.6^{ab} \pm 1.9$	$28.6^{a} \pm 1.0$	$32.6^{bc} \pm 0.3$	$31.5^{abc} \pm 0.8$	$30.1^{ab} \pm 2.0$
Dietary fiber	$12.9^{a} \pm 0.5$	$10.5^{b} \pm 0.6$	$10.5^{b} \pm 0.4$	$13.2^{ac} \pm 0.4$	$14.7^{d} \pm 0.8$	$13.2^{ac} \pm 0.5$	$13.0^{ac} \pm 0.3$	$14.9^{d} \pm 0.5$	$14.4^{cd} \pm 0.4$

	(g/100 g of fresh insect ± SD)								
Moisture	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
	$62.2^{a} \pm 0.2$	$51.2^{b} \pm 0.1$	$59.8^{c} \pm 0.2$	$60.8^{d} \pm 0.3$	$62.7^{a} \pm 0.2$	$60.8^{d} \pm 0.2$	$62.8^{a} \pm 0.4$	$64.2^{e} \pm 0.3$	$65.2^{f} \pm 0.4$

agreement with other research findings (Bordiean et al., 2022; Dreassi et al., 2017; Hong et al., 2020). Only two fatty acids are known to be essential for humans, α-linolenic acid (n-3 fatty acid) and linoleic acid (n-6 fatty acid), which cannot be synthesized by humans and must be obtained through food. The fat content as well as the fatty acid profile of insects can be dependent on feed composition and rearing conditions, as demonstrated by this research. According to the results shown in Table 4, we conclude that the diet followed by the insect during its rearing plays a very important role in the fat content and in the qualitative and quantitative formation of its fatty acids. As in the 9 different samples analyzed, remarkable differences were found between the concentrations of all fatty acid categories (SFA, MUFA, PUFA, n-3, n-6). Also very important were the differences in the concentrations of EPA and DHA with huge differences in their concentrations in some samples. Even greater differences were found in the total n-3 and n-6 fatty acids but also in the ratio between them, which is a very important factor. Total n-3 has a value of 0.131–3.42 % and total n-6 from 7.33 to 10.88 %. Moreover, it is important to mention that sample 3 had lower content of saturated fatty acids (5.44 %), and sample 2 had higher content of MUFA (18.52 %), and PUFA content ranges between 9.18 and 12.64 %. Finally, the α-linolenic content showed dramatically significant differences between the samples and was shown to be affected by the feed substrate significantly, especially for samples 4 and 7 with 25 % and 12.5 % flaxseed in their diet, the results were extremely high (2.843 % and 3.000 %, respectively) compared to the rest of the samples. This effect is evidently due to the enrichment of the substrate with flaxseed, which is rich in α-linolenic acid.

3.3. Minerals

From Table 5 where the results for the minerals in the insect flour are given, it is clear that these are also affected by the food factor given to the insects. In fact, we also notice large significant differences that exist for all metals in many of the samples, and especially in samples 2 and 3. In general, samples 2, 3 and 9 have the best results with fairly high values, while 2, 3 also have the lowest Na values. Sample 3 had the highest concentrations of Mg (5145 mg/kg), P (11,039 mg/kg), and Zn (115 mg/kg). Sample 2 had the lowest Na concentration (452 mg/kg) and the highest K concentration (12,338 mg/kg). Sample 9 had the highest Ca and Cu values (585 and 35.5 mg/kg, respectively). While the highest Fe concentration was in sample 7 (66 mg/kg) and the highest Mn concentrations were in samples 7, 8, 9 (19.4, 19.6, 18.9 mg/kg, respectively). The ranges for the minerals in mg/kg are, Magnesium 2300–5145, Phosphorus 7805–11,039, Calcium 305–585, Sodium 452–1682, Potassium 8865–12,338, Zinc 77.6–115, Iron 31.4–66.0, Copper 10.8–35.3, Manganese 12.5–19.6. From the minimum and maximum values of the mineral and from the statistical results for the significance of the differences in the mean values, it can be seen that there are differences in the results between the samples with different diets. Mainly, based on the contents of minerals and the results for the statistically significant differences, samples 2 and 3 showed distinct differences and stood out from the other samples. While sample 3 presented the highest concentrations of most minerals, with significant differences and a more favorable profile of minerals.

3.4. Amino acid profile

The results for amino acids of *T. molitor* larvae flour, which are listed in Table 6, showed that the diet followed by the larvae during their rearing can affect their concentration. Also, according to recent research, the larval amino acid profile is significantly affected both qualitatively and quantitatively when the food substrate is changed during larval rearing (Kröncke & Benning, 2023). While some amino acids show relatively consistent results across the samples, others exhibit more pronounced variation. For instance, amino acids like arginine, glutamic acid and phenylalanine demonstrate relatively consistent levels across samples with no significant differences. On the other hand, amino acids such as alanine, glycine, threonine, leucine, histidine, lysine, proline, serine, tryptophan, cystine and tyrosine showcase more significant differences in their content across the samples. Thus, it is crucial to consider each amino acid individually to gain a comprehensive understanding of the similarities and dissimilarities observed among the samples. Among the amino acids analyzed, Sample 8 has the lowest amount of Alanine at 3.03 g/100 g, while Sample 9 contains the highest level at 4.17 g/100 g. Arginine ranges from a minimum of 2.10 g/100 g in Samples 2, 4, and 5 to a maximum of 2.42 g/100 g in Sample 6. Aspartic acid shows a minimum of 3.88 g/100 g in Sample 2 and a maximum of 4.67 g/100 g in Sample 6. Valine ranges from 2.42 g/100 g (minimum, Sample 5) to 2.99 g/100 g (maximum, Sample 3). Glutamic acid varies from 5.24 g/100 g (minimum, Sample 2) to 5.89 g/100 g (maximum, Samples 4 and 5). The minimum value for Glycine is 2.56 g/100 g in Sample 2, while the maximum value of 3.11 g/100 g is found in Sample 6. Threonine ranges from 1.27 g/100 g (minimum, Sample 5) to 2.08 g/100 g (maximum, Sample 3). Isoleucine varies from 1.64 g/100 g (minimum, Sample 5) to 2.25 g/100 g (maximum, Sample 3). Leucine shows a minimum of 3.62 g/100 g in Sample 5 and a maximum of 5.32 g/100 g in Sample 3. Histidine ranges from 0.98 g/100 g (minimum, Sample 5) to 1.58 g/100 g (maximum, Sample 3). Cystine ranges from 0.20 g/100 g (minimum, Sample 1) to 0.33 g/100 g (maximum, Sample 6). Lysine varies from 2.13 g/100 g (minimum, Sample 1) to 3.11 g/100 g (maximum, Sample 3). Methionine shows a minimum value of 0.31 g/100 g in Sample 1 and a maximum value of 0.46 g/100 g in Sample 3. Proline ranges from 2.73 g/100 g (minimum, Sample 9) to 3.48 g/100 g (maximum, Sample 3). Serine varies from 1.57 g/100 g (minimum, Sample 5) to 2.34 g/100 g (maximum, Sample 3). Tryptophan ranges from 0.37 g/100 g (minimum, Sample 2) to 0.69 g/100 g (maximum, Sample 6). Tyrosine shows a minimum of 2.60 g/100 g in Sample 9 and a maximum of 4.28 g/100 g in Sample 3. Lastly, Phenylalanine ranges from 1.40 g/100 g (minimum, Sample 5) to 1.57 g/100 g (maximum, Sample 4). When comparing the amino acid profiles of the samples, it is apparent that there are variations in amino acid composition and levels. Some samples show higher total

Table 4
Fatty acid content (g/100 g) of insect flour (± SD).

(g/100 g of insect flour ± SD)									
Fatty acids	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Capric, C10:0	0.015^{a} ± 0.001	0.017^{ab} ± 0.001	0.003^{c} ± 0.001	0.023^{bd} ± 0.002	0.024^{d} ± 0.002	0.034^{e} ± 0.003	0.028^{de} ± 0.002	0.029^{de} ± 0.002	0.029^{de} ± 0.004
Undecylic, C11:0	0.0019^{af} ± 0.0003	0.0014^{ab} ± 0.0003	0.0008^{b} ± 0.0001	0.0032^{cd} ± 0.0002	0.0025^{acf} ± 0.0004	0.0052^{e} ± 0.0003	0.0034^{d} ± 0.0002	0.0047^{e} ± 0.0002	0.0032^{cd} ± 0.0003
Lauric, C12:0	0.106^{a} ± 0.002	0.081^{b} ± 0.001	0.051^{c} ± 0.001	0.080^{b} ± 0.001	0.081^{b} ± 0.002	0.079^{b} ± 0.001	0.076^{b} ± 0.003	0.081^{b} ± 0.002	0.090^{d} ± 0.004
Tridecylic, C13:0	0.022^{ab} ± 0.003	0.012^{cd} ± 0.002	0.006^{d} ± 0.001	0.026^{b} ± 0.005	0.005^{d} ± 0.001	0.024^{ab} ± 0.001	0.026^{b} ± 0.003	0.026^{b} ± 0.002	0.017^{ac} ± 0.003
Myristic, C14:0	0.865^{a} ± 0.041	1.428^{b} ± 0.036	0.783^{acd} ± 0.051	0.683^{cde} ± 0.039	0.802^{ac} ± 0.054	0.646^{de} ± 0.055	0.624^{e} ± 0.058	0.734^{acde} ± 0.047	0.714^{cde} ± 0.060
Myristoleic, C14:1	0.167^{a} ± 0.015	0.351^{bcd} ± 0.038	0.176^{a} ± 0.012	0.285^{b} ± 0.021	0.295^{bc} ± 0.018	0.395^{d} ± 0.032	0.329^{bcd} ± 0.022	0.320^{bc} ± 0.025	0.357^{cd} ± 0.030
Pentadecylic, C15:0	0.038^{a} ± 0.002	0.015^{b} ± 0.001	0.006^{c} ± 0.001	0.022^{d} ± 0.002	0.035^{ae} ± 0.004	0.023^{d} ± 0.002	0.024^{d} ± 0.001	0.031^{ef} ± 0.002	0.027^{df} ± 0.003
Pentadecenoic, C15:1	0.006^{ab} ± 0.002	0.004^{a} ± 0.001	0.003^{a} ± 0.001	0.010^{bc} ± 0.002	0.011^{bcd} ± 0.001	0.016^{d} ± 0.003	0.011^{bcd} ± 0.002	0.011^{bcd} ± 0.001	0.015^{bcd} ± 0.002
Palmitic, C16:0	5.426^{a} ± 0.121	3.736^{b} ± 0.109	3.401^{b} ± 0.201	4.505^{c} ± 0.230	4.774^{ac} ± 0.214	4.848^{ac} ± 0.160	5.012^{ac} ± 0.155	4.865^{ac} ± 0.244	4.987^{ac} ± 0.461
Palmitoleic, C16:1n7cis	0.629^{ab} ± 0.007	1.245^{c} ± 0.005	0.484^{de} ± 0.030	0.595^{a} ± 0.005	0.585^{a} ± 0.027	0.464^{de} ± 0.015	0.513^{d} ± 0.019	0.673^{b} ± 0.008	0.427^{e} ± 0.035
Margaric, C17:0	0.049^{a} ± 0.001	0.023^{b} ± 0.001	0.021^{b} ± 0.001	0.066^{c} ± 0.003	0.047^{a} ± 0.005	0.058^{c} ± 0.002	0.059^{c} ± 0.003	0.065^{c} ± 0.002	0.059^{c} ± 0.005
Heptadecenoic, C17:1	0.017^{a} ± 0.002	0.030^{b} ± 0.003	0.018^{a} ± 0.001	0.019^{a} ± 0.001	0.015^{a} ± 0.004	0.021^{a} ± 0.002	0.018^{a} ± 0.003	0.017^{a} ± 0.001	0.019^{a} ± 0.004
Stearic, C18:0	1.160^{ab} ± 0.098	0.842^{c} ± 0.044	1.007^{ac} ± 0.073	1.366^{bde} ± 0.100	1.290^{bd} ± 0.114	1.574^{ef} ± 0.061	1.632^{f} ± 0.056	1.472^{def} ± 0.089	1.585^{ef} ± 0.119
Elaidic, C18:1n9 trans	0.017^{a} ± 0.002	0.123^{b} ± 0.009	0.099^{c} ± 0.004	0.013^{a} ± 0.002	0.019^{ad} ± 0.002	0.029^{d} ± 0.002	0.018^{ad} ± 0.003	0.013^{a} ± 0.002	0.015^{a} ± 0.003
Oleic, C18:1n9 cis	11.849^{abc} ± 0.191	16.663^{d} ± 0.204	13.795^{e} ± 0.789	10.570^{a} ± 0.122	12.709^{ce} ± 0.622	10.599^{a} ± 0.284	11.262^{ab} ± 0.350	12.406^{bce} ± 0.422	11.673^{abc} ± 0.810
Linoelaidic, C18:2n6 trans	0.014^{abc} ± 0.002	0.011^{ab} ± 0.001	0.007^{a} ± 0.002	0.029^{d} ± 0.002	0.017^{bc} ± 0.005	0.018^{bc} ± 0.002	0.020^{c} ± 0.001	0.013^{abc} ± 0.002	0.015^{bc} ± 0.004
Linoleic, C18:2n6 cis	9.146^{a} ± 0.161	8.593^{ab} ± 0.156	9.505^{a} ± 0.553	5.954^{c} ± 0.252	7.528^{de} ± 0.424	6.671^{cd} ± 0.221	7.666^{be} ± 0.247	7.952^{be} ± 0.185	7.163^{de} ± 0.555
γ-Linolenic, C18:3n6	0.617^{a} ± 0.082	1.227^{b} ± 0.199	1.352^{bc} ± 0.095	1.117^{b} ± 0.136	1.200^{b} ± 0.068	1.669^{d} ± 0.053	1.327^{bc} ± 0.090	1.402^{bcd} ± 0.041	1.574^{cd} ± 0.135
α-Linolenic, C18:3n3	0.386^{a} ± 0.013	0.066^{b} ± 0.002	0.061^{b} ± 0.004	2.843^{c} ± 0.294	0.330^{a} ± 0.034	0.236^{d} ± 0.018	3.000^{c} ± 0.144	0.362^{a} ± 0.016	0.252^{d} ± 0.031
Arachidic, C20:0	0.071^{ab} ± 0.008	0.046^{c} ± 0.002	0.056^{ac} ± 0.003	0.085^{bd} ± 0.005	0.128^{e} ± 0.014	0.086^{bd} ± 0.006	0.103^{d} ± 0.007	0.105^{d} ± 0.003	0.126^{e} ± 0.009
Eicosenoic, C20:1n9	0.038^{ab} ± 0.001	0.031^{b} ± 0.001	0.025^{b} ± 0.002	0.026^{b} ± 0.002	0.030^{b} ± 0.007	0.034^{b} ± 0.002	0.031^{b} ± 0.003	0.070^{c} ± 0.002	0.052^{a} ± 0.014
Eicosadienoic, C20:2n6	0.066^{a} ± 0.005	0.013^{b} ± 0.001	0.014^{b} ± 0.001	0.089^{c} ± 0.004	0.107^{e} ± 0.009	0.099^{cde} ± 0.003	0.092^{cd} ± 0.003	0.108^{e} ± 0.004	0.104^{de} ± 0.008
Henicosanoic, C21:0	0.003^{a} ± 0.001	0.011^{bc} ± 0.001	0.008^{ab} ± 0.001	0.006^{ab} ± 0.001	0.004^{a} ± 0.001	0.018^{d} ± 0.002	0.006^{ab} ± 0.001	0.006^{ab} ± 0.002	0.015^{cd} ± 0.004
Homo-γ–Linolenic, C20:3n6	0.005^{abc} ± 0.001	0.004^{ab} ± 0.001	–	0.006^{bc} ± 0.002	0.002^{a} ± 0.001	0.006^{bc} ± 0.001	0.002^{a} ± 0.001	0.008^{c} ± 0.001	0.006^{bc} ± 0.002
Arachidonic, C20:4n6	0.003^{a} ± 0.001	0.004^{a} ± 0.001	0.003^{a} ± 0.001	0.026^{b} ± 0.003	0.006^{a} ± 0.002	0.006^{a} ± 0.001	0.023^{b} ± 0.002	0.002^{a} ± 0.001	0.006^{a} ± 0.002
Eicosatrienoic, C20:3n3	0.039^{a} ± 0.003	–	–	0.094^{cd} ± 0.006	0.071^{e} ± 0.009	0.104^{c} ± 0.004	0.133^{b} ± 0.004	0.082^{de} ± 0.002	0.073^{e} ± 0.011
Behenic, C22:0	–	–	–	–	–	–	–	–	–
Eicosapentaenoic, C20:5n3 EPA	0.222^{cde} ± 0.017	0.054^{a} ± 0.002	0.055^{a} ± 0.002	0.198^{cd} ± 0.021	0.150^{b} ± 0.015	0.251^{ef} ± 0.007	0.235d^{ef} ± 0.010	0.185^{bc} ± 0.010	0.270^{f} ± 0.027
Erucic, C22:1n9	0.007^{a} ± 0.001	0.010^{a} ± 0.001	0.012^{a} ± 0.001	0.008^{a} ± 0.001	0.033^{b} ± 0.005	0.056^{c} ± 0.003	0.043^{d} ± 0.002	0.011^{a} ± 0.002	0.008^{a} ± 0.001
Docosadienoic, C22:2	0.026^{a} ± 0.004	0.002^{b} ± 0.001	0.003^{b} ± 0.001	0.113^{c} ± 0.005	0.056^{d} ± 0.005	0.089^{e} ± 0.004	0.085^{e} ± 0.006	0.078^{e} ± 0.004	0.111^{c} ± 0.009
Tricosanoic, C23:0	0.087^{ab} ± 0.012	0.013^{c} ± 0.002	0.024^{c} ± 0.001	0.199^{d} ± 0.024	0.140^{b} ± 0.020	0.286^{e} ± 0.021	0.036^{ac} ± 0.005	0.232^{de} ± 0.014	0.229^{d} ± 0.038
Lignoceric, C24:0	0.004^{a} ± 0.001	0.087^{b} ± 0.006	0.071^{c} ± 0.006	0.016^{d} ± 0.002	0.004^{a} ± 0.001	0.027^{e} ± 0.002	0.014^{d} ± 0.001	0.005^{a} ± 0.001	0.004^{a} ± 0.001
Nervonic, C24:1n9	0.037^{ab} ± 0.001	0.059^{e} ± 0.001	0.034^{a} ± 0.002	0.068^{c} ± 0.001	0.056^{e} ± 0.005	0.051^{de} ± 0.003	0.043^{bd} ± 0.002	0.055^{e} ± 0.002	0.039^{ab} ± 0.006
Docosahexaenoic, C22:6n3 DHA	0.030^{a} ± 0.001	0.030^{a} ± 0.002	0.014^{b} ± 0.001	0.100^{c} ± 0.005	0.047^{d} ± 0.004	0.028^{a} ± 0.002	0.057^{d} ± 0.004	0.047^{d} ± 0.003	0.035^{a} ± 0.008
Total Omega-3	0.68^{a} ± 0.02	0.150^{b} ± 0.003	0.131^{c} ± 0.007	3.23^{d} ± 0.27	0.60^{ae} ± 0.06	0.62^{e} ± 0.03	3.42^{d} ± 0.32	0.67^{ae} ± 0.03	0.63^{ae} ± 0.08
Total Omega-6	9.88^{ab} ± 0.42	9.85^{ab} ± 0.36	10.88^{a} ± 0.65	7.33^{c} ± 0.49	8.92^{b} ± 0.85	8.56^{bc} ± 0.37	9.22^{b} ± 0.33	9.56^{ab} ± 0.25	8.98^{b} ± 0.75
Saturated	7.85^{a} ± 0.11	6.31^{bc} ± 0.20	5.44^{b} ± 0.32	7.08^{ac} ± 0.33	7.34^{a} ± 0.48	7.71^{a} ± 0.35	7.64^{a} ± 0.24	7.66^{a} ± 0.30	7.89^{a} ± 0.42

(*continued on next page*)

Table 4 (*continued*)

(g/100 g of insect flour ± SD)									
Fatty acids	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Monounsaturated	12.77^{abc} ± 0.25	18.52^{d} ± 0.25	14.65^{e} ± 0.84	11.59^{a} ± 0.34	13.75^{ce} ± 0.54	11.66^{a} ± 0.33	12.27^{ab} ± 0.27	13.58^{bce} ± 0.20	12.61^{abc} ± 0.71
Polyunsaturated	10.55^{ab} ± 0.24	10.00^{abc} ± 0.36	11.01^{b} ± 0.66	10.57^{ab} ± 0.45	9.51^{ac} ± 0.52	9.18^{c} ± 0.26	12.64^{d} ± 0.24	10.24^{abc} ± 0.19	9.61^{ac} ± 0.55
EPA + DHA	0.25^{ab} ± 0.02	0.084^{c} ± 0.001	0.069^{c} ± 0.003	0.30^{d} ± 0.02	0.20^{e} ± 0.02	0.28^{bd} ± 0.01	0.29^{bd} ± 0.01	0.23^{ae} ± 0.01	0.31^{d} ± 0.03

Table 5
Concentration of minerals in mg per kg of insect flour (± SD).

Minerals	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Magnesium (Mg)	2300^{a} ± 44	3757^{b} ± 96	5145^{c} ± 90	2600de ± 50	2510^{e} ± 42	2738^{d} ± 49	2472^{ae} ± 51	2531^{e} ± 48	2524^{e} ± 114
Phosphorus (P)	7805^{a} ± 185	8977^{c} ± 202	11039^{d} ± 217	8249^{ab} ± 175	8620b^{c} ± 243	9062^{c} ± 199	8978^{c} ± 297	8140^{ab} ± 221	10639^{d} ± 198
Calcium (Ca)	347^{d} ± 11	403^{a} ± 12	555^{b} ± 16	305^{c} ± 18	344^{cd} ± 17	570^{b} ± 13	353^{d} ± 12	381^{ad} ± 15	585^{b} ± 14
Sodium (Na)	1264^{c} ± 55	452^{a} ± 25	689^{b} ± 41	1320^{cd} ± 59	1434^{d} ± 65	1657^{e} ± 58	1358^{cd} ± 68	1359^{cd} ± 63	1682^{e} ± 74
Potassium (K)	8961^{b} ± 327	12338^{a} ± 212	10230^{c} ± 252	8865^{b} ± 295	9084^{bd} ± 220	10238^{c} ± 279	9415^{bd} ± 333	9846^{cd} ± 323	9411^{bd} ± 289
Zinc (Zn)	103^{ac} ± 2.5	77.6^{b} ± 3.5	115^{d} ± 2.5	106^{cd} ± 3.4	101^{ac} ± 2.5	106^{cd} ± 4.4	94.7^{a} ± 2.5	98.0^{ac} ± 4.0	102^{ac} ± 3.3
Iron (Fe)	36.3^{ab} ± 1.0	31.4^{c} ± 1.0	51.5^{d} ± 2.0	37.8^{bf} ± 1.4	46.1^{g} ± 3.0	32.8^{ac} ± 1.4	66.0^{e} ± 1.2	41.4^{fg} ± 1.6	42.3^{fg} ± 1.3
Copper (Cu)	22.0^{a} ± 1.0	10.8^{b} ± 1.0	18.8^{ac} ± 1.0	19.2^{ac} ± 1.2	17.5^{c} ± 1.2	20.2^{ac} ± 1.0	20.0^{ac} ± 1.0	21.1^{a} ± 1.5	35.3^{d} ± 1.3
Manganese (Mn)	13.4^{a} ± 0.50	14.7^{a} ± 1.0	14.9^{a} ± 0.50	14.2^{a} ± 1.5	14.4^{a} ± 1.5	12.5^{a} ± 0.50	19.4^{b} ± 1.4	19.6^{b} ± 1.0	18.9^{b} ± 0.65

Table 6
Amino acid content in g/100 g of insect flour (± SD).

Amino acid	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Alanine	4.03^{a} ± 0.22	3.11^{b} ± 0.18	3.24^{b} ± 0.22	3.22^{b} ± 0.26	3.09^{b} ± 0.17	4.02^{a} ± 0.23	3.63^{ab} ± 0.21	3.03^{b} ± 0.25	4.17^{a} ± 0.28
Arginine	2.15^{a} ± 0.16	2.10^{a} ± 0.09	2.29^{a} ± 0.16	2.10^{a} ± 0.18	2.10^{a} ± 0.16	2.42^{a} ± 0.18	2.22^{a} ± 0.10	2.23^{a} ± 0.06	2.17^{a} ± 0.11
Aspartic acid	4.30^{ab} ± 0.18	3.88^{a} ± 0.22	3.97^{a} ± 0.19	4.49^{ab} ± 0.22	4.11^{ab} ± 0.21	4.67^{b} ± 0.30	4.46^{ab} ± 0.25	4.21^{ab} ± 0.16	4.49^{ab} ± 0.21
Valine	2.78^{ab} ± 0.15	2.79^{ab} ± 0.13	2.99^{a} ± 0.07	2.58^{b} ± 0.14	2.42^{b} ± 0.14	2.73^{ab} ± 0.11	2.75^{ab} ± 0.09	2.55^{b} ± 0.13	2.76^{ab} ± 0.21
Glutamicacid	5.69^{a} ± 0.28	5.24^{a} ± 0.13	5.72^{a} ± 0.24	5.89^{a} ± 0.37	5.89^{a} ± 0.36	5.68^{a} ± 0.29	5.31^{a} ± 0.37	5.46^{a} ± 0.32	5.88^{a} ± 0.40
Glycine	2.98^{ab} ± 0.18	2.56^{a} ± 0.15	2.61^{a} ± 0.12	2.78^{ab} ± 0.17	2.69^{ab} ± 0.14	3.11^{b} ± 0.21	2.90^{ab} ± 0.19	2.86^{ab} ± 0.10	2.94^{ab} ± 0.18
Threonine	1.53^{a} ± 0.06	1.45^{ab} ± 0.08	2.08^{c} ± 0.10	1.42^{ab} ± 0.10	1.27^{b} ± 0.09	1.50^{a} ± 0.06	1.46^{ab} ± 0.06	1.45^{ab} ± 0.05	1.45^{ab} ± 0.06
Isoleucine	1.87^{abc} ± 0.07	1.99^{bcd} ± 0.03	2.25^{d} ± 0.21	1.69^{ab} ± 0.12	1.64^{a} ± 0.05	2.13^{cd} ± 0.05	1.93^{abc} ± 0.15	1.86^{abc} ± 0.09	1.88^{abc} ± 0.05
Leucine	3.88^{a} ± 0.11	4.88^{b} ± 0.08	5.32^{c} ± 0.08	3.82^{a} ± 0.16	3.62^{a} ± 0.21	3.93^{a} ± 0.15	3.74^{a} ± 0.12	3.78^{a} ± 0.15	3.83^{a} ± 0.12
Histidine	1.19^{b} ± 0.08	1.45^{ac} ± 0.12	1.58^{c} ± 0.10	1.11^{bd} ± 0.03	0.98^{d} ± 0.02	1.29^{ab} ± 0.04	1.25^{ab} ± 0.05	1.15^{bd} ± 0.06	1.11^{bd} ± 0.09
Cystine	0.20^{a} ± 0.03	0.29^{abc} ± 0.05	0.31^{bc} ± 0.05	0.25^{abc} ± 0.05	0.22^{ab} ± 0.03	0.33^{c} ± 0.03	0.32^{bc} ± 0.01	0.30^{abc} ± 0.03	0.29^{abc} ± 0.01
Lysine	2.13^{a} ± 0.14	2.77^{bc} ± 0.08	3.11^{c} ± 0.11	2.50^{ba} ± 0.20	2.17^{a} ± 0.16	2.50^{ab} ± 0.12	2.46^{ab} ± 0.07	2.36^{a} ± 0.12	2.14^{a} ± 0.13
Methionine	0.31^{a} ± 0.03	0.45^{c} ± 0.01	0.46^{c} ± 0.05	0.41^{bc} ± 0.04	0.31^{a} ± 0.01	0.35^{ab} ± 0.04	0.41^{bc} ± 0.01	0.34^{ab} ± 0.01	0.38^{abc} ± 0.01
Proline	2.86^{ab} ± 0.10	2.84^{ab} ± 0.07	3.48^{c} ± 0.08	2.92^{ab} ± 0.04	2.65^{a} ± 0.06	2.99^{b} ± 0.13	2.81^{ab} ± 0.14	2.97^{b} ± 0.15	2.73^{ab} ± 0.12
Serine	1.67^{ab} ± 0.06	1.87^{b} ± 0.13	2.34^{c} ± 0.03	1.81^{ab} ± 0.14	1.57^{a} ± 0.06	1.81^{ab} ± 0.15	1.93^{b} ± 0.06	1.69^{ab} ± 0.06	1.73^{ab} ± 0.11
Tryptophan	0.65^{a} ± 0.03	0.37^{b} ± 0.03	0.40^{b} ± 0.02	0.64^{ac} ± 0.03	0.62^{ac} ± 0.02	0.69^{a} ± 0.04	0.66^{a} ± 0.02	0.57^{c} ± 0.02	0.62^{ac} ± 0.02
Tyrosine	2.88^{ac} ± 0.12	3.92^{b} ± 0.03	4.28^{b} ± 0.03	2.62^{a} ± 0.17	2.70^{ac} ± 0.16	2.99^{c} ± 0.13	3.06^{c} ± 0.15	2.78^{ac} ± 0.16	2.60^{a} ± 0.11
Phenylalanine	1.49^{a} ± 0.08	1.48^{a} ± 0.12	1.50^{a} ± 0.06	1.57^{a} ± 0.12	1.40^{a} ± 0.07	1.54^{a} ± 0.10	1.54^{a} ± 0.09	1.50^{a} ± 0.05	1.47^{a} ± 0.07
Total amino acids	**42.6^{ab} ± 2.7**	**43.4^{ab} ± 2.1**	**47.9^{a} ± 2.2**	**41.8^{ab} ± 3.1**	**39.4^{b} ± 2.9**	**44.7^{ab} ± 3.2**	**42.8^{ab} ± 3.2**	**41.1^{ab} ± 3.0**	**42.6^{ab} ± 2.9**

amino acid content, indicating a potentially richer amino acid profile. Sample 3 stands out with a total amino acid content of 47.9 g/100 g, followed by sample 6 with 44.7 g/100 g. These samples show a higher total amino acid concentration, indicating a more favorable amino acid profile. In addition, Sample 6 shows relatively higher levels of essential amino acids such as valine, leucine, histidine and lysine, further contributing to its potentially better amino acid profile. On the other hand, sample 5 has the lowest total amino acid content with 39.4 g/100 g, followed by sample 4 with 41.82 g/100 g. These samples show comparatively lower amino acid concentrations, indicating a potentially lower overall amino acid profile. Sample 4, shows lower levels of essential amino acids such as valine, isoleucine, leucine and lysine, contributing to its lower amino acid profile. These findings demonstrate the significant variability in the amino acid content of the samples, highlighting the importance of the nutrition of *T. molitor* larvae during their rearing and the important role of the food substrate.

3.5. Heavy metal content

Finally, from the data in Table 7 it is evident that the feed substrate

Table 7
Concentration of heavy metals and trace elements in mg per kg of insect flour (± SD).

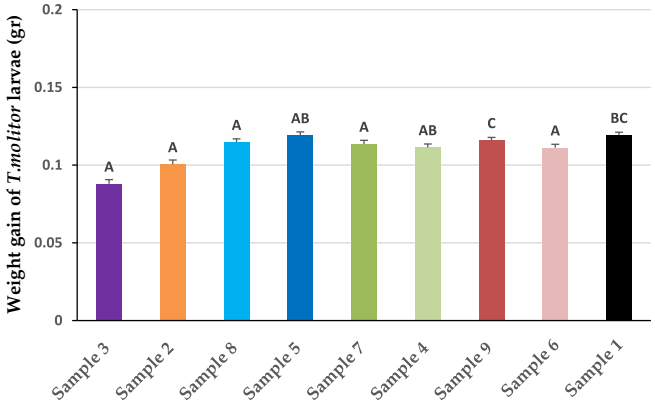
Heavy Metals	Samples 1	Samples 2	Samples 3	Samples 4	Samples 5	Samples 6	Samples 7	Samples 8	Samples 9	LOD* (mg/kg)
Lead (Pb)	<LOD	0.164^{a} ± 0.002	0.011^{b} ± 0.001	0.043^{c} ± 0.001	0.349^{d} ± 0.007	0.030^{e} ± 0.001	0.017^{b} ± 0.001	0.117^{f} ± 0.003	0.041^{c} ± 0.002	**0.0050**
Mercury (Hg)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.0030**
Cadmium (Cd)	0.092^{b} ± 0.009	0.183^{a} ± 0.007	0.183^{a} ± 0.019	0.059^{de} ± 0.005	0.124^{c} ± 0.009	0.064^{e} ± 0.001	0.059^{d} ± 0.007	0.052^{de} ± 0.002	0.039^{d} ± 0.005	**0.0010**
Arsenic (As)	<LOD	<LOD	0.019^{a} ± 0.002	<LOD	<LOD	0.026^{a} ± 0.002	<LOD	<LOD	0.023^{a} ± 0.004	**0.015**
Chromium (Cr)	0.334^{ac} ± 0.018	0.318^{a} ± 0.011	0.364^{cd} ± 0.018	0.362^{cd} ± 0.012	0.257^{b} ± 0.011	0.387^{d} ± 0.015	0.317^{a} ± 0.010	0.326^{ac} ± 0.012	0.320^{a} ± 0.017	**0.0025**
Molybdenum (Mo)	1.118^{a} ± 0.016	0.701^{b} ± 0.037	0.710^{b} ± 0.044	0.780^{b} ± 0.031	0.324^{c} ± 0.007	1.180^{a} ± 0.030	1.333^{d} ± 0.021	0.605^{e} ± 0.027	1.446^{f} ± 0.032	**0.025**
Selenium (Se)	0.097^{ab} ± 0.017	0.031^{b} ± 0.013	0.038^{b} ± 0.012	0.187^{ac} ± 0.025	0.219^{c} ± 0.046	0.244^{cd} ± 0.032	0.256^{cd} ± 0.027	0.362^{e} ± 0.030	0.323^{de} ± 0.055	**0.015**
Nickel (Ni)	1.849^{a} ± 0.030	3.254^{b} ± 0.043	2.852^{c} ± 0.160	1.051^{d} ± 0.024	0.630^{e} ± 0.009	1.793^{a} ± 0.019	1.173^{d} ± 0.043	0.707^{e} ± 0.029	1.036^{d} ± 0.007	**0.020**
Cobalt (Co)	0.044^{ab} ± 0.001	0.053^{bc} ± 0.001	0.052^{bc} ± 0.001	0.041^{a} ± 0.002	0.023^{d} ± 0.004	0.075^{e} ± 0.005	0.067^{ef} ± 0.003	0.042^{a} ± 0.006	0.060^{cf} ± 0.002	**0.020**
Aluminum (Al)	2.11^{a} ± 0.06	4.68^{b} ± 0.06	2.95^{c} ± 0.11	1.53^{d} ± 0.04	1.42^{d} ± 0.15	4.94^{e} ± 0.11	2.99^{c} ± 0.09	1.66^{d} ± 0.02	3.18^{c} ± 0.10	**0.20**

* **Limit of Detection (LOD).**

has significantly affected the heavy metal content in the samples. As the only factor that changed during the rearing of the different samples was their diet. Concentrations for most heavy metals determined in the sample flours varied, with the greatest differences in the most toxic where Pb (<LOD up to 0.349 mg/kg), Cd (0.039–0.183 mg/kg) and As (<LOD to 0.026 mg/kg). However, in most results the levels were quite low according to Regulation (EC) No. 915/2023, while Hg is below the LOD for all samples. Cr levels ranged from 0.257 to 0.387 mg/kg, indicating significant differences in some samples. While for the rest of the metals the concentration ranges are as follows, Mo 0.324–1.446 mg/kg, Se 0.031–0.362 mg/kg, Ni 0.630–3.254 mg/kg, Co 0.023–0.075 mg/kg and Al 1.42–4.94 mg/kg, also demonstrating distinct differences in heavy metal concentrations in the examined samples. Specifically, the presence of lead, cadmium, arsenic, chromium, molybdenum, selenium, nickel, cobalt and aluminum varied significantly between samples, with some substrates such as seaweed (*Posidonia oceanica*) increasing the levels of many metals such as arsenic, chromium, selenium and aluminum. Lead levels were highest in larvae fed wheat bran with 25 % and 12.5 % carob flour, as well as 100 % oats, indicating that carob flour and oats contributed significantly to lead absorption, as no lead was detected for the sample with the standard diet (sample 1). Cadmium concentrations were higher in larvae fed on oats and sunflower seed-wheat bran mixture. Arsenic was detected exclusively in the samples whose diets contained sunflower seeds and seaweed, highlighting these substrates as possible sources of arsenic. Chromium content increased in seaweed fed larvae, with the highest concentration found in sample 6, highlighting the role of seaweed in increasing chromium levels. Molybdenum showed great variability, with the highest levels in larvae fed wheat bran with 12.5 % seaweed and the lowest in those fed wheat bran with 25 % carob. Selenium and cobalt levels were also significantly higher in samples fed on seaweed food substrates. Nickel content was higher in sample 2 with the oat diet, indicating oat as the main source of nickel, while aluminum levels were higher in larvae fed wheat bran with 25 % seaweed. These findings demonstrate that the food substrate substantially affects the heavy metal content in mealworms, with specific substrates such as seaweed, oats and sunflower seeds significantly altering heavy metal uptake at higher concentrations. These findings are important for assessing larval safety and assessing the contribution of their diet to contamination with heavy metals.

3.6. Larval weight and growth

The food substrate in which the larvae were reared appeared to be a significant factor influencing larval weight gain ($F = 15.63$, $df = 8951$, $P < 0.0001$). According to the variations in larval weight gain observed among some samples (Scheme 1). Based on the results, the larval weight gain for samples 3 and 2 was significantly lower than the rest of the samples and compared to sample 1 (standard diet). However, sample 2 had a higher weight gain than sample 3. In the remaining samples, no significant difference was recorded between them or with the standard diet (Sample 1). Enrichment of the standard diet with sunflower seeds (Sample 3) caused an adverse effect on the weight gain of *T. molitor* larvae. In previous work, they tested the effect of a standard diet supplemented with 7.5 % (*w*/w) sunflower seeds and again reported negative results (Rossi et al., 2022). Possibly, diets enriched with oil-rich seeds may adversely affect the growth of *T. molitor* larvae. In the current study, oats (Sample 2) were also used as a single substrate and proved to be a less profitable food source for larvae compared to the standard diet. While, in another study they showed that oats (bran and flakes) together with wheat bran and other ingredients was a profitable diet to increase biomass of *T. molitor* larvae (Kröncke & Benning, 2022). In the case of carob flour (Sample 5 and Sample 8) it proved to be a profitable food source for the larvae. In a study it was shown that the weight of larvae fed a mixture of wheat bran with 25 %, 50 % and 75 % of whole carob pods was similar to the control (Antonopoulou et al., 2022). Our results showed that a much lower concentration of 12.5 % (Sample 8) can also be equally effective in supporting larval weight gain. The use of flaxseed (Sample 4 and sample 7) as a dietary supplement had beneficial effects on larvae growth. Similarly in one study they examined larvae weight



Scheme 1. Average individual weight gain (gr ± SE) of *Tenebrio molitor* larvae reared on different substrates. Columns followed by the same letter are not significantly different ($P < 0.05$, ANOVA, Tukey - Kramer HSD test).

gain when fed basic diets supplemented with flaxseed flour (10 %) and found significant positive results (Francardi et al., 2017). Previous studies have not evaluated the effect of *P. oceanica* leaves (Sample 6 and Sample 9). Interestingly, this substrate proved highly suitable while when used at the lower concentration (12.5 %) (Sample 9) the results were as positive as when used at the increased concentration (25 %) (Sample 6). In fact, it was recently reported that seaweed is rich in protein, vitamins, minerals, fiber and other nutrients (Peñalver et al., 2020). Therefore, our results suggest that seaweed may offer a valuable alternative low-cost food substrate for rearing *T. molitor* and their potential should be further evaluated.

4. Conclusions

Undoubtedly, edible insects present nutritional profiles of high nutritional value, which in several studies are comparable to those of traditional protein sources and often surpass them. They have a high protein and fat content, but also a sufficient amount of vitamins and minerals. The protein content of insects on a dry basis varies between 7 and 91 %, and many species contain about 60 % protein of high biological value. After protein, fat is the second most abundant macronutrient in the insect nutritional profile, ranging from 13 % for insects of the order Orthoptera (grasshoppers, crickets) to 33 % for those of the order Coleoptera (beetles, beetle larvae) (van Huis, 2016). At the same time, significant amounts of monounsaturated and polyunsaturated fatty acids and essential fatty acids in some cases have been detected. Also, through analysis of the amino acid profile, essential amino acids were found, at acceptable values. Due to the rich composition of the nutritional profile of edible insects, these insects can also be used for the production of animal feed, providing a solution to the existing issues. Research results have shown that *T. molitor* larvae can be used as an alternative protein source in poultry nutrition, as their nutritional profile is comparable to that of conventional feed (Hussain et al., 2017).

The results of the present study are comparable to the results of other studies in both protein content and total fat and fatty acid profile. All groups of larvae (Samples 1–9) showed significant differences between them in their nutritional value and the formation of their nutritional profile. However, some of them showed a significant difference in larval weight and the content of some heavy metals. So, insects pose a risk of the presence of chemical and toxic contaminants such as heavy metals, which is mainly due to the substrate on which the insects are grown. Since several studies have shown the accumulation of heavy metals in insects, their concentration levels in them and in breeding substrates need to be determined for the safe use of insects as food and feed.

In conclusion, the differences recorded in the results obtained for the different mealworm samples point to the significant effect of diet on their growth, nutritional profile and safety. For this reason, it is considered necessary that the diet of insects, especially those intended for human consumption, consists of high-quality ingredients in order for their nutritional profile to be of high value and meet the requirements of human safety and nutrition. The same would legitimately happen with regard to the feeding of insects intended for animal feed, so that they can provide all the necessary nutrients to animals and be a viable and reliable alternative to conventional animal feed. The macronutrients of the samples as well as the fatty acid profile, the mineral content and the amino acid profile showed significant differences between the different groups, both qualitatively and quantitatively. So, their diet during rearing is a very important factor, which can significantly shape and influence the nutritional profile and value of the larvae and its produced flour. Therefore, great importance should be given to this parameter, especially when these insects are intended for human consumption, so that they represent a safe and nutritious food and are a rich source of both macronutrients and micronutrients, with as few as possible contaminants.

The use of edible insects to produce food for human consumption is still in its early stages. However, the number of studies being carried out for this purpose is increasing. The breeding of edible insects is also in its early stages and is being carried out on a small scale so far. Thus, in the future, in order to make possible the inclusion of insects in foods intended for consumption by humans and animals, their mass production must be achieved, as well as their availability throughout the year. Mass production should be done in such a way that it is economically viable. Also, it would be beneficial to study the optimal conditions for the growth of insects, so that they are produced with as high nutrients as possible. It is also considered necessary to create a system that will ensure the health safety and quality of insect products, from production to consumption. This fact can help to strengthen the already existing legal framework. The pulverization of insects as a processing method can contribute both to the safety of the product from the growth of microorganisms (due to low water activity) and to their easier acceptance by consumers. Developing consumer-appealing insect products is still a challenge, especially when it comes to Western countries where consumers are prejudiced against them. Insect products that closely resemble conventional foods would help in their acceptance and consumption by the public, as well as educating consumers about the various benefits of insect consumption. It is particularly important to conduct analysis on different insect species beyond those currently approved, as insects may be found that have even higher nutritional value and significant benefits. Additional research and analysis of the already known edible insects are also necessary, for the results obtained and presented to be thorough and for progress to be made in the field of entomophagy. Despite the difficulties that exist, edible insects are an innovative approach to dealing with the problems of the modern world, and in the future, they could be part of the diet of both humans and farmed animals.

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CRediT authorship contribution statement

Konstantina Papastavropoulou: Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Anastasia Koupa:** Writing – review & editing, Methodology. **Evangelia Kritikou:** Writing – review & editing, Methodology. **Marios Kostakis:** Writing – review & editing, Software, Methodology. **Sofia Dervisoglou:** Methodology, Data curation. **Andreas Roussos:** Methodology. **Dionysios Perdikis:** Writing – original draft, Methodology. **Nikolaos S. Thomaidis:** Writing – review & editing, Supervision. **Emel Oz:** Writing – review & editing. **Fatih Oz:** Writing – review & editing. **Charalampos Proestos:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Haizhou Wu:** Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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