

## Article

# Nutritional Composition and Safety Parameters of Mealworms (*Tenebrio molitor*) Reared on Substrates Derived from By-Products

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**Abstract:** Mealworms provide a viable option for transforming agricultural and food processing by-products that can be converted into a valuable source of high-quality protein and fat suitable for both animals and humans. Hence, our investigation was aimed at employing sprouted and green potatoes, wheat bran, and by-products from brewers' production as a comprehensive substrate for rearing mealworms. The nutritional value (fat and protein content) and composition of amino acids (AAs) and fatty acids (FAs) were tested in lyophilized and milled larvae. The results showed that the highest amount of protein was detected in sample 3L (59.18 ± 0.07%), grown on brewers' spent grain, whereas sample 2S (with wheat bran) comes out with the highest fat content (34.22 ± 0.491%). It was found that the amount of FAs in the larvae depended on the substrate used: the statistically highest amount of monounsaturated FAs was detected in the sample with sprouted potatoes (1L), the highest content of omega-3 FAs was found in the control sample (4CL), with agar–agar gels, and the sample with brewers spent grains (3L) showed a statistically higher amount of oleic acid. Meanwhile, the highest content of total polyunsaturated FAs (36.23%) was detected in the sample with wheat bran (2L). During the study, 16 distinct AAs have been analyzed, and sample 3L has demonstrated the highest content in 11 instances. It is noteworthy that the sample containing brewers' spent grain exhibited the highest peroxide levels, indicating oxidation. Meanwhile, the determined number of volatile fatty acids did not exceed the regulatory limits for meat. In summary, it can be asserted that adjusting the substrate is essential for extracting specific amino acids and FAs. However, this could potentially affect the content of peroxides. This adaptation enables the desired chemical composition in larvae, making it suitable for specific purposes such as animal or human nutrition and health enhancement.

**Keywords:** fatty acid; amino acid; sustainability; peroxide; volatile fatty acids; by-products



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## 1. Introduction

As the growing interest in mealworms is evident, it is clear that food safety considerations are receiving widespread attention [1]. However, together with a shift towards an economic, safe, and sustainable outcome, there is an increasing trend of attempting to rear

mealworms using by-products from production and agriculture: crop failures, rapeseed meal, wheat bran, brewer's spent grain, sugar beet molasses, grape pomace, apple pomace, pumpkin oil seed meal, and sunflower meal [2]. The impact of these by-products on the mealworms themselves can vary, depending on the chosen raw materials and their proportions [3]. The rearing of *Tenebrio molitor* larvae (Linnaeus, 1758), a member of the darkling beetle family (*Tenebrionidae*), using plant-based by-products offers multiple benefits. These include an effective means of generating biomass, utilizing food waste, producing high-quality proteins, and achieving both reduced environmental impact and increased cost efficiency [4–6]. When rearing mealworms, it should be taken into account not only that they are grown quickly with the lowest costs but also that the resulting biomass is of high quality. When analyzing the nutritional value of mealworms, besides the quantity of proteins and fats, it is crucial to consider the specific AAs and FAs present.

It is projected that the world's population will reach nearly 9.8 billion people by 2050 [7], and if agricultural systems remain as they are now, the production of edible crops would need to increase by 119% [8]. Consequently, environmental sustainability concerns underscore the importance of developing alternative protein strategies, including new sources of high-quality proteins and fats [9]. In scientific sources, there is a lot of published information about the high protein percentage in mealworm larvae [10]. However, to determine the quality of proteins, it is important to consider the concentration of each AA [11]. When choosing a substrate for growing mealworms, it is important to consider the composition of AAs because it can influence the growth rate and productivity of the larvae [10]. Meanwhile, the AAs composition of mealworms is important for humans in several key ways, such as health and nutritional value [12]. Essential AAs are important for both human and animal nutrition and health because the body cannot produce them and must obtain them through food [13]. Ravzanaadii et al., while rearing mealworms on traditional substrate (wheat bran), have found that the composition of AA meets the needs of not only domestic animals but also humans. In general, concentrations of lysine and threonine were higher, but lower amounts of methionine and cysteine were found in mealworms [14]. Another study conducted by Kröncke et al. showed that by choosing a substrate that is characterized by a high protein content and a suitable amino acid composition, the total protein content increases (70.9–74.1% dry weight), while the fat content decreases analogously [10]. Indeed, the drying method can also influence the composition of amino acids, as it was noted by Selaledi and Mabelebele that mealworm larvae dried in the sun and oven had higher levels of essential AA compared to lyophilized ones [15].

The composition of FAs in mealworms is important for several reasons, first because it influences the nutritional value [16]. Various FAs have different nutritional properties, and their presence in food can affect human health. For example, omega-3 FAs, found in fish, flaxseeds, and nuts, are known for their beneficial effects on heart health [17]. It is known that excessive consumption of saturated fatty acids (SFA) can increase the risk of heart and cardiovascular diseases, whereas unsaturated fatty acids can be beneficial for health [18]. Dreassi et al. studied the variation in fatty acid composition in *T. molitor* larvae depending on the substrate used [19]. They have found that despite the different fat content of the six different rearing substrates used, the fat content of *T. molitor* larvae was similar (>34% larvae). The results of the mentioned authors showed that the composition of FAs differs in larvae grown on different feeding substrates [19].

However, regardless of the nutritional value, it is equally important to monitor the formation of chemical toxins that can have a negative impact on human health [20]. Although the majority of chemical toxins, including biogenic amines, polycyclic aromatic hydrocarbons, heavy metals, glycoalkaloids, pesticides, etc., have already been examined and the results were published by the authors, it is necessary to pay attention to peroxides and volatile fatty acids, which have not been studied yet using these by-products [21]. The larvae primarily consume organic materials, which are converted into energy and other substances during digestion [22]. Various compounds are formed during digestion, includ-

ing fatty acids, which may be classified as volatile compounds. The FAs may result from lipid breakdown processes within the larvae's bodies or may be produced by microbial activity that affects organic materials present in the larvae's food or digestive tract [23]. Therefore, volatile compounds can originate as by-products of larval digestion or as a result of microbial metabolism.

The formation of peroxides can occur due to various factors, including environmental conditions, diet, and microbial activity [24]. Most peroxides are formed as a result of lipid oxidation, which can occur in the presence of high levels of oxygen or due to the activity of peroxidase enzymes [25]. These enzymes are naturally occurring in mealworms or their food constituents. Additionally, microorganisms can contribute to the formation of peroxides in the larval environment by promoting lipid oxidation or directly producing peroxides [26]. Yu-Ho et al. have conducted a study examining oxidative stability and have found that the accumulation of primary oxidation products during storage in intact mealworm oil increased the peroxide value extremely rapidly to 185.82 meq/kg after 50 days of storage, concluding that processing corrects the peroxide value [27].

Therefore, the aim of our study was to find local by-products or food residues that could be used in the mass production of larvae as the main substrate while maintaining a reduced amino and fatty acid ratio. Already published research data on larval fecundity, survival, etc. using brewers' sprain grain and wheat bran as substrate were encouraging [28,29]. In addition, sprouted potatoes from farms that were no longer suitable for food were included in the experiment. Our research hypothesis posits that by using by-products from production or farms as feed for mealworms, it is possible to obtain a product of high nutritional value. In this study, we have found out the influence of the substrate on the nutritional value, amino acid, and fatty acid composition of mealworms. In this research, we addressed a research gap by investigating the peroxide and volatile fatty acid content of mealworms, which had not been widely examined, and by analyzing potential factors influencing their formation. Additionally, we expanded upon the chemical safety and nutritional value analyses conducted in a previous study [21]. Furthermore, we explored the use of sprouted potatoes as a substrate, which is particularly relevant for our region but has not been tested by other researchers.

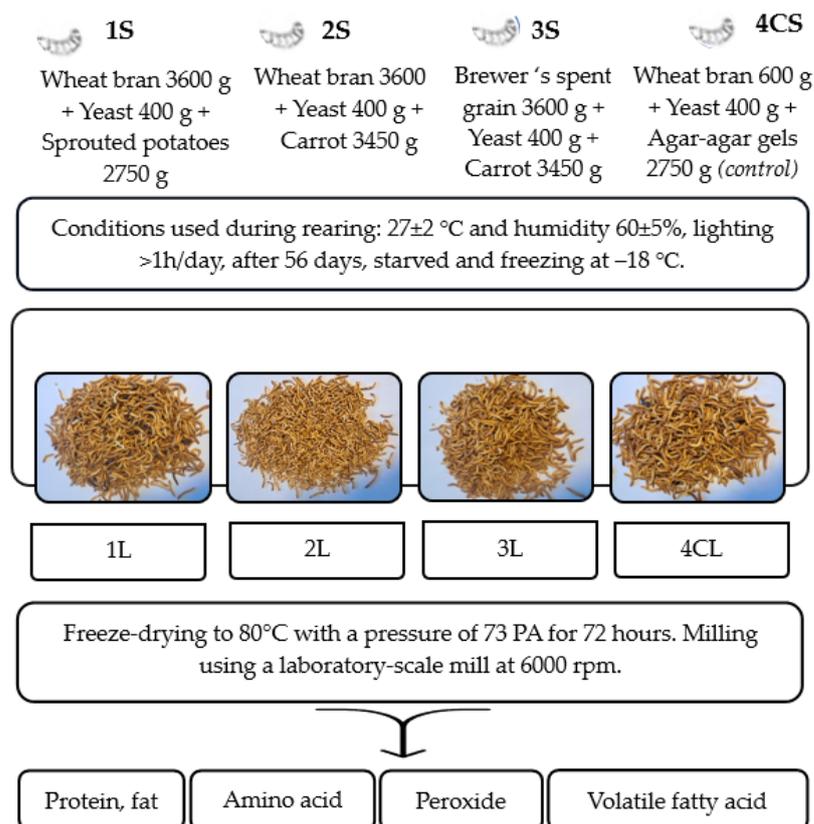
## 2. Materials and Methods

### 2.1. Rearing of *Tenebrio molitor*

An experiment involving the cultivation of mealworm larvae on different diets was carried out at the insect rearing research and development facility of Divaks company in Vilnius, Lithuania (Figure 1) [30].

During the experiment,  $27 \pm 2$  °C was maintained in the climate chamber. The relative humidity was  $60 \pm 5\%$ , and there was no lighting, except for viewing and analyzing the larvae (up to an hour per day). At the beginning of the experiment, mealworm eggs were collected from adult beetles of various ages using 40 cm × 60 cm insect rearing boxes with beetle breeding trays (Beekenkamp, Westland, The Netherlands). Wheat flour (Kaunos grūdai, Kaunas, Lithuania) was used as the substrate for egg laying and carrots as a source of moisture. Eggs were collected every 3–4 days using a 0.5 mm sieve. After collecting 17 g of eggs, corresponding to approximately 30,000 individuals, they were placed in one of the aforementioned 40 cm × 60 cm boxes with 1.5 kg of dry feed. Different substrate combinations were used for larval rearing. The first combination (1S) was made using a dry feed mixture (4 kg) of wheat bran (Fasma, Radviliškis, Lithuania) and dry brewer's yeast (Ekoproduktas, Panevėžys, Lithuania) in a 9:1 ratio. Additionally, farm waste was incorporated, including green/sprouted potatoes from local farmers (2.75 kg). The dry mass of the second combination (2S) mainly consisted of grain processing by-products. The mixture (approximately 4 kg) included wheat bran (Fasma, Radviliškis, Lithuania) and dry brewer's yeast (Ekoproduktas, Panevėžys, Lithuania) in a 9:1 ratio, along with carrots (Sanitex, Lithuania), totaling 3.45 kg per box. The third substrate (3S) consisted of brewery by-products (4 kg), featuring brewer's grain (Eurokorma, Vilnius, Lithuania) and dry brewer's yeast (Ekoproduktas, Panevėžys, Lithuania), in a 9:1 ratio, and carrots (Sanitex,

Kaunas, Lithuania) (totaling 3.45 kg in a box). Finally, the control diet (4CS) utilized in this bioassay was composed of dry feed from a mixture (4 kg) of wheat bran (Fasma, Radviliškis, Lithuania) and dry brewer's yeast (Ekoproduktas, Panevėžys, Lithuania) in a 9:1 ratio, along with agar-agar gels (10 g/l) (2.75 kg) (Carl Roth, Karlsruhe, Germany). Wet substrate, that is, carrots and potatoes, was given three times a week. Larvae were reared for 56 days using this method. Following this duration, the larvae were isolated from frass and remnants of their feed by employing a 2 mm sieve. Subsequently, they were placed in a climate chamber for a 24 h fasting period. After this fasting period, the unfed larvae were screened once more, killed at  $-18\text{ }^{\circ}\text{C}$ , and then moved for further examination.



**Figure 1.** Chart illustrating the complete research procedure. 1L—larvae (wheat bran + brewer's yeast + green potatoes); 2L—larvae (wheat bran + brewer's yeast + carrot); 3L—larvae (brewers' spent grain + brewer's yeast + carrot); 4CL—larvae, control (wheat bran + brewer's yeast + agar-agar gels); 1S—substrate (wheat bran + brewer's yeast + sprouted potatoes); 2S—substrate (wheat bran + brewer's yeast + carrot); 3S—substrate (brewers' spent grain + brewer's yeast + carrot); and 4CS—substrate, control (wheat bran + brewer's yeast + agar-agar gels).

## 2.2. Preparation of Samples

Mealworms and perishable substrate ingredients: sprouted (green) potatoes, carrots, and agar-agar gels were fast frozen in a Liebherr fast freezer (LGv 5010 MediLine, Richmond, BC, Canada) for 8 h at  $-35\text{ }^{\circ}\text{C}$ . Freeze drying was performed in a lyophilizer (Harvest Right, North Salt Lake, UT, USA) up to  $80\text{ }^{\circ}\text{C}$ . A pressure of 73 PA was used for freeze drying; the process of lyophilization lasted approximately 72 h.

Subsequently, lyophilized larvae and substrate were subjected to milling using a laboratory-scale mill (Fritsch Mill Pulverisette 14, Idar-Oberstein, Germany) at 6000 rpm.

## 2.3. Determination of Protein and Fat Content

The testing was conducted at an accredited laboratory, specifically the Chemical Science Laboratory of the Food Institute at Kaunas University of Technology in Lithuania [31].

The fat content was determined following the standard LST ISO 1443:2000, titled “Meat and meat products—Determination of total fat content” [32]. The sample is boiled in dilute hydrochloric acid (4 N) to release the lipid fractions, then filtered, dried, and extracted with n-hexane, the fat being retained on the filter.

The protein content was conducted at an accredited laboratory: the National Food and Veterinary Risk Assessment Institute located in Kaunas, Lithuania [33]. The protein content was determined according to the Kjeldahl reference method: COMMISSION REGULATION (EC) No. 152/2009. These methods are outlined in Annex III, Part C, of the regulation [34]. The protein conversion factor was 6.25.

#### 2.4. Determination of Amino Acids

The methodology was carried out according to the study by Juknienė et al. [35]. AAs were determined according to the COMMISSION REGULATION (EC) No. 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (text with EEA relevance) [34]. The hydrolysis of the samples was performed as described in COMMISSION REGULATION No. 152/2009. Briefly, about 100 mg of the sample were hydrolyzed with a 6M HCl solution containing 0.1% *w/v* phenol in the laboratory oven at 110 °C for 23 h. The resulting mixture was cooled, pH adjusted to 2.2, and diluted to 250 mL with citrate buffer (containing 0.1% *w/v* phenol and 5% *v/v* thiodiglycol). The resulting sample solution was used for derivatization. Concentrations of AAs were determined using a GCMS-QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph with a mass spectrometer. The concentration of individual analytes was determined using a calibration curve. To 50 µL of the sample solution, 50 µL of internal standard (~500 µM of norleucine), 120 µL of 0.1 M HCl, 40 µL of 2M NaOH, 200 µL of methanol–pyridine mixture (MeOH: Pyridine—4:1), and 500 µL of chloroform were added. The derivatization procedure was performed according to Ichihara et al., with some modifications [36]. Derivatization was performed using 50 µL of isobutylchloroformate. Afterward, 40 µL of 12.5 M NaOH was added, and the mixture was rederivatized with 50 µL of isobutyl chloroformate. The mixture was centrifuged at 13.2 krpm, and the organic layer was dried with anhydrous sodium sulfate before the analysis. A Capillary Rxi<sup>®</sup>-5MS column (Restek, Bellefonte, PA, USA) (length 30 m, coating thickness 0.25 µm, and inner diameter of 0.25 mm) was used for the analysis. The mass spectrometer operated in single-ion monitoring mode. The analyte was injected in splitless mode. The following parameters were used: MS ion source temperature: 220 °C, MS interface temperature: 300 °C, helium (carrier gas) flow: 0.99 mL/min, injector: 250 °C, and oven temperature: 100 °C (0.5 min) and 10 °C min<sup>-1</sup> to 310 °C (4 min).

#### 2.5. Determination of Fatty Acids

The methodology used to determine fatty acids was according to Juknienė et al. without any changes [35]. The composition analysis of fatty acids (FAs) was carried out per established procedures. The samples were prepared following the guidelines outlined in the standard LST EN ISO 12966—2:2017 Part 2 [37], which covers the preparation of methyl esters of FAs. To determine the fatty acid methyl esters, a gas chromatograph GC-MS (PerkinElmer Clarus 680, Waltham, MA, USA) and a mass spectrometer (PerkinElmer Clarus SQ8T) were utilized. The chromatographic column temperature was set at 60 °C for 1 min, followed by an increase at a rate of 12 °C per minute until reaching 250 °C, where it was held for 10 min. The spectrometer temperature was programmed to increase at a rate of 5 °C per minute up to 300 °C, and it was maintained at this temperature for 20 min. The evaporator was maintained at a temperature of 250 °C. The calibration curve for this analysis was prepared using the standard Supelco 37 Component FAME Mix provided by Merck & Co., Inc. (Rahway, NJ, USA).

## 2.6. Determination of Peroxide Value

The analysis was conducted at an accredited laboratory, the Chemical Science Laboratory of the Food Institute at Kaunas University of Technology in Lithuania [23]. The peroxide value was determined following the standard ISO 27107:2010 for animal and vegetable fats and oils. The determination of peroxide value and potentiometric end-point was performed [38]. The samples were homogenized to ensure uniformity. For this, 100 g of the homogenized sample is poured into 120 mL of diethyl ether and stirred for 2 h on a magnetic stirrer. Samples were filtered using a paper filter and then left to evaporate the diethyl ether. Then, 5 g of the samples are accurately weighed into a clean, dry reaction vessel. The samples were dissolved in a 50 mL mixture of isooctane (40 mL) and glacial acetic acid (60 mL), and then iodide was added. The iodide liberated by the peroxides was determined volumetrically using a sodium thiosulfate standard solution (0.01 N). The endpoint of the titration was determined electrochemically using the automatic titrator 916 Ti-Touch (Metrohm, Herisau, Switzerland). The peroxide value in milliequivalents of active oxygen per kilogram was calculated according to the following formula:

$$\frac{(V - V_0) \cdot C_{thio} \cdot F \cdot 1000}{m}$$

where  $V$ —is the volume of sodium thiosulfate solution used for the determination, in milliliters;  $V_0$ —is the volume of the sodium thiosulfate standard solution used for the blank test in milliliters;  $F$ —is the titer of the 0.01 N sodium thiosulfate;  $m$ —is the mass of the test portion in grams;  $c_{thio}$  is the concentration of the sodium thiosulfate, in mols per liter.

## 2.7. Determination of Volatile Fatty Acid Content

The tests were carried out in an accredited laboratory, the Chemical Science Laboratory, of the Food Institute at Kaunas University of Technology in Lithuania [27]. Volatile fatty acid content was determined, according to the statement of the minister of agriculture of the Republic of Lithuania on the approval of technical regulations for the assessment of the freshness of meat and poultry (FMAP) [39]. The technical regulation for meat and poultry freshness assessment was prepared in accordance with 29 April 2004 European Parliament and Council Regulation (EC) No. 853/2004 establishing specific hygiene requirements for foodstuffs of animal origin (OJ 2004 special edition, Chapter 3, Volume 45, p. 14) [40], as last amended in 11 January 2012 COMMISSION REGULATION (EU) No. 16/20012 (OJ 2012 L 8, p. 29) [41]. This determination was performed in triplicate for each sample. For this, 25 g of crushed larvae was placed into a 0.75–1.0-L capacity round-bottomed flask, to which 150 mL of a 2% sulfuric acid solution were added. The contents of the flask were stirred, and then the flask was tightly sealed and subjected to steam distillation until 200 mL of distillate was collected. Simultaneously, a control experiment was conducted under identical conditions. This was performed to ascertain the presence of volatile fatty acids that may be present in the sulfuric acid. The resulting larval distillate was titrated using a 0.1 mol/L potassium hydroxide solution. The amount of volatile fatty acids (mg) in mealworms was calculated according to the formula suitable for poultry:

$$X = \frac{5.61 \times (V_1 - V_2) \times K \times 100}{g} \quad (1)$$

where 5.61—0.1 mol/L titer of potassium hydroxide solution, mg/mL;  $V_1$ —0.1 mol/L the amount of potassium hydroxide solution used to neutralize the volatile fatty acids in 200 mL of mealworms extract, mL;  $V_2$ —0.1 mol/L amount of potassium hydroxide solution used to neutralize volatile fatty acids in 200 mL control extract, mL;  $K$ —correction factor for the molar concentration of potassium hydroxide (1.0–0.1 mol/L for potassium hydroxide solution); and  $g$ —mass of the mealworms, g.

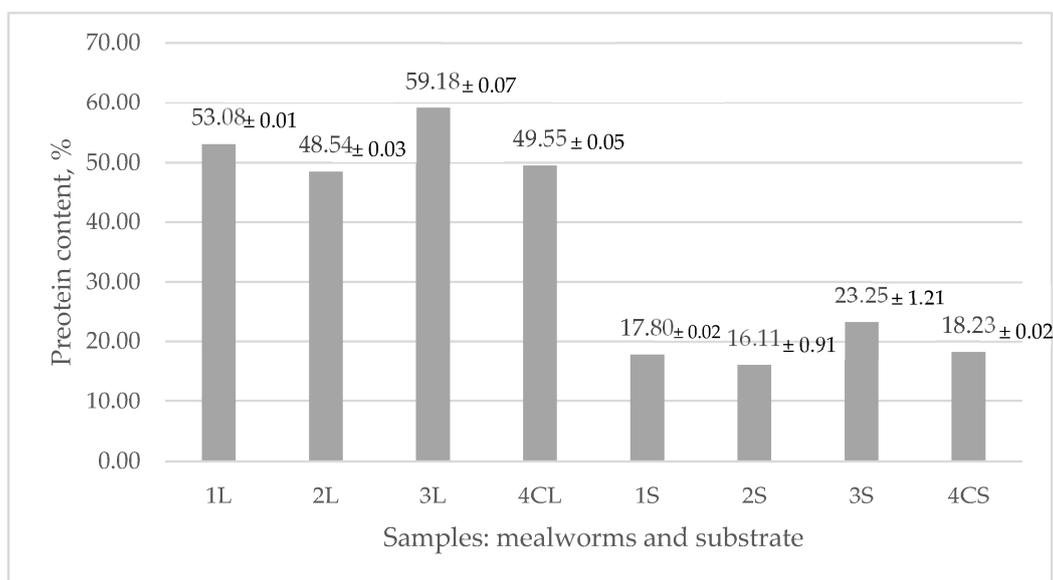
## 2.8. Statistics

Data analysis was performed using IBM SPSS Statistics 29.0.0.0 (241) (Des Plaines, IL, USA, JAV). The means and standard deviations for the examined variables within the compared groups were computed. To assess differences between the study groups, an ANOVA with the Fisher's LSD test was employed. Statistical significance was determined when the  $p$ -value was less than 0.05. Comparisons were conducted separately between larvae and substrate, as well as among them. The entire experiment was repeated three times.

## 3. Results and Discussion

### 3.1. Protein Content

Larvae reared on different substrates (1S, 2S, 3S, and 4CS) showed variations in protein content. Specifically, larvae from group 3L, reared on substrate 3S, exhibited the highest protein content at  $59.08 \pm 0.07\%$  ( $p < 0.05$ ), while larvae from group 2S had the lowest protein content at  $16.10 \pm 0.1\%$  ( $p < 0.05$ ) (Figure 2). These results have suggested that the choice of substrate significantly affected the protein content of mealworm larvae. These data are important for optimizing mealworm larvae as a protein source for various applications, such as animal feed and human consumption.



**Figure 2.** Protein content in substrate and mealworms. 1L—larvae (wheat bran + brewer's yeast + green potatoes); 2L—larvae (wheat bran + brewer's yeast + carrot); 3L—larvae (brewers' spent grain + brewer's yeast + carrot). 4CL—larvae, control (wheat bran + brewer's yeast + agar-agar gels); 1S—substrate (wheat bran + brewer's yeast + sprouted potatoes); 2S—substrate (wheat bran + brewer's yeast + carrot); 3S—substrate (brewers' spent grain + brewer's yeast + carrot); 4CS—substrate, control (wheat bran + brewer's yeast + agar-agar gels).

Lienhard et al. using brewer's spent grain have determined only 29.4% of protein in larvae, while in our study, we found  $48.59 \pm 0.03\%$  of protein when supplementing with carrots and brewer's yeast [29]. Mancini et al. applied former food products to *T. molitor* rearing. Their results showed that when rearing mealworms on brewery-spent grains, a protein content of 51.34% was determined, while in our study, a very similar percentage of  $48.54 \pm 0.03\%$  was found [42]. Bordiean et al. have studied the influence of different diets on the nutritional value of mealworms and have determined the protein content of  $47.9 \pm 0.10\%$  using a diet with wheat bran (on a dry weight basis). Whereas in our study, 3L larvae grown on a similar substrate (additionally inserted brewer's yeast + carrot) showed a protein content of  $59.18 \pm 0.07\%$  [16].

Azagoh and colleagues conducted a study determining the protein content in lyophilized *T. molitor* larvae and have found an extremely high protein content, as high as  $68.8 \pm 0.1\%$ , while in our analogous 3L sample with wheat bran, a 9.62% lower protein content was detected, and it was similar to the amount found in fish (63%) or soy meal (55) [43–45].

Mlček et al. have examined the influence of feed on the breeding of mealworms and, at the same time, reviewed the changes in nutritional value depending on the substrate used [46]. The results of the mentioned study showed that, using ware potatoes as a substrate, the amount of protein determined in the larvae reached  $54.9 \pm 0.3$  g/100 g (corresponding to 54.9%). In our study, using green and sprouted potatoes,  $53.08 \pm 0.01\%$  protein was found, so the results were similar to those of Mlček et al.'s study [46].

Our findings have indicated that the protein content in mealworms was influenced by the protein content present in the chosen substrate during their growth ( $p < 0.05$ ).

### 3.2. Composition of Amino Acids

Nine amino acids—histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine—are considered indispensable nutrients, and as mammals do not synthesize them, these are commonly referred to as the essential AA [47]. These that are particularly noteworthy in terms of health benefits are those that contribute to overall protein quality and have positive effects on human health; they play crucial roles in protein synthesis, immune function, and overall health [48,49]. Leucine, for example, is a branched-chain amino acid that is known to stimulate muscle protein synthesis [50]. Aspartic acid and glutamic acid are important for neurotransmitter function, while tyrosine is a precursor for neurotransmitters and has antioxidant properties [51]. Dietary AAs play a crucial role in human health. For instance, branched-chains may have adverse effects on blood pressure, while leucine has been demonstrated to play a crucial role in muscle function, underscoring its significance, as reviewed by Flynn et al. [52]. Stull conducted a scientific study investigating the amino acid composition of *T. molitor* larvae. The findings revealed that larvae subjected to a diet of wheat bran, oats, brewer's yeast, and carrots displayed the presence of all essential AA [53].

In our study (Table 1), analogously to the one conducted by Fasce et al., certain effects of diet on the compositions of AAs and FAs were observed [28]. The larvae consistently exhibited higher levels of valine, leucine, isoleucine, cystine, and threonine compared to their respective substrates ( $p < 0.05$ ). Methionine and phenylalanine levels were higher in larvae compared to the substrate, except for phenylalanine in larvae 4CL ( $p < 0.05$ ). Lysine and tyrosine levels were generally higher in larvae, especially in larvae 3L ( $p < 0.05$ ). Additionally, the control samples (4CS and 4CL) consistently exhibited lower amino acid values compared to the experimental conditions.

Ravzanaadii et al. have analyzed the nutritional value of mealworm and found that the content of isoleucine was 3.556 g/100 g, while in our study, it was 1.6–2.2 times lower than the researched value of only 1.61–2.14 g/100 g [14]. Meanwhile, the amount of leucine was determined to be 3.405 g/100 g, and in our study, it was 3.02–3.79 g/100 g, so the amount was very similar or even higher when using by-products. Lysine content in mealworms was also similar in both experiments; according to Ravzanaadii et al., it was 2.906, and our analogous results with the 3L sample had  $2.92 \pm 0.152$ . It is important to note that the amount of valine (2.439) in our results was higher in almost all cases, except for sample 2L (2.25–3.14 g/100 g), if compared to the two mentioned studies [14].

Observing the amino acid composition of lyophilized larvae and substrates, it is evident that larvae in 3L exhibited elevated levels of multiple AAs compared to other larvae and substrates. Specifically, 11 out of the 16 identified AAs demonstrated the highest concentrations in larvae in 3L. Therefore, it can be suggested that larvae in 3L could potentially have a very positive impact on consumers health. Individual AA contributes differently to health, and a balanced intake of various AAs is essential for overall well-being.

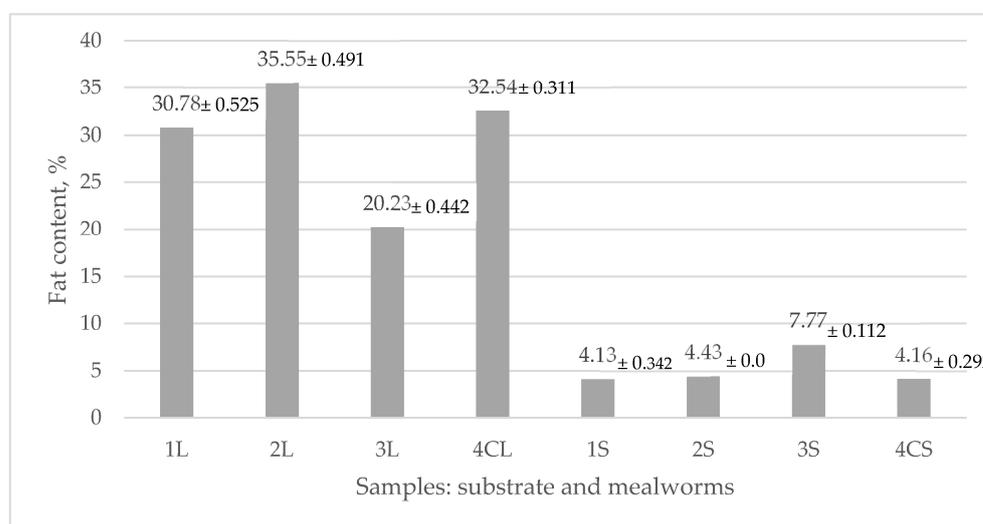
**Table 1.** Amino acid composition in lyophilized larvae and substrate, g/100 g of dry matter, average ± standard error, n = 3.

Samples	Larvae				Substrate			
	1L	2L	3L	4CL (control)	1S	2S	3S	4CS (control)
Valine	2.88 ± 0.134 a	2.25 ± 0.013 b	3.14 ± 0.130 a	2.93 ± 0.174 a	0.84 ± 0.089 a	0.74 ± 0.061 a	1.19 ± 0.025 b	0.83 ± 0.038 a
Leucine	3.54 ± 0.130 a	3.02 ± 0.002 b	3.79 ± 0.183 a	3.45 ± 0.262 ab	1.09 ± 0.060 a	1.00 ± 0.071 a	1.53 ± 0.009 b	1.10 ± 0.041 a
Isoleucine	2.01 ± 0.104 a	1.61 ± 0.011 b	2.14 ± 0.096 a	2.02 ± 0.111 a	0.63 ± 0.085 ac	0.47 ± 0.059 b	0.79 ± 0.007 c	0.52 ± 0.042 ab
Threonine	1.39 ± 0.082	1.27 ± 0.045	1.45 ± 0.002	1.34 ± 0.111	0.45 ± 0.004 a	0.39 ± 0.019 b	0.52 ± 0.013 c	0.41 ± 0.017 b
Methionine	0.60 ± 0.027 a	0.46 ± 0.044 b	0.62 ± 0.023 a	0.57 ± 0.026 ab	0.18 ± 0.011 a	0.24 ± 0.001 b	0.30 ± 0.017 c	0.26 ± 0.012 b
Phenylalanine	1.63 ± 0.065 ab	1.63 ± 0.039 ab	1.83 ± 0.097 a	1.61 ± 0.089 b	0.72 ± 0.034 a	0.66 ± 0.045 a	1.11 ± 0.010 b	0.69 ± 0.034 a
Lysine	2.73 ± 0.060 a	2.46 ± 0.016 b	2.92 ± 0.152 a	2.80 ± 0.075 a	0.80 ± 0.048 a	0.87 ± 0.044 ab	1.07 ± 0.001 c	0.95 ± 0.042 b
Histidine	1.60 ± 0.116 abc	1.49 ± 0.032 b	1.81 ± 0.116 cd	1.89 ± 0.009 d	0.83 ± 0.005 a	0.61 ± 0.021 b	0.71 ± 0.008 c	0.67 ± 0.002 d
Aspartic acid	3.92 ± 0.132 a	3.21 ± 0.035 b	4.30 ± 0.257 a	3.78 ± 0.306 ab	1.22 ± 0.056 a	1.10 ± 0.051 a	1.47 ± 0.048 b	1.19 ± 0.060 a
Glutamic acid	5.88 ± 0.238 a	3.73 ± 0.121 b	6.49 ± 0.903 a	6.17 ± 0.046 a	3.86 ± 0.254 a	2.80 ± 0.199 b	3.67 ± 0.171 a	3.00 ± 0.081 b
Glycine	2.41 ± 0.062 a	2.87 ± 0.067 b	2.66 ± 0.091 b	2.43 ± 0.090 a	0.83 ± 0.054	0.74 ± 0.061	0.81 ± 0.003	0.76 ± 0.03
Serine	1.55 ± 0.116 ab	1.39 ± 0.041 a	1.73 ± 0.002 b	1.68 ± 0.111 b	0.75 ± 0.003 a	0.66 ± 0.033 b	0.74 ± 0.013 a	0.67 ± 0.004 b
Alanine	3.35 ± 0.109 a	3.38 ± 0.094 ab	3.74 ± 0.166 b	3.40 ± 0.148 ab	0.80 ± 0.05 a	0.72 ± 0.046 a	0.99 ± 0.029 b	0.79 ± 0.045 a
Proline	2.94 ± 0.106 ab	2.67 ± 0.020 a	2.98 ± 0.143 ab	3.09 ± 0.174 b	1.12 ± 0.043 a	1.06 ± 0.063 a	1.88 ± 0.029 b	1.13 ± 0.025 a
Tyrosine	3.25 ± 0.037 a	3.47 ± 0.144 a	3.88 ± 0.160 b	3.54 ± 0.070 a	0.50 ± 0.013 a	0.64 ± 0.034 b	0.85 ± 0.026 c	0.40 ± 0.020 d
Cystine	0.38 ± 0.005 a	0.20 ± 0.005 b	0.37 ± 0.013 a	0.38 ± 0.007 a	0.34 ± 0.014 a	0.50 ± 0.001 b	0.50 ± 0.001 b	0.48 ± 0.013 b

a,b,c,d—means marked with different letters in the row (in the groups Larvae and Substrate, separately) differed significantly ( $p < 0.05$ , Fisher’s LSD criterion); 1L—larvae (wheat bran + brewer’s yeast + green potatoes); 2L—larvae (wheat bran + brewer’s yeast + carrot); 3L—larvae (brewers’ spent grain + brewer’s yeast + carrot); 4CL—larvae, control (wheat bran + brewer’s yeast + agar-agar gels); 1S—substrate (wheat bran + brewer’s yeast + sprouted potatoes); 2S—substrate (wheat bran + brewer’s yeast + carrot); 3S—substrate (brewers’ spent grain + brewer’s yeast + carrot); 4CS—substrate, control (wheat bran + brewer’s yeast + agar-agar gels).

### 3.3. Fat Content

The research results suggested that choosing a substrate with the highest fat content does not necessarily lead to the highest fat accumulation in the larvae, in contrast to proteins (Figure 3). We have noticed that larvae reared on different substrates exhibit variations in fat content. Mealworms from group 2L, reared on substrate 2S, had the significantly ( $p < 0.01$ ) highest fat content at  $34.22 \pm 0.491\%$  compared with larvae from group 3L, reared on substrate 3S, which had the lowest fat content at  $21.12\%$ .



**Figure 3.** Fat content in substrate and mealworms. 1L—larvae (wheat bran + brewer’s yeast + green potatoes); 2L—larvae (wheat bran + brewer’s yeast + carrot); 3L—larvae (brewers’ spent grain + brewer’s yeast + carrot); 4CL—larvae, control (wheat bran + brewer’s yeast + agar-agar gels); 1S—substrate (wheat bran + brewer’s yeast + sprouted potatoes); 2S—substrate (wheat bran + brewer’s yeast + carrot); 3S—substrate (brewers’ spent grain + brewer’s yeast + carrot); 4CS—substrate, control (wheat bran + brewer’s yeast + agar-agar gels).

Dreassi et al. conducted a research study examining the impact of various diets on the lipid and FAs content of mealworms [19]. According to the authors, the fat content in the substrate did not exhibit a statistically significant influence on the fat content observed in

the larvae, analogously as in our study. The aforementioned researchers have identified the highest fat content in larvae cultivated on an oat flour substrate ( $48.31 \pm 3.81\%$ ), whereas in our investigation using wheat by-products (in sample 2L), fat content was  $34.22 \pm 0.491\%$ , showing a difference of 14% [19]. One of the hypotheses for the difference in fat content between our study and the aforementioned study, and what could have influenced it, is that different research methods, larval treatments, and substrates were used.

Meanwhile, in another study, performed by Bordiean et al., 11-week-old mealworm larvae grown on experimental diets were examined. When grown on wheat bran, the fat content ( $26.1 \pm 0.82\%$ ) was 5.6% less than in our 1L sample ( $31.78 \pm 0.525\%$ ) [16].

In the aforementioned study by Mlček et al., the influence of the substrate on the fat content of mealworm larvae was also determined. The findings indicate that, in the case of using ware potatoes, as opposed to our sprouted potatoes that are no longer suitable for human consumption (1L), the fat content was measured at  $31.2 \pm 0.4$  g/100 g, while in our study, it was  $31.78 \pm 0.02\%$  [46]. It is noteworthy that the results exhibit no statistically significant difference. This comparison shows that even with by-products that are no longer fit for human consumption and should be disposed of, mealworms process them into quality fats.

In the study, we can confirm the conclusions by Bordiean et al. that the lower the protein content in the diet, the higher the fat content is found in the larvae [16].

### 3.4. Fatty Acid Content

The results of our study (Table 2) have shown that larvae in group 3L exhibited a significantly higher percentage of C16:0 ( $35.14 \pm 0.113\%$ ) compared to other larval groups, while C12:0 was notably abundant in larvae from group 3L ( $4.05 \pm 0.001\%$ ), showcasing the sensitivity of this SFA to specific dietary components. Larvae in group 1L displayed a distinct elevation in C16:1 ( $3.69 \pm 0.011\%$ ), underlining the influence of their unique dietary substrate on monounsaturated fatty acid content. C18:2 cis W-6 exhibited significant variations among larvae samples, with 1L and 4CL larvae demonstrating higher percentages compared to other samples, illustrating the specific modulation of this PUFA by distinct substrates ( $p < 0.05$ ).

The essential omega-3 fatty acid in 2L, C20:3-w3 with  $13.46 \pm 0.22\%$ , exhibited notable differences ( $p < 0.001$ ) among larvae groups, emphasizing the role of substrate composition in shaping the nutritional quality of larvae. Larvae from group 4CL displayed a higher percentage of DHA ( $4.19 \pm 0.003\%$ ), also known as essential alpha-linolenic acid, suggesting a potential association between the choice of substrate and the synthesis or accumulation of this essential fatty acid.

Dabbou et al. from Cambridge University have analyzed the meat of chickens fed with a substrate containing the mealworm *T. molitor*. The results have demonstrated that breast meat showed higher percentages of oleic and  $\alpha$ -linolenic acids and lower atherogenicity and thrombogenicity indexes. Therefore, it can be assumed that changing the composition of FAs in feed can have a positive effect on animal health [54].

According to Stephanie Venn-Watson et al., higher circulating concentrations of pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) in humans are associated with a lower risk of cardiometabolic disease, lower mortality, and better physiological condition [55]. Our results have shown the fatty acid profile of substrate and larvae fed with experimental diets. The highest amounts of C17:0 fatty acid were determined in the sample 2L ( $9.60 \pm 0.009\%$ ), while they were not detected in the substrate at all, and this is a significantly lower amount compared to the larvae ( $0 < 0.01$ ).

Mancini et al. tested different treatments for mealworms in their study, focusing on fatty acid composition and other parameters [42]. The results have shown that the lowest amount detected in the sample (C18:1) was observed when cooked at  $70^\circ\text{C}$  for 30 min ( $25.11\%$ ), while the highest amount ( $40.68\%$ ) was found in uncooked mealworms. In our study (Table 2), the lyophilization method was used, and for comparison, the sample 3L contained the least amount of C18:1 acid ( $33.303 \pm 0.067\%$ ), while the highest amount,

according to Simone Mancini et al., was  $39.24 \pm 0.002\%$ . Hence, the upper limit of detection aligned closely with our findings [42].

To summarize, larvae in 3L exhibited a beneficial fatty acid composition, especially in oleic acid. The unique fatty acid profiles identified across different larval groups emphasize the significance of customizing larval diets to achieve specific fatty acid compositions. These discoveries provide valuable insights for enhancing insect-rearing methodologies with the goal of producing larvae with tailored nutritional profiles suitable for diverse applications, including animal feed or human consumption.

These data suggest that the nutritional composition of mealworms, specifically in terms of FAs, is distinct from the substrate they are cultivated on (Table 3). Particularly, larval samples demonstrated significantly higher levels of C12:0, C16:1, C18:1 cis, C18:2 trans, and C20:1, compared to substrate ( $p < 0.001$ ). The observed variations may have implications for the potential use of mealworms as a sustainable and nutrient-rich food source. Further research and exploration into the factors influencing these fatty acid profiles could contribute to the optimization of mealworm cultivation for specific nutritional purposes.

These findings underscore the significance of diet in modulating the nutritional quality of insect larvae. Understanding the interplay between feeding substrates and resulting fatty acid compositions is pivotal, especially in the context of employing insect larvae as a sustainable protein source or in animal feed formulations.

According to Dreassi et al., although fat content in mealworms did not depend on the substrate and was similar ( $>34\%$  in larvae and  $>30\%$  in pupae), FA compositions differed in both larvae and pupae grown on different feeding substrates [19]. This is confirmed by the results of our study (Table 4), as we found 52.64% SFA in the sample 3L, while a significantly lower amount (only 17.33%) was detected in the sample 4CL. However, the amount of SFA detected in the substrate had no significant effect on the amount found in mealworms ( $p < 0.01$ ).

Polyunsaturated fatty acids (PUFA) are considered to be one of the most important cellular components, affecting the functioning and normal development of many organisms [56]. Petermann et al. have studied the roles of unsaturated fatty acids in the nervous system and concluded that PUFA levels are associated with diseases of the central nervous system such as depression, autism spectrum disorders, obesity, and neurodegenerative diseases [57]. Meanwhile, in our study, the highest amount of PUFA was found in the larvae in 3L sample with wheat bran.

In Table 4, we can see that the content of unsaturated fatty acids (MUFA + PUFA) of all larvae varied from 73.65% to 78.48%, except for sample 3L, which had only 46.07% of total fatty acid content.

Omega-3s, integral components of cell membrane phospholipids, fulfill crucial functions in the body [58]. They, polyunsaturated FAs (which include omega-3 and omega-6 FAs), not only serve as an energy source but also contribute to the formation of eicosanoids, exerting diverse effects on the cardiovascular, pulmonary, immune, and endocrine systems [59,60]. Omega-3 FAs are especially valued since the ratio of omega-3 to -6 in the Western diet is particularly inappropriate (15/1–16.7/1), and it promotes the pathogenesis of many diseases, such as cardiovascular, cancer, inflammatory, and autoimmune diseases [61] (recommended 4:1) [62]. Distinct differences were observed in omega-3 and omega-6 FAs, as well as the omega-6/3 FAs ratio, indicating varying nutritional profiles between larvae and substrate samples. Among the four plant by-products used for larval feeding, we have determined the recommended optimal ratio of omega-6 to omega-3 FAs in the three respective experimental groups (samples 2L, 3L, and 4CL). The amount of omega-3 FAs was significantly higher in mealworms compared to the substrate ( $p < 0.01$ ), while omega-6, on the contrary, was significantly lower ( $p < 0.01$ ). The most noteworthy finding was the exceptionally high omega-6/omega-3 FAs ratio in larvae sample 1L, indicating a significant imbalance in the essential FAs. This result could be attributed to the specific composition of the larval diet, specifically wheat bran, brewer's yeast, and sprouted potatoes.

**Table 2.** Fatty acid composition in lyophilized larvae and substrate, % of total FAs content, average ± standard error, *n* = 3.

Samples	Larvae				Substrate			
	1L	2L	3L	4CL	1S	2S	3S	4CS
C12:0	3.14 ± 0.001 a	0.41 ± 0.001 b	4.05 ± 0.001 c	2.25 ± 0.001 d	-	-	0.43 ± 0.008 b	-
C14:1 cis	-	-	0.96 ± 0.001 b	-	-	-	-	-
C16:0	17.05 ± 0.002 a	8.38 ± 0.003 b	35.14 ± 0.113 c	14.3 ± 0.665 d	19.72 ± 0.083 a	17.50 ± 0.062 b	24.63 ± 0.003 c	18.92 ± 0.044 d
C16:1	3.69 ± 0.011 a	0.61 ± 0.001 b	0.68 ± 0.001 c	2.62 ± 0.114 d	-	-	0.29 ± 0.001 b	-
C17:0	1.29 ± 0.002	9.60 ± 0.009	2.35 ± 0.001	0.34 ± 0.001	-	-	-	-
C18:0	4.80 ± 0.012 a	2.23 ± 0.001 b	10.47 ± 0.023 c	0.41 ± 0.012 d	1.22 ± 0.001 a	2.11 ± 0.003 b	2.34 ± 0.012 c	1.35 ± 0.094 d
C18:1 cis	39.24 ± 0.002 a	33.85 ± 0.001 b	33.30 ± 0.067 c	36.51 ± 0.654 d	19.25 ± 0.043 a	18.30 ± 0.072 b	16.10 ± 0.073 c	18.83 ± 0.063 d
C18:2 trans	-	-	1.42 ± 0.002 b	0.42 ± 0.033c	-	-	-	-
C18:2 cis W-6	26.45 ± 0.004 a	12.40 ± 0.005 b	6.70 ± 0.050 c	22.83 ± 0.548 d	53.99 ± 0.06 a	49.54 ± 0.004 b	49.54 ± 0.052 b	53.91 ± 0.041 c
c18:3 alfa	-	-	0.36 ± 0.001 b	-	-	-	-	-
c18:3 gama	-	1.34 ± 0.001 b	0.53 ± 0.006 c	0.61 ± 0.003 d	-	-	0.52 ± 0.042 b	-
C20:1	2.01 ± 0.001 a	1.15 ± 0.001 b	0.94 ± 0.001 c	1.55 ± 0.021 d	5.39 ± 0.001 a	10.17 ± 0.051 b	7.74 ± 0.032 c	5.35 ± 0.032 d
C22:0	0.14 ± 0.003 a	3.55 ± 0.003 b	0.64 ± 0.014 c	0.02 ± 0.001 d	-	1.18 ± 0.001 b	1.18 ± 0.001 c	-
C20:3 w6	0.35 ± 0.012 a	3.72 ± 0.002 b	0.90 ± 0.003 c	1.33 ± 0.001 d	-	-	-	-
C22:1 w9	0.18 ± 0.002 a	2.41 ± 0.001 b	-	2.68 ± 0.003 d	-	-	-	-
C20:3w-3	1.03 ± 0.011 a	13.46 ± 0.221 b	-	2.06 ± 0.001 d	0.39 ± 0.041 a	0.39 ± 0.012 a	1.18 ± 0.062 b	0.14 ± 0.003 c
C22:4 w6	0.32 ± 0.012 a	2.53 ± 0.011 b	0.22 ± 0.004 c	5.06 ± 0.085 d	-	-	0.13 ± 0.001 b	-
C20:5 w3	0.32 ± 0.001 a	2.80 ± 0.001 b	0.05 ± 0.001 c	2.81 ± 0.006 d	0.03 ± 0.001 a	0.29 ± 0.004 b	-	-
DHA	-	1.59 ± 0.001 b	1.28 ± 0.001 c	4.19 ± 0.003 d	-	-	2.7 ± 0.008 b	1.53 ± 0.008 c

—not detected; a,b,c,d—means marked with different letters in the row (in the groups Larvae and Substrate, separately) differed significantly (*p* < 0.05, Fisher’s LSD criterion); 1L—larvae (wheat bran + brewer’s yeast + green potatoes); 2L—larvae (wheat bran + brewer’s yeast + carrot); 3L—larvae (brewers’ spent grain + brewer’s yeast + carrot); 4CL—larvae, control (wheat bran + brewer’s yeast + agar-agar gels); 1S—substrate (wheat bran + brewer’s yeast + sprouted potatoes); 2S—substrate (wheat bran + brewer’s yeast + carrot); 3S—substrate (brewers’ spent grain + brewer’s yeast + carrot); 4CS—substrate, control (wheat bran + brewer’s yeast + agar-agar gels).

**Table 3.** Comparison of fatty acids in lyophilized larvae and substrate, % of total FAs content, average ± standard error, *n* = 3.

Sample	Fatty Acids												
	C16:1	C17:0	C18:0	C18:1 cis	C18:2 trans	c18:3 gama	C20:1	C20:3 w6	C22:1 w9	C20:3 w3	C22:4 w6	C20:5 w3	
1-15 Larvae	2.46 ± 1.411 ***	1.90 ± 1.373 ***	3.39 ± 3.821 *	4.48 ± 3.960 *	35.72 ± 2.472 ***	0.46 ± 0.613 ***	0.62 ± 0.504 **	1.41 ± 0.431 ***	1.58 ± 1.341 **	1.32 ± 1.294 **	4.14 ± 5.674 *	2.03 ± 2.027 **	1.49 ± 1.371 **
Substrate	0.11 ± 0.201	0.07 ± 0.132	0 ± 0	1.75 ± 0.51	18.12 ± 1.273	0 ± 0	0.14 ± 0.233	7.16 ± 2.084	0 ± 0	0 ± 0	0.53 ± 0.411	0.03 ± 0.062	0.08 ± 0.135

In the groups, larvae and substrate separately differed significantly (\* *p* < 0.05; \*\* *p* < 0.01; \*\*\* *p* < 0.001, Fisher’s LSD criterion).

**Table 4.** Fatty acid ratio in lyophilized larvae and substrate, % of total FAs content,  $n = 3$ .

Samples	Larvae				Substrate			
	1L	2L	3L	4CL	1S	2S	3S	4CS
Total SFA	26.42 a	24.15 b	52.64 c	17.33 d	20.94 a	20.79 b	28.59 c	20.25 d
Total MUFA	45.12 a	38.02 b	35.89 c	43.37 d	24.64 a	28.47 b	24.13 c	24.18 d
Total PUFA	28.47 a	36.24 b	10.18 c	35.11 d	54.42a	50.74 b	44.63 c	54.05 d
Omega-6 FA	27.12 a	18.65 b	7.82 c	29.22 d	54.00 a	49.54 b	42.92 c	53.91 d
Omega-3 FA	0.32 a	4.39 b	1.69 c	6.99 d	0.03 a	0.29 b	2.65 c	1.53 d
Omega-6/3 FA	85.05 a	4.25 b	4.632 c	4.18 d	1838.83 a	170.51 b	16.20 c	35.34 d

a,b,c,d—means marked with different letters in the row (in the groups larvae and substrate separately) differed significantly ( $p < 0.05$ , Fisher's LSD criterion); SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids; 1L—larvae (wheat bran + brewer's yeast + green potatoes); 2L—larvae (wheat bran + brewer's yeast + carrot); 3L—larvae (brewers' spent grain + brewer's yeast + carrot); 4CL—larvae, control (wheat bran + brewer's yeast + agar-agar gels); 1S—substrate (wheat bran + brewer's yeast + sprouted potatoes); 2S—substrate (wheat bran + brewer's yeast + carrot); 3S—substrate (brewers' spent grain + brewer's yeast + carrot); 4CS—substrate, control (wheat bran + brewer's yeast + agar-agar gels).

Diets composed entirely of 100% bread and 100% oat flour demonstrated SFA, PUFA content, and an  $n-6/n-3$  ratio deemed more suitable for human consumption, according to Dreassi et al. [19]. Meanwhile, in our study, the healthiest combination would be 2L and 3L larvae, considering the SFA and ratio of omega-6 and -3 FAs.

In the aforementioned study, Mancini et al. have determined the amount of SFA, MUFA, PUFA, and omega-6/3 ratio in mealworms [42].

The highest amount of SFA was found in uncooked mealworms—30.37%, the lowest—13.76%, while in our study, the highest amount (52.64%) was found in sample 3L, whereas the lowest one was found in sample 4CL with 17.33% of total FAs. The amount of MUFA in the aforementioned study was 42.83% in unexposed larvae, while in our study, it was determined to be as high as 45.117% in sample 1L. So lyophilization and a suitable substrate with wheat bran, brewer's yeast, and sprouted potatoes, gave a better result than it would have been without processing. The highest amount (36.24%) of PUFA was found in sample 2L, while using the same substrate—brewery-spent grains and bread as former foodstuffs—only with a different processing method. The determined amount varied from 14.55 to 52.38%, according to Mancini et al. The ratio of omega-6 to -3 fatty acids in samples 2L, 3L, and 4CL was balanced, whereas in a study conducted by Mancini et al., the closest ratio was found in untreated (5.49%) or oven cooked larvae at 70 °C for 30 min. (1.57%). Therefore, in comparison, we can say that the selection of our substrates and the method of processing contributed to a balanced ratio of omega FAs [42].

The data shown in Table 4 could be crucial for future research exploring the influence of substrate components on larval development and health. Additionally, FAs may also impact human health and nutrition.

### 3.5. Peroxide Value and Volatile Fatty Acid Content

Volatile fatty acids and lipid peroxides can form due to high levels of oxidation [63]. When fats and oils undergo oxidation, they can form lipid peroxides, which are initial oxidation products [64]. Subsequently, these peroxides can break down into volatile fatty acids. Oxidation can occur due to various factors, including heat, light, air, and other oxidation-promoting agents [65]. In comparison, with the legal act ISO 27107:2010, which is intended for animal and vegetable fats and oils, if the amount of peroxides exceeds 10 meq/kg, it is suggested that fats are oxidized [66]. Kröncke et al., in their study, found that freeze-dried methods are not suitable for mealworms because they exhibit significantly higher oxidation than the other drying methods [22]. So, one of the hypotheses that there is an increase in peroxides in mealworms (Table 5) is due to the selected processing method. In our research, all larvae were kept under similar conditions, but it was found that in sample 3L with brewers' spent grain, the amount of peroxide exceeded the recommended limit and the content was 100 times higher than in samples 1L, 2L, and 4L ( $p < 0.01$ ). However, a statistically significant difference between samples 1L, 2L, and 3L was not

detected. The higher content of peroxides and volatile acids found in larvae grown on brewers' spent grain could be explained by various factors. The possible reasons may include specific differences in chemical composition between brewers' spent grain and other growth substrates, which influence larval chemical interactions and metabolism. In our previous study, we noticed that brewers' spent grain had a significantly higher amount of fats and oils, which, if exposed to air or other oxidizing factors, could start oxidation processes leading to the formation of peroxides [67].

The volatile fatty acid content was found to be significantly higher in the sample 3L, analogously to the peroxide content. It was even tens of times higher than in samples from other cultivation conditions ( $p < 0.01$ ), and the reasons may also be the same. According to the previously mentioned statement by FMAP [37], the amount of volatile fatty acids in mealworms should not exceed 0.35 mL. In our study, the maximum amount of volatile fat was found in the sample 3L (346 mg), which corresponds to 0.35 mL, so the larvae are considered to be completely fresh.

**Table 5.** Peroxide value and volatile fatty acid content in lyophilized larvae,  $n = 3$ .

	Peroxide Value, mg	Volatile Fatty Acid Content, meq/kg of Fat
1L	0.1 ± 0.0 a	44.67 ± 1.33 a
2L	0.1 ± 0.0 a	39 ± 1.0 a
3L	14.87 ± 0.3 b	346 ± 4.0 b
4CL	0.17 ± 0.3 a	43 ± 1.0 a

a,b—means marked with different letters in the column (in the groups of larvae separately) differed significantly ( $p < 0.05$ , Fisher's LSD criterion). 1L—larvae (wheat bran + brewer's yeast + green potatoes); 2L—larvae (wheat bran + brewer's yeast + carrot); 3L—larvae (brewers' spent grain + brewer's yeast + carrot); 4CL—larvae, control (wheat bran + brewer's yeast + agar-agar gels).

Thus, it can be concluded that not only the treatment method but also the substrate itself can influence the oxidative processes, leading to the formation of a higher number of peroxides in the sample 3L compared to larvae grown on other substrates. The volatile fatty acid content may not exceed regulatory limits because their quantity may depend on other factors such as the composition and quantity of lipid acids, which vary among different growth conditions. Additionally, metabolic processes may occur that enable larvae to control the volatile fatty acid content, even if the level of peroxides is higher. However, it should also be taken into account that there are no adapted research methods or regulations for mealworm analysis, so meat/poultry standards were used.

#### 4. Conclusions

The highest protein content was found in sample 3L when grown on brewers spent grain. Out of the 16 analyzed amino acids, larvae 3L exhibited the highest levels in 11 instances, including essential ones. Sample 2S with wheat bran had the significantly highest fat content ( $34.22 \pm 0.491\%$ ) compared with other samples. By analyzing the amount of fat in the larvae and in the substrate used, we did not find any significant dependence. Sample 3L exhibited a beneficial fatty acid composition, especially concerning omega-3 FAs and oleic acid. Sample 2L had the highest total content of PUPA (36.24%) and total content of SFA (24.15%), while sample 1L had the highest total content of MUFA (45.18%). The worst omega-6/3 FA ratio was detected in sample 1L with sprouted potatoes, while in the rest of the samples (2L, 3L, and 4CL), the ratio was very close to the scientific recommendation of 1:4. Thus, the results showed that the amount of both FAs and AAs in the larvae depended on the substrate in which they were reared, but sample 3L was the leader in both AAs and FAs. We have also found that the substrate can have an influence on peroxides and volatile fatty acid content since, during cultivation and storage under analogous conditions, except for the monthly substrate, in sample 3L with the brewer's spent grain, a significantly higher amount of peroxides was formed. The short-term benefit of this study encompasses a better understanding of the dietary conditions of *T. molitor* larvae and their interaction with the substrate, which can contribute to improving their breeding conditions and nutritional value. The long-term benefit entails the emergence of new opportunities for the efficient conversion of low-cost industrial by-products through

the utilization of *T. molitor*. However, for future research, it should be taken into account whether the growth of *T. molitor* larvae under specific conditions creates opportunities for the formation of toxic elements and the reproduction of pathogenic microorganisms. Also, the exact source of peroxide formation should be found out in order to prevent its formation in the future.

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