*sustainability*

MDPI

*Article*

# Utilization of Spent Coffee Grounds as a Feed Additive for Enhancing the Nutritional Value of *Tenebrio molitor* Larvae

**Konstantina Kotsou [1], Theodoros Chatzimitakos [1], Vassilis Athanasiadis [1], Eleni Bozinou [1], Christos G. Athanassiou [2] and Stavros I. Lalas [1,*]**

[1] Department of Food Science and Nutrition, University of Thessaly, Terma N. Temponera Str., 43100 Karditsa, Greece; kkotsou@agr.uth.gr (K.K.); tchatzimitakos@uth.gr (T.C.); vaathanasiadis@uth.gr (V.A.); empozinou@uth.gr (E.B.)

[2] Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, School of Agricultural Sciences, University of Thessaly, Phytokou Str., 38446 Volos, Greece; athanassiou@uth.gr

* Correspondence: slalas@uth.gr; Tel.: +30-24410-64783

**Abstract:** Increasing demand for sustainable protein sources has spurred interest in the exploration of alternative protein sources with a reduced environmental impact. This study investigates the use of spent coffee grounds (SCG), a widely available by-product, as a feed additive for *Tenebrio molitor* larvae, aiming to contribute to the circular economy and enhance the nutritional quality of the insects. The larvae were fed with a mixture of bran (the conventional feed) and SCGs (10 and 25% *w*/*w*). Larval viability, growth, and nutritional composition, including protein, fat, carbohydrates, ash, carotenoids, vitamins A and C, and polyphenols, were evaluated. Increasing the proportion of SCGs in the larvae's feed led to an enhanced nutritional value of the larvae. In particular, crude protein increased by 45.26%, vitamin C showed an increase of 81.28%, and vitamin A showed an increase of 822.79%, while polyphenol content increased by 29.01%. In addition, the oil extracted from these larvae showed enhanced nutritional value and greater resistance to oxidation. The results highlight the promising use of SCGs as a feed additive for *T. molitor* larvae, offering a sustainable approach to enhance their nutritional value. Delving deeper into the results, the addition of 10% SCGs resulted in a 45.26% increase in crude protein compared to the SCG0 sample. Concurrently, increasing SCGs in the dietary substrate led to an increase in vitamin content; in sample SCG25, vitamin C content increased by 81.28% while vitamin A content increased by 822.79% compared to the control sample. Moreover, there was a large increase in polyphenol content with the SCG25 sample showing the highest value, which was a 29.01% increase over the control sample.

**Keywords:** carotenoids; coffee by-products; edible insects; fat; fatty acids; proteins; proximate composition; vitamin A; vitamin C; yellow mealworm larvae



**Citation:** Kotsou, K.; Chatzimitakos, T.; Athanasiadis, V.; Bozinou, E.; Athanassiou, C.G.; Lalas, S.I. Utilization of Spent Coffee Grounds as a Feed Additive for Enhancing the Nutritional Value of *Tenebrio molitor* Larvae. *Sustainability* **2023**, *15*, 16224. https://doi.org/10.3390/su152316224

Academic Editor: Dimitris Skalkos

Received: 5 October 2023
Revised: 12 November 2023
Accepted: 21 November 2023
Published: 23 November 2023



## 1. Introduction

In light of the Earth's increasing population, the widespread consumption of natural resources, and the resulting production of significant amounts of agricultural by-products, there is a continuous and urgent concern for sustainable practices [1,2]. In the scientific and industrial sectors, ongoing efforts are made to establish sustainable methods for managing these discarded by-products in order to reduce the consumption of natural resources [3]. Among the various by-products generated and discarded annually, one noteworthy example is the residue produced after the roasting and brewing of coffee beans [4]. Coffee is the most widely consumed beverage globally and ranks as the second-largest commercial product, following petroleum [5]. According to the study by Getachew et al. [6], the improper disposal of coffee grounds, due to their high tannin and caffeine content, could pose environmental risks. Consequently, research efforts have increased to investigate the coffee grounds' properties and potential applications.

According to previous research, it has been elucidated that spent coffee grounds (SCG) contain bioactive compounds, notably polyphenols, which are well-documented for their antioxidant and antimicrobial attributes [7–9]. These by-products also encompass a variety of phenolic compounds, including caffeic acid, chlorogenic acid, and its isomer, neochlorogenic acid, known for their diverse biological activities, including anticancer, antilipidemic, antidiabetic, antiviral, and antipyretic effects, among others [10–12]. Despite their unsuitability for human consumption, coffee by-products have found utility as an ingredient in poultry feed, demonstrating their compatibility with animal consumption [13]. This multifaceted application of coffee by-products underscores their pivotal role in promoting a circular economy. Consequently, it prompts further exploitation regarding potential additional avenues for their sustainable utilization.

The expanding global population and the aforementioned patterns of resource consumption have led the scientific community toward the exploration of novel, innovative food sources [14]. Among the most promising solutions, the rearing and utilization of insects have garnered significant attention [15]. One promising insect species in this context is the larvae of *Tenebrio molitor* (TM) (Coleoptera: Tenebrionidae), commonly referred to as yellow mealworm larvae, which is frequently used as feed for fish, poultry, and pigs [16]. These larvae have gained recognition for their exceptional nutritional profile, characterized by high protein content, essential amino acids, fats, and essential fatty acids [17,18]. Notably, the TM insect participates inherently in a circular economy model by being reared on substrates derived from various by-products, thus contributing to a sustainable ecosystem. Various by-products have been subjected to examination in order to assess their effects on TM larvae growth. Among these substrates are rice bran, corncob, and potato peels [19] along with a range of industrial by-products, including chicken feed, wheat and rye bran, rapeseed meal and cake, flax cake, and *Silybum marianum* cake [20,21].

The digestive capabilities of mealworm larvae have garnered attention for their ability to process a diverse array of substrates and by-products [22]. Furthermore, these larvae have been documented as having negligible or limited quantities of specific essential nutrients, including vitamins A and C, as well as other bioactive compounds such as polyphenols [23]. Building upon these observations, the aim of our study was the examination of the feasibility of utilizing SCGs as a rearing substrate for TM insects. This investigation serves a dual purpose: firstly, to evaluate the influence of the substrate on the growth and development of the larvae, particularly up to the pupae stage, which follows the larval stage and precedes adulthood in insects [24], and, secondly, to assess the impact of the substrate on the nutritional composition of the larvae. The ultimate objective of this research is the enhancement of the nutritional value of a food source (TM larvae), while simultaneously adhering to principles of cost-effectiveness (by utilizing SCGs, a by-product that is abundantly available, to contribute to the economic viability of insect farming) and ecological sustainability (by repurposing a waste product to contribute to waste reduction and promote a circular economy, aligning with broader sustainability goals). By adopting such an approach, we aspire to contribute to the development of an economically viable and environmentally friendly method for producing a high-quality food resource. This approach represents a novel and environmentally conscious strategy for repurposing waste materials and aligns with the growing emphasis on circular economies in agriculture. Moreover, our dual focus on larval growth and development, coupled with considerations of cost-effectiveness and ecological sustainability, sets our approach apart, contributing to a more comprehensive understanding of the potential of alternative feed sources and their broader implications for sustainable and environmentally friendly agricultural practices. Based on the above, this study aligns with several Sustainable Development Goals (SDGs) by contributing to the global agenda for sustainable development. Firstly, the investigation into utilizing SCGs as a feed additive for TM larvae aligns with SDG 12 (Responsible Consumption and Production) by promoting the efficient and sustainable use of resources, specifically by repurposing a widely available by-product to enhance nutritional value. The emphasis on circular economy principles and waste reduction corresponds to SDG

12 targets. Additionally, the study aligns with SDG 2 (Zero Hunger) by exploring alternative protein sources to meet the increasing demand for sustainable food resources. The enhancement of nutritional content in TM larvae, particularly the substantial increase in crude protein and vitamins, contributes to SDG 3 (Good Health and Well-being) by addressing the nutritional aspect of food security. Furthermore, the research aligns with SDG 15 (Life on Land) by promoting sustainable practices in insect rearing, offering a potential eco-friendly solution to enhance food production while mitigating environmental impact. In summary, this study actively supports the interconnected goals of responsible consumption, zero hunger, good health, and environmental sustainability outlined in the United Nations' Sustainable Development Goals.

## 2. Materials and Methods

### *2.1. Chemicals and Reagents*

High-performance liquid chromatography (HPLC) grade solvents, specifically, hexane, acetone, ethanol, and methanol, were employed in this study and were supplied from Carlo Erba (Val de Reuil, France). Bradford reagent, ascorbic acid, β-carotene, trichloroacetic acid (163.69 M), hydrochloric acid (6.00 N), and iron (III) chloride (162.20 M) were sourced from Sigma-Aldrich (Steinheim, Germany). Gallic acid, Folin–Ciocalteu reagent, sodium anhydrous carbonate, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), and 2,4,6-tri-2-pyridinyl-1,3,5-triazine (TPTZ) were obtained from Penta (Prague, Czech Republic).

### *2.2. Insects and Spent Coffee Grounds (SCG) Material*

The experiments were performed using newly hatched *Tenebrio molitor* (TM) larvae (7 days old). To obtain the above population, a plastic tray (24 × 29.5 × 10 cm) with an opening in the top cover for adequate ventilation was filled with 2 kg of white flour (as an oviposition substrate), and about 500 adult TM were added over a mesh (to avoid cannibalization of the eggs). In addition, agar was also added as a source of moisture to the insect substrate. Insects were maintained under constant conditions, i.e., 26 ± 1 °C, 55 ± 5% relative humidity (RH), and continuous darkness, for 7 days in order to oviposit. The eggs were left to hatch and removed for further use when the larvae reached 7 days old.

The SCGs were obtained from a local coffee store in Karditsa city, Greece. The coffee grounds (60% Arabica and 40% Robusta) were transferred to the laboratory and underwent lyophilization in order to remove water.

### *2.3. Feeding Trial*

In a series of feeding trials, the growth of TM larvae on wheat bran–based feeding substrates with different percentages of dried SCG, namely, 10% (SCG10), 25% (SCG25), 50% (SCG50), 75% (SCG75), and 100% (SCG100), was evaluated, while wheat bran alone (SCG0) was used as a control. The bran was procured from a local shop in Volos, Greece, and had a particle size <2 mm.

In the preliminary experiments, 50 TM larvae were transferred into plastic, cylindrical vials (diameter 6.5 cm, height 8.8 cm, Carl Roth GmbH & Kg, Karlsruhe, Germany) with a shielded opening in the top cover allowing air circulation, together with 8 g of each substrate. As with the oviposition process, agar was provided as a source of moisture, three times a week while rearing conditions were 26 ± 1 °C, 55 ± 5% RH, and continuous darkness. To avoid depletion and inability of larvae to develop and grow, 2 g of food was added to each vial (the food nearly ran out). Each feed substrate was examined in nine replicates. In order to determine larval survival and development, larvae were separated from the substrate and counted, and their total weight was determined on a precision scale (Equinox EAB125i, Adam Equipment Inc., FoxHollow Road, Oxford, UK). This procedure was repeated every week until the first pupa appeared in each vial.

In order to produce sufficient TM population for further experiments, TM larvae were reared on a larger scale. The substrates that showed satisfactory survival and growth results (*vide infra*) were used (i.e., SCG0, SCG10, and SCG25). Into plastic trays (24 × 29.5 × 10 cm),

1500 larvae were introduced along with 300 g of each substrate and reared for eight weeks under the same conditions while using agar as a moisture source. Larvae were separated by sieving from the rearing substrate, fasted for 24 h, weighed, and euthanized by freezing (−20 °C). Larvae were then placed in a freeze-dryer for 24 h and crushed into a fine powder stored in sterile glass vials at −40 °C for further analysis.

### *2.4. Larval Composition Analysis*

#### 2.4.1. Water Content

A Biobase BK-FD10P (Jinan, China) lyophilizer was used to remove the moisture content from all larvae samples. Subsequently, the quantification of moisture content within samples was conducted using a gravimetric method referred to by May et al. [25].

#### 2.4.2. Crude Protein Content

In order to extract the proteins from the sample, 1 g of the sample was immersed in 10 mL of distilled water adjusted to pH 12 with 1 M NaOH. The extraction process was carried out for 60 min at 500 rpm and at room temperature. Subsequently, the mixture underwent centrifugation for 5 min at 4500× *g*, and the supernatant was retracted, so as to be further analyzed. To ensure the complete extraction of proteins, the extraction procedure was repeated two more times on the solid residue.

Quantification of protein content, expressed as a percentage, was conducted using the Bradford method [26]. For protein quantification, 900 µL of Bradford reagent was combined with 100 µL of the sample, and this mixture was allowed to react for a duration of 10 min in the absence of light. Ultimately, the absorbance of the samples was quantified at 595 nm using a spectrophotometer (Shimadzu UV-1700 Pharma Spectrophotometer, Kyoto, Japan).

#### 2.4.3. Carbohydrates Content

For the extraction of the carbohydrates, 1 g of the larval sample was added to 10 mL of distilled water. The mixture was stirred at 500 rpm for 1 h at 50 °C. The mixture was then centrifuged for 5 min at 4500× *g* and the supernatant was retracted and further analyzed. The phenol/sulfuric acid method was used to quantify the amount of carbohydrates [27]. In brief, 0.22 mL of the supernatant was transferred to a plastic tube and 0.65 mL of concentrated sulfuric acid and 0.13 mL of 5% *w*/*v* aqueous phenol solution were immediately added. The mixture was placed in a water bath at 90 °C for 5 min and then allowed to cool to room temperature for 5 min. The absorbance of the solution was measured at 495 nm using a spectrophotometer while a calibration curve was prepared using D(+)-glucose as a standard.

#### 2.4.4. Ash Content

The ash content of the samples was calculated gravimetrically [26]. Approximately 5 g of the sample was placed in porcelain crucibles and placed in an oven. The temperature of the oven was increased to 550 °C with a rate of 5 °C/min, and the samples were heated until no black residues were visible. The crucibles were then placed in a desiccator and left to cool to room temperature. Then the weight of the crucibles was recorded and the ash content was determined.

#### 2.4.5. Total Fat, Fatty Acids, and Calculated Oxidizability Value (COX)

For the determination of the fat content of the larvae, a defatting process was carried out. More specifically, 1 g of sample was mixed with 10 mL of *n*-hexane in a glass vial with a screw cap. Extraction was performed by stirring at 40 °C and 600 rpm for 60 min. To isolate the supernatant, the solution was centrifuged at 4500× *g* for 10 min. The supernatant was transferred to a pre-weighed flask. The extraction process was carried out two more times on the solid residue and the supernatants were pooled. Finally, the solvent was removed using a rotary evaporator at 40 °C. For the identification and quantification of the fatty

acids of the samples as well as the calculation of the various indices, the method used in our previous research [26] was used.

#### 2.4.6. Energy Content

The determination of the energy of TM larvae was based on the standard Equation (1):

$$\text{Energy (kcal/100 g)} = (9 \times \%\text{ crude fat}) + (4 \times \%\text{ crude protein}) + (4 \times \%\text{ carbohydrates}) \quad (1)$$

#### 2.4.7. Vitamin C Content

The screening and quantification of the amount of vitamin C samples was performed using a modified colorimetric analysis [28]. An accurately weighed amount of 5 g of powder from each sample of *T. molitor* was mixed with 27 mL of a distilled water:methanol mixture (60:40, $v/v$) and 3 mL of a 10% $w/v$ trichloroacetic acid solution. The mixture was stirred vigorously for 60 sec and then 20 mL of *n*-hexane was added. The final solution was stirred at room temperature for 30 min and then centrifuged for 10 min at 10,000× $g$. The lower aqueous phase was withdrawn and used as a sample. Next, 1 mL of the sample was mixed with 0.5 mL of Folin–Ciocalteu reagent (10% $v/v$) and allowed to incubate for 10 min. Absorbance was measured at 760 nm and quantification was performed with an ascorbic acid calibration curve.

#### 2.4.8. β-Carotene–Vitamin A Content

A previously described technique was used to estimate the β-carotene and Vitamin A content [29]. First, 1 g of each sample was added to the extraction stage along with 10 mL of ethanol, and the mixture was stirred at room temperature for 30 min at 300 rpm. The mixture was then centrifuged for 5 min at 3600× $g$ while being periodically shaken in an ice bath. Using a standard β-carotene calibration curve and the resulting extract's absorbance at 450 nm, the amount of carotenoid concentration was calculated (range: 0–50 mg/L, equation: y = 0.0182x + 0.0119, $R^2$ = 0.9982).

#### 2.4.9. Ferric-Reducing Antioxidant Power (FRAP) Assay

The FRAP method was used to determine the antioxidant capacity of the samples according to Makris and Kefalas [30]. The extracted solution as described in 2.4.7 was used in this assay. 50 µL of the extracts were mixed with 50 µL of $FeCl_3$ solution (4 mM in 0.05 M HCl) and incubated at 37 °C for 30 min. Then 900 µL of the TPTZ solution (1 mM in 0.05 M HCl) was added and the absorbance was measured at 620 nm. A calibration curve (concentration range: 50–500 µmol/L in 0.05 M HCl) was generated using ascorbic acid as a standard compound.

#### 2.4.10. Total Polyphenol Content (TPC) Determination

A previously documented technique was used to calculate the extract's total polyphenol content (TPC) [31]. The extracted solution as described in 2.4.7 was used in this assay. A total of 100 µL of the extract was mixed with an equal amount of Folin–Ciocalteu reagent and left for 2 min. Following the addition of 800 mL of $Na_2CO_3$ solution (5% $w/v$), the mixture was incubated for 20 min at 40 °C without light. Using a standard calibration curve, the absorbance was measured at 740 nm to calculate the TPC, which was represented as mg of gallic acid equivalents (GAE) per g of dry weight (dw) (range: 0–100 mg/L, equation: y = 0.0138x − 0.0044, $R^2$ = 0.9996) with gallic acid, and the concentration of total polyphenol ($C_{TP}$) was determined. To calculate the extraction yield of total polyphenol ($Y_{TP}$), the following Equation (2) was used:

$$Y_{TP}(\text{mg GAE/g dw}) = \frac{C_{TP} \times V}{w} \quad (2)$$

where $V$ represents the volume of the extraction medium (in L) and $w$ represents the dry weight of the sample (in g).

### *2.5. Statistical Analysis*

For the determination of larval survival and growth, a total of nine measurements were taken. For the proximate composition analysis, three samples were used, each analyzed in triplicate, resulting in a total of nine measurements. Results were expressed as the mean values of the nine measurements using standard deviation (SD). The data was examined to see if they were frequently distributed using the Kolmogorov–Smirnov test. The presence of statistically significant differences ($p < 0.05$) among the samples was assessed using one-way ANOVA (Analysis of Variance) with a post-hoc Tukey HSD (Honestly Significant Difference) Test Calculator (Tukey HSD used with Tukey–Kramer formula). For the statistical analysis, SPSS (version 29) (SPSS Inc., Chicago, IL, USA) software was used.

## 3. Results and Discussion

The primary focus of the study was to investigate the impact of SCGs as a feed additive on the nutritional value of *Tenebrio molitor* larvae. While acknowledging the importance of chemical composition analysis of the feed, the scope of the study was more centered on *T. molitor* and on the nutritional aspects, such as protein, vitamins, and polyphenols, rather than an exhaustive examination of all chemical components of the feed. Moreover, SCGs have been explored in various studies for their nutritional value, as well as toxic compounds such as ochratoxin A. While the presence of ochratoxin A is a legitimate concern in coffee-related products, existing safety standards may provide insights into the likelihood of contamination. The SCGs were obtained from coffee stores, intended for human consumption. As such, it was taken for granted that the raw materials were thoroughly and carefully examined, so as to be available in the market.

### *3.1. Survival and Growth of TM Larvae*

A number of factors need to be considered in order to evaluate a feed substrate for its suitability for insect rearing [32]. One of the main factors that needs to be examined is the survival of the insects. The selection of bran as a dietary control was based on the fact that it is a widely used substrate that promotes rapid growth and ensures high survival and high larval weight gain [33]. From preliminary experiments, it was learned that larvae in the SCG50, SCG75, and SCG100 samples did not survive more than one week after SCG addition, while those in the SCG0, SC10, and SCG25 samples survived to constitute the examined samples. The % survival rates of the larvae in the three remaining substrates (i.e., SCG0, SCG10, and SCG25) at each week are reported in Table 1. As can be seen, as the SCG content in the larval feeding substrate increases, the number of larvae decreases, albeit to a small extent (i.e., up to 10% in the eighth week). This may be due to the fact that in SCGs several compounds are present, some of which may induce toxicity in the larvae, such as ochratoxin A [34]. However, it is also known that TM larvae can self-select their diet preferences [35]. Therefore, the reduced survival may also be due to reduced feed consumption from their dietary preferences.

**Table 1.** Survival (%) of *T. molitor* larvae (±SD) fed for eight weeks with wheat bran (control) (SCG0) and bran fortified with different rates of spent coffee grounds, 10% (SCG10) and 25% (SCG25)) ($n = 9$).

| Diets | 2nd Week | 3rd Week | 4th Week | 5th Week | 6th Week | 7th Week | 8th Week |
|---|---|---|---|---|---|---|---|
| SCG0 | 97 ± 2 | 95 ± 3 | 94 ± 3 | 93 ± 4 | 91 ± 3 | 90 ± 2 $^{a}$ | 89 ± 2 $^{a}$ |
| SCG10 | 96 ± 4 | 95 ± 4 | 93 ± 4 | 92 ± 3 | 92 ± 3 | 87 ± 5 $^{a,b}$ | 86 ± 5 $^{a}$ |
| SCG25 | 96 ± 2 | 95 ± 2 | 93 ± 3 | 91 ± 4 | 90 ± 3 | 83 ± 3 $^{b}$ | 80 ± 3 $^{b}$ |

In all cases, values represent means ± SD. Within each evaluation interval (Week 2–8), means followed by the same superscript letter are not significantly different ($p > 0.05$). Where no letters exist, no statistically significant differences were noted.

The second parameter that must be examined for new feed substrates is the weight gain of the larvae. Results from the weight of the larvae fed with SCGs are shown in Figure 1. As can be seen, a statistically significant increase ($p < 0.0.5$) in the weight gain

of the larvae was recorded at weeks 5 and 6 for both SCG percentages, compared to the control larvae. However, in the next two weeks, although a 7.8% increase in the weight gain of the larvae fed with SCG25 was recorded, this was not statistically significantly different ($p > 0.0.5$) compared to the control sample. Although no significant increase in the weight of the larvae was recorded, it is noteworthy that the weight of the larvae did not decline due to selective feeding [35]. This result opens new avenues in insect rearing, given that both domestically and scientifically SCGs and their various extracts act as a repellent for insects such as mosquitoes [36,37].

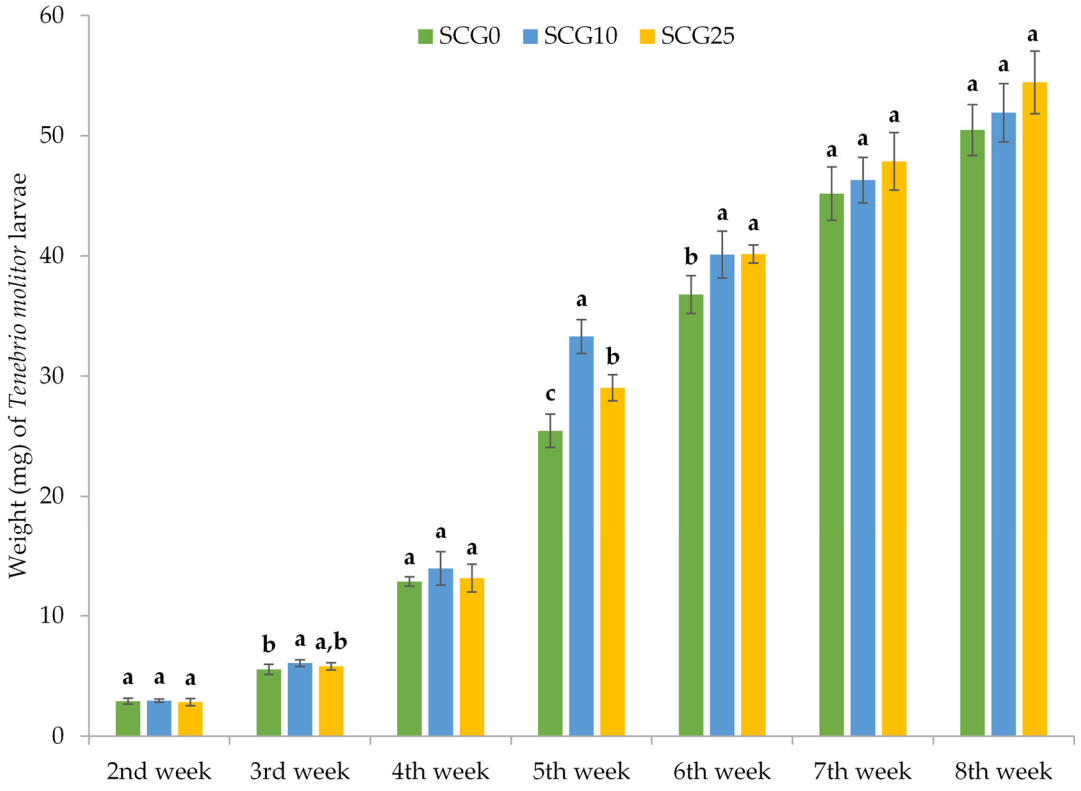


**Figure 1.** Individual weight (mg) of *T. molitor* larvae fed for eight weeks with wheat bran (control) (SCG0) and bran fortified with different rates of spent coffee grounds, 10% (SCG10) and 25% (SCG25)) ($n = 9$). Statistically significant differences ($p < 0.05$) are denoted with different letters (e.g., a–c).

Although the microbiological safety of products is often studied for food products, this analysis was not carried out in this case. As such, any potential risk to larvae or subsequent consumers would need to be addressed in further research or product development. The present study does not present a ready-to-eat food product but a potential additional ingredient, TM flour, for the preparation of a new product. Our focus on nutritional improvements through SCG supplementation does not negate the significance of safety considerations in food product development. However, we believe that addressing these concerns falls within the purview of future research endeavors that specifically aim to develop ready-to-eat food products using TM flour.

### *3.2. Evaluation of the Nutritional Value of the Larvae*

The first step was to determine the water content of the samples. The larvae fed with bran, considered as the control sample, exhibited a moisture content of approximately 30%, while the larvae fed with varying proportions of SCGs displayed a moisture content of approximately 17.7%. TM larvae present a high water content [38], sometimes ranging from 60.80 to 72.60%. Nevertheless, when the water content is so high, very low percentages of crude protein, in the range of 23.50–16.50%, respectively, also occur [39]. Consequently, the

water content in the present study may not reach such high values and this fact may be attributed to their rich protein content, which is presented afterwards.

#### 3.2.1. Proximate Composition

The high crude protein content has instigated substantial interest in yellow mealworm larvae within both the scientific community and the food industry. Consequently, the impact of SCGs on the crude protein content of the larvae was investigated. Detailed proximate composition data for TM larvae, fed with different SCG-based diets, is presented in Table 2. Commonly, TM larvae reared on bran, their primary dietary substrate, typically exhibit a crude protein content ranging from 13.68% to 27.60% [40,41]. However, it is crucial to acknowledge that the variability in crude protein content among larvae is influenced by geographic and rearing conditions, as evident in the diverse results. Although SCGs contain a small amount of crude protein [42], their use as a feed additive resulted in a significant boost in the crude protein levels of larvae. Specifically, the SCG10 sample exhibited a crude protein content of 47.34%, which was a 45.26% increase over the control sample, while the SCG25 sample exhibited a substantial 30.86% increase over the SCG0 group, as its amount of crude protein was 42.65%; both samples' differences were statistically significant ($p < 0.05$). Last but not least, it is worth mentioning that SCG10 was 10.99% more enhanced in crude protein content than SCG25.

**Table 2.** Proximate composition of *T. molitor* larvae fed for eight weeks with wheat bran (control) (SCG0) and bran fortified with different rates of spent coffee grounds, 10% (SCG10) and 25% (SCG25)) ($n = 9$).

| % Composition of Dry Weight | SCG0 | SCG10 | SCG25 |
|---|---|---|---|
| Crude protein | $32.59 \pm 0.43^{c,*}$ | $47.34 \pm 0.17^{a}$ | $42.65 \pm 0.26^{b}$ |
| Crude fat | $22.77 \pm 1.29^{a}$ | $20.2 \pm 1.07^{a}$ | $21.93 \pm 1.55^{a}$ |
| Carbohydrates | $25.72 \pm 0.04^{a}$ | $13.41 \pm 0.77^{c}$ | $19.42 \pm 0.21^{b}$ |
| Crude ash | $1.89 \pm 0.07^{c}$ | $3.51 \pm 0.04^{a}$ | $2.44 \pm 0.03^{b}$ |
| Energy (kcal/100 g) | $438.17 \pm 13.49^{a}$ | $424.8 \pm 13.39^{a}$ | $445.65 \pm 15.83^{a}$ |

* Within each line, statistically significant differences ($p < 0.05$) are denoted with different superscript letters (e.g., a–c).

Given the ongoing exploration of TM larvae as potential substitutes for conventional sources of crude protein like meat, it is important to underscore the magnitude of this increase. For context, beef typically contains ~21.35 g/100 g of crude protein content, while chicken contains ~19.40 g/100 g [43]. In comparison, these contents are 52.64% and 67.99% lower compared to the control sample, and 121.73% and 144.02% lower compared to the SCG10 sample. This underscores the substantial nutritional advantage of TM larvae over conventional protein sources. Notably, the high (47.34%) crude protein content in SCG10 makes TM larvae close to the highest recorded percentage of crude protein in these larvae (50%) [44]. While amino acid profiling provides valuable insights into the specific constituents of proteins, emphasis was placed on the overall protein content, which serves as a pertinent and meaningful indicator of the nutritional enhancement achieved through SCG supplementation. The fundamental premise lies in recognizing that protein content itself is a critical determinant of nutritional value. As such, since our study aimed to contribute to the discourse on sustainable protein sources, with a focus on the circular economy and reduced environmental impact, the demonstrated increase in protein content, independent of the amino acid profile, underscores the potential of SCGs as a viable and sustainable feed additive.

Table 2 shows a noticeable reduction in the percentage of carbohydrates across various samples, which is in line with the observed increment in protein percentages. Specifically, samples SCG10 and SCG25 displayed a substantial decrease of 47.86% and 24.49% (statistically significant at $p < 0.05$) in carbohydrate content when compared to the SCG0 group.

It is noteworthy that the nutritional evaluation of mealworms primarily emphasizes their crude protein content, often omitting essential components like carbohydrates. Nevertheless, in this study, we examined the carbohydrate content as it constitutes a fundamental component of a balanced diet [45]. Beyond their significance in human nutrition, carbohydrates play a pivotal role in the growth and development of insects. A substantial portion of carbohydrates in insects is attributed to chitin [27], which serves as the main material composing the exoskeleton of TM throughout all developmental stages [46]. However, it is observed that as protein levels in the samples increase, there is a concomitant and significant decrease in carbohydrate content. This observation was somewhat expected, as carbohydrates provide the larvae with the requisite energy for their development and the execution of their metamorphosis [35]. Although chitin is undoubtedly a significant component in insect exoskeletons and plays a crucial role in growth and development, its quantification was not carried out since it was not aligned with the primary objectives of the study. In this study, the emphasis was on assessing the overall nutritional changes in TM larvae when supplemented with SCGs. Focus extended beyond individual components to encompass macronutrients, micronutrients, and the holistic nutritional value of the larvae. As far as the human diet is concerned, the consumption of limited carbohydrates can help reduce overall caloric intake without affecting the intake of essential nutrients, e.g., proteins and minerals [47]. Finally, low-carbohydrate diets improve cardiovascular risk factors and prevent or treat diabetes [48].

The determination of crude ash content is of paramount importance as it provides insights into the mineral composition of a food product, a key aspect of its nutritional profile. As elucidated in Table 2, the percentage of crude ash in the SCG-fed samples exhibits a wide variation, from 1.89% in SCG0 to 3.51% in SCG10. Notably, the inclusion of SCGs in the larval diet resulted in a statistically significant ($p < 0.05$) elevation in mineral content. Specifically, SCG10 displayed an 85.14% increase, while SCG25 demonstrated a 29.10% rise in comparison to the control sample. Furthermore, SCG10 emerged as the leading sample in terms of crude ash content, aligning with its prominent position in relation to crude protein levels. It is noteworthy that the highest recorded crude ash value in previous studies on TM larvae was found to be 3.81% [41], a content comparable to that of SCG10. Conversely, the control sample was found to contain 1.89%, which is consistent with prior studies reporting values within the range of 1.23% to 2.20% [40,49]. Collectively, these findings underscore the substantial increase in mineral content attributed to the inclusion of SCGs in the larval diet, with SCG10 achieving or surpassing values reported in prior research.

Another characteristic of TM larvae is their notably high fat content [50]. Extensive investigations have been conducted to elucidate their fat content, reporting a fat content between 20% and 45% [51,52]. According to our results, the observed fat percentages ranged between 20.20% (SCG10) and 22.77% (SCG0). These results were somewhat expected, given the inverse relationship between crude protein and fat percentages, whereby higher crude protein content typically corresponds to lower fat content. The fat percentage exhibited an 11.29% reduction in SCG10 and a 3.69% decrease in SCG25 compared to SCG0. It is important to highlight that while dietary fat serves as a crucial component of a balanced diet, it necessitates prudent consumption. This is underscored by research associating the consumption of high-fat diets with the development of severe health conditions [53].

The percentage of fat in a food product is of paramount significance, but equally critical is the assessment of its oil content and value, which is determined via relevant indicators [54]. Fatty acids encompass diverse health-promoting effects, including the prevention of cardiovascular disease, exhibited by polyunsaturated and monounsaturated fatty acids [55]. The composition of fatty acids in the samples is presented in Table 3, revealing substantial quantities of important fatty acids such as palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2 ω-6). Concerning C16:0, higher values were observed in SCG25, which exhibited increases of 8.76% and 10.82% over SCG0 and SCG10, respectively. Additionally, C18:1 is known for its anti-cancer properties [56], and our data

in Table 3 demonstrates a significant ($p < 0.05$) increase in C18:1 content as SCG content rises in the TM larval feeding substrates, reaching a peak of 45.87% in SCG25. Similarly noteworthy is the fatty acid C18:2, known for its protective effects against cardiovascular diseases [57]. While the C18:2 content of the samples is high, it is noteworthy that an increase in SCGs in the feed does not appear to increase C18:2 levels. Instead, our findings indicate a significant ($p < 0.05$) decrease of 31.77% between the SCG0 and SCG25 samples. These results collectively underscore the diversity of fatty acids present in TM larvae and their potential health benefits, while shedding light on the nuanced impact of coffee content on specific fatty acid compositions. Nevertheless, it should be pointed out that a high saturated fat intake is associated with atherosclerosis and coronary artery diseases [58] and, as can been seen in Table 3, TM larvae are quite rich in saturated fatty acids.

**Table 3.** Fatty acid composition of *T. molitor* larvae fed for eight weeks with wheat bran (control) (SCG0) and bran fortified with different rates of spent coffee grounds, 10% (SCG10) and 25% (SCG25)) ($n = 9$).

| Fatty Acid (%) | Diets | | |
|---|---|---|---|
| | SCG0 | SCG10 | SCG25 |
| C12:0 | 0.08 ± 0.00 $^{b,*}$ | 0.10 ± 0.00 $^{a}$ | nd ** |
| C14:0 | 2.06 ± 0.08 $^{b}$ | 2.77 ± 0.19 $^{a}$ | 2.36 ± 0.06 $^{b}$ |
| C16:0 | 19.3 ± 1.37 $^{a}$ | 18.94 ± 0.53 $^{a}$ | 20.99 ± 1.26 $^{a}$ |
| C18:0 | 0.21 ± 0.01 $^{b}$ | 0.23 ± 0.01 $^{a}$ | nd |
| C18:1 | 23.98 ± 1.39 $^{b}$ | 24.83 ± 1.86 $^{b}$ | 34.98 ± 1.64 $^{a}$ |
| C18:2 (ω-6) | 53.87 ± 4.04 $^{a}$ | 52.67 ± 3.53 $^{a}$ | 40.88 ± 1.92 $^{b}$ |
| C20:0 | 0.50 ± 0.03 $^{b}$ | 0.45 ± 0.03 $^{b}$ | 0.78 ± 0.02 $^{a}$ |
| ∑ SFA $^{1}$ | 22.15 ± 1.49 $^{a}$ | 22.5 ± 0.76 $^{a}$ | 24.13 ± 1.34 $^{a}$ |
| ∑ MUFA $^{2}$ | 23.98 ± 1.39 $^{b}$ | 24.83 ± 1.86 $^{b}$ | 34.98 ± 1.64 $^{a}$ |
| ∑ PUFA $^{3}$ | 53.87 ± 4.04 $^{a}$ | 52.67 ± 3.53 $^{a}$ | 40.88 ± 1.92 $^{b}$ |
| ∑ UFA $^{4}$ | 77.85 ± 5.43 $^{a}$ | 77.5 ± 5.39 $^{a}$ | 75.87 ± 3.57 $^{a}$ |
| PUFA:SFA ratio | 2.43 ± 0.02 $^{a}$ | 2.34 ± 0.08 $^{a}$ | 1.69 ± 0.01 $^{b}$ |
| MUFA:PUFA ratio | 0.45 ± 0.01 $^{c}$ | 0.47 ± 0.00 $^{b}$ | 0.86 ± 0.00 $^{a}$ |
| COX $^{5}$ | 5.79 ± 0.43 $^{a}$ | 5.67 ± 0.38 $^{a}$ | 4.56 ± 0.21 $^{b}$ |
| IA $^{6}$ | 0.35 ± 0.00 $^{b}$ | 0.39 ± 0.01 $^{a}$ | 0.4 ± 0.00 $^{a}$ |
| IT $^{7}$ | 0.55 ± 0.00 $^{b}$ | 0.57 ± 0.02 $^{b}$ | 0.62 ± 0.01 $^{a}$ |
| HH $^{8}$ | 3.63 ± 0.01 $^{a}$ | 3.55 ± 0.13 $^{a}$ | 3.25 ± 0.03 $^{b}$ |
| HPI $^{9}$ | 2.82 ± 0.02 $^{a}$ | 2.57 ± 0.07 $^{b}$ | 2.49 ± 0.01 $^{b}$ |

* Within each line, statistically significant differences ($p < 0.05$) are denoted with different superscript letters (e.g., a–c). ** nd: not detected. [1] SFAs, saturated fatty acids (%): SUM of C12:0, lauric acid; C14:0, myristic acid; C16:0, palmitic acid; C18:0, stearic acid; C20:0, arachidic acid. [2] MUFAs, monounsaturated fatty acids (%): SUM of C18:1, oleic acid. [3] PUFAs, polyunsaturated fatty acids (%): SUM of C18:2, ω-6, linoleic acid. [4] UFAs, unsaturated fatty acids (%): SUM of MUFAs and PUFAs. [5] COX, calculated oxidizability value. [6] IA, Index of atherogenicity. [7] IT, Index of thrombogenicity. [8] HH, Hypocholesterolemic/hypercholesterolemic ratio. [9] HPI, Health-promoting index.

Furthermore, the COX value, an important indicator in assessing the oxidative stability of oil, was calculated. A lower COX value signifies enhanced oxidative stability and, consequently, an extended shelf life of the oil product [59]. In our case, we observed a decrease in the COX value as the SCG content increased, underscoring the fact that SCG consumption by TM larvae promotes a higher shelf life of their oil. Next, other indicators pertinent to the overall quality of oils were also examined. These indicators encompassed the atherogenicity index (IA), thrombogenicity index (IT), and health promotion index (HPI). A lower IA value is indicative of a healthier food product. For instance, high IA values, such as 4.08, are reported for milk [60], a value which is ten times higher than the values recorded for TM larvae oil. In our case, no statistically significant differences ($p < 0.05$) were recorded for SCG-fed larvae. A similar principle applies to the IT index, where lower values are more favorable for human health. For instance, in a study focused on seaweed, another innovative food product alongside insects, the IT value ranged from

0.04 to 2.94 [61]. In contrast, our samples exhibited a maximum value of 0.62 (SCG25). The above comparison further contextualizes the health benefits, highlighting the potential of SCG-fed larvae as a nutritionally favorable and innovative food source. Last but not least, HPI is relevant as the consumption of foods with high values of this index has a positive effect on cardiovascular diseases [62]. Comparing the present HPI data with the corresponding HPI value of meat (2.91) [63], it is obvious that the data are fully comparable while proving that the nutritional value of TM larvae does not differ significantly from conventional food products. This not only supports the larvae's potential as a sustainable alternative but also underscores its role in promoting cardiovascular health. In essence, the analysis of these indices provides a nuanced and comprehensive assessment of the nutritional quality and health implications of TM larvae reared on SCGs. The results not only contribute to the broader understanding of insect-based nutrition but also position SCG-fed larvae as a promising and innovative source of nutrition with potential benefits for human health.

#### 3.2.2. Vitamin C and A Content of TM Larvae

Vitamins C and A belong to the group of essential vitamins, each serving distinct roles crucial for proper human physiological function [64]. TM larvae inherently exhibit vitamin deficiencies [65]. Hence, this study endeavors to naturally enhance their vitamin content via dietary modifications. Results are presented in Table 4. A substantial increase in vitamin content within the examined larvae was recorded as the quantity of SCGs in their dietary substrates increased. While SCGs themselves lack increased ascorbic acid content, they do contain a quantity of up to 8.18 ± 0.39 mg of vitamin C equivalent (VCE)/g SCG [66]. Evidently, this is efficiently absorbed by the larvae during their developmental stages. The elevation in ascorbic acid content was high, exhibiting a statistically significant ($p < 0.05$) rise of 43.63% with the inclusion of 10% SCGs and an even more pronounced increase of 81.28% with the addition of 25% SCGs. This underscores the capacity of the larvae to increase their vitamin C levels, despite prior deficiencies, merely by consuming food waste. Equally noteworthy is the enhancement in β-carotene content, a vital nutrient for humans because it contributes to various therapeutic effects in managing numerous diseases, such as cancer, cardiovascular disorders, COVID-19, cystic fibrosis, and bronchiectasis [67,68]. Larvae fed with this diet can increase their content up to 8.42 µg/g, marking a substantial increase from the control sample containing 0.91 µg/g. The same applies for vitamin A. Larvae fed on bran exhibit a vitamin A content of approximately 2.72 µg RAE/100 g. Intriguingly, the addition of up to 25% SCGs increases this content by 822.79%. Comparatively, chicken contains a mere 6.00 µg/100 g of vitamin A [69]. This underscores the nutritional potential of mealworm larvae, revealing their richness in proteins and essential nutrients, including vitamins, in comparison to conventional protein sources such as chicken.

**Table 4.** Content of *T. molitor* larvae fed for eight weeks with wheat bran (control) (SCG0) and bran fortified with different rates of spent coffee grounds, 10% (SCG10) and 25% (SCG25)), in vitamin C, β-carotene, and vitamin A ($n = 9$).

| Diets | Vitamin C (µg/g) | β-Carotene (µg/g) | Vitamin A (µg RAE/100 g) |
|---|---|---|---|
| SCG0 | 218.09 ± 2.09 $^{c,*}$ | 0.91 ± 0.06 $^{c}$ | 2.72 ± 0.19 $^{c}$ |
| SCG10 | 313.25 ± 3.24 $^{b}$ | 4.36 ± 0.56 $^{b}$ | 13.02 ± 1.68 $^{b}$ |
| SCG25 | 395.35 ± 1.98 $^{a}$ | 8.42 ± 0.34 $^{a}$ | 25.1 ± 1 $^{a}$ |

* Within each column, statistically significant differences ($p < 0.05$) are denoted with different superscript letters (e.g., a–c).

#### 3.2.3. Antioxidant Properties of the Larvae's Extracts

Table 5 provides a summary of the results of the assessment of the antioxidant activity of mealworm larvae. The results unveil a consistent pattern. As the amount of SCGs in the dietary substrate increases, both the antioxidant activity and polyphenol concentration exhibit an increase. More specifically, the addition of 10% SCGs results in a statistically

significant ($p < 0.05$) increase of 9.5% in antioxidant activity, and this effect becomes even more pronounced with the addition of 25% SCGs, where the increase rises to 91.86%. Furthermore, statistically significant differences ($p < 0.05$) are evident in polyphenol content among the samples. A comparative analysis between SCG0 and SCG10 reveals a 23.34% increase, while comparing SCG0 and SCG25 demonstrates a more substantial increase (29.01%). These findings are comparable with prior studies, proposing that SCGs exhibit antioxidant activity and contain polyphenols (approximately 34.43 mg GAE/g) [7,8,70]. Hence, it can be inferred from these results that the nutrients present in the larvae feed substrate are readily absorbed by the larvae, resulting in additive nutritional resources. TM larvae with enhanced antioxidant properties could be used to develop dietary supplements targeting specific health problems, such as immune system support, anti-ageing, or cardiovascular health. So, these larvae can be processed into various food products like protein powders, protein bars, and snacks giving them extra significant properties. However, attention should be paid to the European Union's indications on the consumption of TM larvae. In particular, the Commission states that primary sensitization and allergic reactions to flour proteins from TM larvae may be induced in people with allergies to crustaceans and dust mites [71].

**Table 5.** Antioxidant properties (FRAP assay) and total polyphenols (TPC) of *T. molitor* larvae fed with different rates of spent coffee grounds, 10% (SCG10) and 25% (SCG25)) ($n = 9$).

| Diets | FRAP (μmol AAE/g) | TPC (mg GAE/g) |
|---|---|---|
| SCG0 | $130.8 \pm 2.48^{c,*}$ | $43.22 \pm 1.8^{b}$ |
| SCG10 | $143.23 \pm 1.87^{b}$ | $53.31 \pm 1.48^{a}$ |
| SCG25 | $250.95 \pm 3.53^{a}$ | $55.76 \pm 0.24^{a}$ |

* Within each column, statistically significant differences ($p < 0.05$) are denoted with different superscript letters (e.g., a–c).

## 4. Conclusions

Coffee consumption generates substantial waste. Our present study reaffirms the suitability of these by-products as animal feed, while opening new avenues for their use in rearing TM larvae. Although, a minor decrease in their survivability was recorded, without having a toll on the weight gain of the larvae, the substrate comprising 75% wheat bran and 25% SCGs proved highly beneficial for TM larvae, enhancing their protein, vitamin, and polyphenol contents, as well as their antioxidant capacity. Our findings underscore the utilization potential of SCGs, promoting the sustainability and ecological impact of coffee consumption. In fact, by adding SCGs in the feed substrate of TM larvae, four goals of the United Nations Sustainable Development Goals (SDGs) can be achieved. Specifically, goal 2 can be achieved through the production of alternative animal feed, combined with the achievement of goal 12 for reducing food industry by-products and promoting a circular economy. TM larvae can be raised using food industry waste, and no negative effects were observed in their growth, development, and metamorphosis. Additionally, goal 2, producing new protein-rich foods, can be achieved with a 45.26% increase in crude protein content in the larvae after their rearing with 10% SCGs. Furthermore, with the increase in protein content, there was an 81.28% increase in vitamin C and an 822.79% increase in vitamin A in the samples that were reared with 25% SCGs. This study further substantiates the notion that insects can effectively consume a wide array of products and efficiently absorb the nutrients offered in their diet. Moreover, it is important to highlight that TM larvae possess the potential to become one of the most nutritionally-rich sources of animal-derived food. These points warrant increased consideration for their consumption either in their natural form or as additives in low–nutritional value products like bread, cakes, and biscuits. Moreover, it must be stated that the present study does not present a ready-to-eat food product but a potential additional ingredient, TM flour, for the preparation of a new product. Therefore, since the utilization of SCGs was found suitable for insect rearing, we urge other researchers to use it for the creation of more nutritious larvae and add it to food,

whereas future work could address concerns regarding the microbiological and chemical safety of the new food products.

**Author Contributions:** Conceptualization, S.I.L. and K.K.; methodology, S.I.L. and K.K.; software, T.C. and V.A.; validation, K.K., E.B., T.C. and V.A.; formal analysis, K.K., T.C., V.A. and E.B.; investigation, K.K. and E.B.; resources, S.I.L.; data curation, T.C., V.A. and S.I.L.; writing—original draft preparation, K.K. and T.C.; writing—review and editing, K.K., T.C., V.A., E.B. and S.I.L.; visualization, K.K.; supervision, C.G.A. and S.I.L.; project administration, S.I.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All the data are contained within the article.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| COX | Calculated Oxidizability Value |
| HH | Hypocholesterolemic/Hypercholesterolemic Ratio |
| HPI | Health-Promoting Index |
| IA | Index of Atherogenicity |
| IT | Index of Thrombogenicity |
| MUFA | Monounsaturated Fatty Acids |
| PUFA | Polyunsaturated Fatty Acids |
| SCG | Spent Coffee Grounds |
| SFA | Saturated Fatty Acids |
| TM | *Tenebrio molitor* |
| UFA | Unsaturated Fatty Acids |

## References

1. Kaza, S.; Yao, L.; Bhada-Tata, P.; Van Woerden, F. *What a Waste 2.0 Introduction—"Snapshot of Solid Waste Management to 2050." Overview Booklet*; Urban Development Series; World Bank Group: Washington, DC, USA, 2018; pp. 1–38.
2. FAO 2011. *The Methodology of the FAO Study: "Global Food Losses and Food Waste—Extent, Causes and Prevention"*; Vol. SIK Report; FAO: Rome, Italy, 2013; ISBN 9789172903234.
3. Patwa, N.; Sivarajah, U.; Seetharaman, A.; Sarkar, S.; Maiti, K.; Hingorani, K. Towards a Circular Economy: An Emerging Economies Context. *J. Bus. Res.* **2021**, *122*, 725–735. [CrossRef]
4. Mussatto, S.I.; Carneiro, L.M.; Silva, J.P.A.; Roberto, I.C.; Teixeira, J.A. A Study on Chemical Constituents and Sugars Extraction from Spent Coffee Grounds. *Carbohydr. Polym.* **2011**, *83*, 368–374. [CrossRef]
5. Chatzimitakos, T.; Athanasiadis, V.; Kotsou, K.; Palaiogiannis, D.; Bozinou, E.; Lalas, S.I. Optimized Isolation Procedure for the Extraction of Bioactive Compounds from Spent Coffee Grounds. *Appl. Sci.* **2023**, *13*, 2819. [CrossRef]
6. Getachew, A.T.; Chun, B.S. Influence of Pretreatment and Modifiers on Subcritical Water Liquefaction of Spent Coffee Grounds: A Green Waste Valorization Approach. *J. Clean. Prod.* **2017**, *142*, 3719–3727. [CrossRef]
7. Seo, H.S.; Park, B.H. Phenolic Compound Extraction from Spent Coffee Grounds for Antioxidant Recovery. *Korean J. Chem. Eng.* **2019**, *36*, 186–190. [CrossRef]
8. Panusa, A.; Zuorro, A.; Lavecchia, R.; Marrosu, G.; Petrucci, R. Recovery of Natural Antioxidants from Spent Coffee Grounds. *J. Agric. Food Chem.* **2013**, *61*, 4162–4168. [CrossRef] [PubMed]
9. Smyrnakis, G.; Stamoulis, G.; Palaiogiannis, D.; Chatzimitakos, T.; Athanasiadis, V.; Lalas, S.I.; Makris, D.P. Recovery of Polyphenolic Antioxidants from Coffee Silverskin Using Acid-Catalyzed Ethanol Organosolv Treatment. *ChemEngineering* **2023**, *7*, 72. [CrossRef]
10. Santana-Gálvez, J.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. Chlorogenic Acid: Recent Advances on Its Dual Role as a Food Additive and a Nutraceutical against Metabolic Syndrome. *Molecules* **2017**, *22*, 358. [CrossRef]
11. Kang, N.J.; Lee, K.W.; Shin, B.J.; Jung, S.K.; Hwang, M.K.; Bode, A.M.; Heo, Y.S.; Lee, H.J.; Dong, Z. Caffeic Acid, a Phenolic Phytochemical in Coffee, Directly Inhibits Fyn Kinase Activity and UVB-Induced COX-2 Expression. *Carcinogenesis* **2009**, *30*, 321–330. [CrossRef]

12. Haider, K.; Haider, M.R.; Neha, K.; Yar, M.S. Free Radical Scavengers: An Overview on Heterocyclic Advances and Medicinal Prospects. *Eur. J. Med. Chem.* **2020**, *204*, 112607. [CrossRef]
13. Zarrinbakhsh, N.; Rodriguez, A.; Misra, M.; Mohanty, A. Characterization of Wastes and Coproducts from the Coffee Industry for Composite Material Production. *BioRes* **2016**, *11*, 7637–7653. [CrossRef]
14. Bordiean, A.; Krzyżaniak, M.; Stolarski, M.J. Bioconversion Potential of Agro-Industrial Byproducts by *Tenebrio molitor*—Long-Term Results. *Insects* **2022**, *13*, 810. [CrossRef] [PubMed]
15. Aidoo, O.F.; Osei-Owusu, J.; Asante, K.; Dofuor, A.K.; Boateng, B.O.; Debrah, S.K.; Ninsin, K.D.; Siddiqui, S.A.; Chia, S.Y. Insects as Food and Medicine: A Sustainable Solution for Global Health and Environmental Challenges. *Front. Nutr.* **2023**, *10*, 1113219. [CrossRef] [PubMed]
16. Thrastardottir, R.; Olafsdottir, H.T.; Thorarinsdottir, R.I. Yellow Mealworm and Black Soldier Fly Larvae for Feed and Food Production in Europe, with Emphasis on Iceland. *Foods* **2021**, *10*, 2744. [CrossRef]
17. Zielińska, E.; Baraniak, B.; Karaś, M.; Rybczyńska, K.; Jakubczyk, A. Selected Species of Edible Insects as a Source of Nutrient Composition. *Food Res. Int.* **2015**, *77*, 460–466. [CrossRef]
18. Jantzen da Silva Lucas, A.; Menegon de Oliveira, L.; da Rocha, M.; Prentice, C. Edible Insects: An Alternative of Nutritional, Functional and Bioactive Compounds. *Food Chem.* **2020**, *311*, 126022. [CrossRef]
19. Andreadis, S.S.; Panteli, N.; Mastoraki, M.; Rizou, E.; Stefanou, V.; Tzentilasvili, S.; Sarrou, E.; Chatzifotis, S.; Krigas, N.; Antonopoulou, E. Towards Functional Insect Feeds: Agri-Food by-Products Enriched with Post-Distillation Residues of Medicinal Aromatic Plants in *Tenebrio molitor* (Coleoptera: Tenebrionidae) Breeding. *Antioxidants* **2022**, *11*, 68. [CrossRef]
20. Bordiean, A.; Krzyżaniak, M.; Stolarski, M.J.; Peni, D. Growth Potential of Yellow Mealworm Reared on Industrial Residues. *Agric.* **2020**, *10*, 599. [CrossRef]
21. Bordiean, A.; Krzyżaniak, M.; Aljewicz, M.; Stolarski, M.J. Influence of Different Diets on Growth and Nutritional Composition of Yellow Mealworm. *Foods* **2022**, *11*, 3075. [CrossRef]
22. Biasato, I.; Gasco, L.; De Marco, M.; Renna, M.; Rotolo, L.; Dabbou, S.; Capucchio, M.T.; Biasibetti, E.; Tarantola, M.; Sterpone, L.; et al. Yellow Mealworm Larvae (*Tenebrio molitor*) Inclusion in Diets for Male Broiler Chickens: Effects on Growth Performance, Gut Morphology, and Histological Findings. *Poult. Sci.* **2018**, *97*, 540–548. [CrossRef]
23. Rumpold, B.A.; Schlüter, O.K. Nutritional Composition and Safety Aspects of Edible Insects. *Mol. Nutr. Food Res.* **2013**, *57*, 802–823. [CrossRef] [PubMed]
24. Truman, J.W. The Evolution of Insect Metamorphosis. *Curr. Biol.* **2019**, *29*, R1252–R1268. [CrossRef] [PubMed]
25. May, J.C.; Wheeler, R.M.; Grim, E. The Gravimetric Method for the Determination of Residual Moisture in Freeze-Dried Biological Products. *Cryobiology* **1989**, *26*, 277–284. [CrossRef] [PubMed]
26. Kotsou, K.; Chatzimitakos, T.; Athanasiadis, V.; Bozinou, E.; Rumbos, C.I.; Athanassiou, C.G.; Lalas, S.I. Enhancing the Nutritional Profile of *Tenebrio molitor* Using the Leaves of *Moringa oleifera*. *Foods* **2023**, *12*, 2612. [CrossRef] [PubMed]
27. Son, Y.; Hwang, I.; Nho, C.; Kim, S.; Kim, S. Determination of Carbohydrate Composition in Mealworm. *Foods* **2021**, *10*, 640. [CrossRef] [PubMed]
28. Al-Amiery, A.A.; Al-Majedy, Y.K.; Kadhum, A.A.H.; Mohamad, A.B. Hydrogen Peroxide Scavenging Activity of Novel Coumarins Synthesized Using Different Approaches. *PLoS ONE* **2015**, *10*, e0132175. [CrossRef]
29. Ocampo, E.T.M.; Libron, J.A.M.A.; Guevarra, M.L.D.; Mateo, J.M.C. Phytochemical Screening, Phenolic Acid Profiling and Antioxidant Activity Analysis of Peels from Selected Mango (*Mangifera* spp.) Genotypes in the Philippines. *Food Res.* **2020**, *4*, 1116–1124. [CrossRef]
30. Makris, D.P.; Kefalas, P. Characterization of Polyphenolic Phytochemicals in Red Grape Pomace. *Int. J. Waste Resour.* **2013**, *3*, 2–5. [CrossRef]
31. Cicco, N.; Lanorte, M.T.; Paraggio, M.; Viggiano, M.; Lattanzio, V. A Reproducible, Rapid and Inexpensive Folin-Ciocalteu Micro-Method in Determining Phenolics of Plant Methanol Extracts. *Microchem. J.* **2009**, *91*, 107–110. [CrossRef]
32. Pinotti, L.; Ottoboni, M. Substrate as Insect Feed for Bio-Mass Production. *J. Insects Food Feed* **2021**, *7*, 585–596. [CrossRef]
33. LeCato, G.; Flaherty, B. Description of Eggs of Selected Species of Stored-Product Insects (Coleoptera and Lepidoptera). *J. Kansas Entomol. Soc.* **1974**, *39*, 308–317.
34. Pakshir, K.; Dehghani, A.; Nouraei, H.; Zareshahrabadi, Z.; Zomorodian, K. Evaluation of Fungal Contamination and Ochratoxin A Detection in Different Types of Coffee by HPLC-Based Method. *J. Clin. Lab. Anal.* **2021**, *35*, e24001. [CrossRef] [PubMed]
35. Kröncke, N.; Benning, R. Self-Selection of Feeding Substrates by *Tenebrio molitor* Larvae of Different Ages to Determine Optimal Macronutrient Intake and the Influence on Larval Growth and Protein Content. *Insects* **2022**, *13*, 657. [CrossRef] [PubMed]
36. Hussein, H.; Abouamer, W.; Ali, H.; Elkhadragy, M.; Yehia, H.; Farouk, A. The Valorization of Spent Coffee Ground Extract as a Prospective Insecticidal Agent against Some Main Key Pests of Phaseolus Vulgaris in the Laboratory and Field. *Plants* **2022**, *11*, 1124. [CrossRef]
37. Thanasoponkul, W.; Changbunjong, T.; Sukkurd, R.; Saiwichai, T. Spent Coffee Grounds and Novaluron Are Toxic to Aedes Aegypti (Diptera: Culicidae) Larvae. *Insects* **2023**, *14*, 564. [CrossRef] [PubMed]
38. Kröncke, N.; Wittke, S.; Steinmann, N.; Benning, R. Analysis of the Composition of Different Instars of *Tenebrio molitor* Larvae Using Near-Infrared Reflectance Spectroscopy for Prediction of Amino and Fatty Acid Content. *Insects* **2023**, *14*, 310. [CrossRef]
39. Kröncke, N.; Benning, R. Determination of Moisture and Protein Content in Living Mealworm Larvae (*Tenebrio molitor* L.) Using Near-Infrared Reflectance Spectroscopy (NIRS). *Insects* **2022**, *13*, 560. [CrossRef]

40. Costa, S.; Pedro, S.; Lourenço, H.; Batista, I.; Teixeira, B.; Bandarra, N.M.; Murta, D.; Nunes, R.; Pires, C. Evaluation of *Tenebrio molitor* Larvae as an Alternative Food Source. *NFS J.* **2020**, *21*, 57–64. [CrossRef]
41. Nowak, V.; Persijn, D.; Rittenschober, D.; Charrondiere, U.R. Review of Food Composition Data for Edible Insects. *Food Chem.* **2016**, *193*, 39–46. [CrossRef]
42. Iriondo-Dehond, A.; Uranga, J.A.; Del Castillo, M.D.; Abalo, R. Effects of Coffee and Its Components on the Gastrointestinal Tract and the Brain–Gut Axis. *Nutrients* **2021**, *13*, 88. [CrossRef]
43. Siulapwa, N.; Mwambungu, A.; Lungu, E.; Sichilima, W. Nutritional Value of Four Common Edible Insects in Zambia. *Int. J. Sci. Res.* **2014**, *3*, 876–884.
44. Melo, V.; Garcia, M.; Sandoval, H.; Jiménez, H.D.; Calvo, C. Quality Proteins from Edible Indigenous Insect Food of Latin America and Asia. *Emirates J. Food Agric.* **2011**, *23*, 283–289.
45. Daniel, J.R.; Vidovic, N. *Carbohydrates, Role in Human Nutrition*; Reference Module in Food Science; Elsevier: Amsterdam, The Netherlands, 2018. [CrossRef]
46. Abidin, N.A.Z.; Kormin, F.; Abidin, N.A.Z.; Anuar, N.A.F.M.; Bakar, M.F.A. The Potential of Insects as Alternative Sources of Chitin: An Overview on the Chemical Method of Extraction from Various Sources. *Int. J. Mol. Sci.* **2020**, *21*, 4978. [CrossRef] [PubMed]
47. Dong, T.; Guo, M.; Zhang, P.; Sun, G.; Chen, B. The Effects of Low-Carbohydrate Diets on Cardiovascular Risk Factors: A Meta-Analysis. *PLoS ONE* **2020**, *15*, e0225348. [CrossRef] [PubMed]
48. Santos, F.L.; Esteves, S.S.; da Costa Pereira, A.; Yancy, W.S.J.; Nunes, J.P.L. Systematic Review and Meta-Analysis of Clinical Trials of the Effects of Low Carbohydrate Diets on Cardiovascular Risk Factors. *Obes. Rev. Off. J. Int. Assoc. Study Obes.* **2012**, *13*, 1048–1066. [CrossRef]
49. Ghaly, A.E.; Alkoaik, F.N. The Yellow Mealworm as a Novel Source of Protein. *Am. J. Agric. Biol. Sci.* **2009**, *4*, 319–331. [CrossRef]
50. Dreassi, E.; Cito, A.; Zanfini, A.; Materozzi, L.; Botta, M.; Francardi, V. Dietary Fatty Acids Influence the Growth and Fatty Acid Composition of the Yellow Mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Lipids* **2017**, *52*, 285–294. [CrossRef]
51. Ghosh, S.; Lee, S.M.; Jung, C.; Meyer-Rochow, V.B. Nutritional Composition of Five Commercial Edible Insects in South Korea. *J. Asia. Pac. Entomol.* **2017**, *20*, 686–694. [CrossRef]
52. Jajic, I.; Popovic, A.; Urosevic, M.; Krstovic, S.; Petrovic, M.; Guljas, D.; Samardzic, M. Fatty and Amino Acid Profile of Mealworm Larvae (*Tenebrio molitor* L.). *Biotechnol. Anim. Husb.* **2020**, *36*, 167–180. [CrossRef]
53. Pipoyan, D.; Stepanyan, S.; Stepanyan, S.; Beglaryan, M.; Costantini, L.; Molinari, R.; Merendino, N. The Effect of Trans Fatty Acids on Human Health: Regulation and Consumption Patterns. *Foods* **2021**, *10*, 2452. [CrossRef]
54. Yun, J.M.; Surh, J. Fatty Acid Composition as a Predictor for the Oxidation Stability of Korean Vegetable Oils with or without Induced Oxidative Stress. *Prev. Nutr. Food Sci.* **2012**, *17*, 158–165. [CrossRef]
55. Baum, S.J.; Kris-Etherton, P.M.; Willett, W.C.; Lichtenstein, A.H.; Rudel, L.L.; Maki, K.C.; Whelan, J.; Ramsden, C.E.; Block, R.C. Fatty Acids in Cardiovascular Health and Disease: A Comprehensive Update. *J. Clin. Lipidol.* **2012**, *6*, 216–234. [CrossRef] [PubMed]
56. Paszczyk, B.; Polak-śliwińska, M.; Łuczyńska, J. Fatty Acids Profile, Trans Isomers, and Lipid Quality Indices in Smoked and Unsmoked Cheeses and Cheese-like Products. *Int. J. Environ. Res. Public Health* **2020**, *17*, 71. [CrossRef] [PubMed]
57. Atik, I.; Karasu, S.; Sevik, R. Physicochemical and Bioactive Properties of Cold Press Wild Plum (*Prunus spinosa*) and Sour Cherry (*Prunus cerasus*) Kernel Oils: Fatty Acid, Sterol and Phenolic Profile. *Riv. Ital. Sostanze Grasse* **2022**, *99*, 13–20.
58. Wali, J.A.; Jarzebska, N.; Raubenheimer, D.; Simpson, S.J.; Rodionov, R.N.; O'Sullivan, J.F. Cardio-Metabolic Effects of High-Fat Diets and Their Underlying Mechanisms-A Narrative Review. *Nutrients* **2020**, *12*, 1505. [CrossRef] [PubMed]
59. Al-Amrousi, E.F.; Badr, A.N.; Abdel-Razek, A.G.; Gromadzka, K.; Drzewiecka, K.; Hassanein, M.M.M. A Comprehensive Study of Lupin Seed Oils and the Roasting Effect on Their Chemical and Biological Activity. *Plants* **2022**, *11*, 2301. [CrossRef]
60. Nantapo, C.T.W.; Muchenje, V.; Hugo, A. Atherogenicity Index and Health-Related Fatty Acids in Different Stages of Lactation from Friesian, Jersey and Friesian × Jersey Cross Cow Milk under a Pasture-Based Dairy System. *Food Chem.* **2014**, *146*, 127–133. [CrossRef]
61. Kumar, M.; Kumari, P.; Trivedi, N.; Shukla, M.K.; Gupta, V.; Reddy, C.R.K.; Jha, B. Minerals, PUFAs and Antioxidant Properties of Some Tropical Seaweeds from Saurashtra Coast of India. *J. Appl. Phycol.* **2011**, *23*, 797–810. [CrossRef]
62. Chen, J.; Liu, H. Nutritional Indices for Assessing Fatty Acids: A Mini-Review. *Int. J. Mol. Sci.* **2020**, *21*, 5695. [CrossRef]
63. Makhutova, O.N.; Nokhsorov, V.V.; Stoyanov, K.N.; Dudareva, L.V.; Petrov, K.A. Preliminary Estimation of Nutritional Quality of the Meat, Liver, and Fat of the Indigenous Yakutian Cattle Based on Their Fatty Acid Profiles. *Foods* **2023**, *12*, 3226. [CrossRef]
64. Doley, J. *Vitamins and Minerals in Older Adults: Causes, Diagnosis, and Treatment of Deficiency*; Elsevier Inc.: Amsterdam, The Netherlands, 2017; ISBN 9780128092996.
65. Oonincx, D.G.A.B.; Finke, M.D. Insects as a Complete Nutritional Source. *J. Insects Food Feed* **2023**, *9*, 541–543. [CrossRef]
66. Wu, C.T.; Agrawal, D.C.; Huang, W.Y.; Hsu, H.C.; Yang, S.J.; Huang, S.L.; Lin, Y.S. Functionality Analysis of Spent Coffee Ground Extracts Obtained by the Hydrothermal Method. *J. Chem.* **2019**, *2019*, 4671438. [CrossRef]
67. Kapała, A.; Szlendak, M.; Motacka, E. The Anti-Cancer Activity of Lycopene: A Systematic Review of Human and Animal Studies. *Nutrients* **2022**, *14*, 5152. [CrossRef] [PubMed]
68. Anand, R.; Mohan, L.; Bharadvaja, N. Disease Prevention and Treatment Using β-Carotene: The Ultimate Provitamin A. *Rev. Bras. Farmacogn.* **2022**, *32*, 491–501. [CrossRef]

69. Payne, C.L.R.; Scarborough, P.; Rayner, M.; Nonaka, K. A Systematic Review of Nutrient Composition Data Available for Twelve Commercially Available Edible Insects, and Comparison with Reference Values. *Trends Food Sci. Technol.* **2016**, *47*, 69–77. [CrossRef]
70. Andrade, C.; Perestrelo, R.; Câmara, J.S. Bioactive Compounds and Antioxidant Activity from Spent Coffee Grounds as a Powerful Approach for Its Valorization. *Molecules* **2022**, *27*, 7504. [CrossRef]
71. Turck, D.; Bohn, T.; Castenmiller, J.; De Henauw, S.; Hirsch-Ernst, K.I.; Maciuk, A.; Mangelsdorf, I.; McArdle, H.J.; Naska, A.; Pelaez, C.; et al. Safety of Frozen and Dried Formulations from Whole Yellow Mealworm (*Tenebrio molitor* Larva) as a Novel Food Pursuant to Regulation (EU) 2015/2283. *EFSA J.* **2021**, *19*, e06778. [CrossRef]