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The Impact of Farm and Industrial Feed Waste on the Safety Parameters of *Tenebrio molitor* Larvae

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Abstract: The rising global demand for animal-based food has an increasingly detrimental ecological impact, exacerbated by significant food waste (approximately one-third of all food). This research aimed to analyze the possibility of changing the usually balanced feed with sustainable alternatives that remain as a by-product of the production of farms, grain processing, and breweries, thus promoting the sustainability of agriculture. The mealworm larvae were reared on different substrates: (1) agar-agar gels, wheat bran, and brewer's yeast, (2) carrots, wheat bran, and brewer's yeast, (3) sprouted potatoes, wheat bran, and brewer's yeast, and (4) carrots, brewers' spent grain and brewer's yeast. For analysis, the frozen larvae were lyophilized and tested for chemical safety in three accredited laboratories. The results have shown that all tested samples had lower levels of pesticides than the detection limit. In scientific literature, we didn't find studies on polycyclic aromatic hydrocarbons (PAH). In our study, we found PAH in the substrate and these toxins, as our study shows, can also enter the larvae, but no significant accumulation was observed (sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene was 0.0007 mg/kg). Furthermore, the total content of PAH, benzo(a)pyrene and histamine did not exceed recommended levels. We have noticed that the highest concentration of heavy metals (e.g., chromium—1.45 ± 0.02) was found in the sample with the brewer's by-products. While numerous studies utilize plant-derived by-products, the accumulation of glycoalkaloids has not been explored. Among the all glycoalkaloids (tomatidin, tomatine, α-solanine, α-chaconine and solanidin), amounts of α-solanine and α-chaconine were the highest, detected in the sample with sprouted potatoes (175.12 ± 0.21 and 139.32 ± 0.32 mg/kg, respectively). The amount of total putrescine, tyramine, spermine, and spermidine in mealworm larvae was statistically higher compared to the amount detected in the substrate, and histamine level-on the contrary, was statistically significantly lower compared to the amount detected in the substrate. Considering the amount of toxic substances found in the substrate from the by-products, we can assume that mealworms did not accumulate high levels of toxins, which would violate regulations.

Keywords: mealworms; plants by-products; biogenic amines; PHA; heavy metals; glycoalkaloids; pesticides

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1. Introduction

Yellow mealworm has been traditionally consumed outside the European Union (EU) [1]. The frozen, dried, and powder forms mealworms (*Tenebrio molitor* Linnaeus, 1758) family of darkling beetles (*Tenebrionidae*) are the first novel food of insect origin approved by the authorities as a result of the first opinion of the European Food Safety Authority (EFSA) in January 2021 [2,3]. These larvae are notable for their high protein content and abundance of essential amino acids such as phenylalanine, tyrosine, and tryptophan [4].

Demand for animal-origin food is increasing globally and is projected to grow by 70–80% by 2050 [5]. The *T. molitor* larvae can be consumed as human food and are industrially grown as animal feed/supplement. In this context, *T. molitor* emerges as a promising partial solution due to its ability to be cultivated at high densities, coupled with a significantly reduced need for water and space when compared to traditional farm animals like beef cattle, chickens, and pigs [5]. The cultivation of mealworms is cost-effective, energy-efficient with a low ecological footprint, and is one of the most important species for converting plant biomass into high-quality protein [6,7]. One can assume that the consumption of mealworm larvae may be economically viable. Firstly, considering the costs of livestock farming, the expenses associated with the cultivation of mealworm larvae could be lower than traditional livestock farming [6,8,9]. Additionally, it is essential to consider the impact of meat and mealworm larvae consumption on healthcare costs. Excessive consumption of meat, rich in saturated fatty acids, poses a risk to cardiovascular health and contributes to the onset of chronic diseases [10–12]. Treating these diseases imposes significant costs on the state in terms of healthcare [13]. Therefore, the integration of mealworm larvae into diets may lead to long-term financial savings in the healthcare sector. One of the well-known aspects to consider is the environment and sustainability, specifically the emission of greenhouse gases. The cultivation of mealworm larvae may result in lower greenhouse gas emissions, contributing to mitigating climate change [14]. In general, the incorporation of mealworm larvae into Western diets could potentially have positive effects on both the economy and healthcare, as well as contribute to environmental sustainability. However, the realization of these benefits depends on numerous factors and can only be adequately explained through in-depth research and analysis.

In the Western world, the consumption of mealworm larvae has not been widely embraced [15]. Several factors contribute to the hesitancy in incorporating mealworms into Western diets: cultural preferences, perceptions, and potential safety concerns [16,17]. Limited research and understanding of the potential risks associated with insect consumption, such as allergic reactions or the presence of contaminants, may contribute to apprehension [18,19]. While studies have been conducted on various aspects of mealworms, there is a notable gap in comprehensive research focusing on chemical safety aspects. One of the ways to encourage wider acceptance of mealworms in Western diets is to address safety concerns through rigorous scientific research. Panel members of EFSA, including Dominique Turck et al., conducted an analysis of biogenic amines by rearing larvae exclusively on the adapted substrate. They claim that the maximum level of histamine found in the larvae was no higher than those used in the fishery sector [20]. They have also concluded that the detectable amount of polycyclic aromatic hydrocarbons in snacks with larvae was below 2 μg/kg, so the maximum residue level is much lower than in different food products, except for infant food and food for special medical purposes [20]. Several studies on the accumulation of heavy metals (Cd, Pb, Ni, As, Hg) in *T. molitor* have been reported [21]. Poma et al. investigated the effect of heavy metals on mealworms and found that concentrations of heavy metals in mealworms were lower than in chicken eggs, fish, and meat [22]. However, Mlček et al. observed a high and potentially dangerous concentration of cadmium in mealworms (157–186 mg kg^{-1}) [23]. Considering this, during the selection of by-product ingredients, it is essential to consider its correlation with the heavy metal content in the feeding substrates.

Additionally, mealworm larvae exhibit relatively rapid growth. As per scientific findings, mealworms can ingest up to 10% of their body weight in feed per day [24], and

they demonstrate a lower sensitivity to changing their substrate to low-value (biowaste) materials in comparison to certain other edible insects [25–27]. Several studies have already been conducted on the cultivation of mealworm larvae using various biowastes. Multiple criteria have been analyzed, including cultivation duration, survival rates, feed consumption, chemical composition, microbial loads, antioxidant status, and economic efficiency [21,27–30]. Cultivation practices involving wheat bran, green and sprouted potatoes, and brewery by-products showcase the versatility of mealworms. Wheat bran is one of the main sources on which mealworm larvae are grown industrially, due to its adequate growth and nutritional value. Wheat bran is the outer layer of wheat, particularly valuable from a nutritional perspective [31]. Wheat bran is removed (separated) during the flour milling process to obtain finer flour used in food production [32].

Brewer's spent grain, considered a brewer's by-product, can be successfully used to grow mealworm larvae as well, is also a valuable strategy, given their ready availability, large quantities, and relative cost-effectiveness [13]. Brewers' by-products are valued because they are readily available, can be obtained in large quantities, and are relatively inexpensive.

Potatoes contain essential nutrients, including vitamin C, potassium, and dietary fiber [33,34]. In Europe, several tons of sprouted and green potatoes are discarded annually, the precise quantity can vary depending on numerous factors, including consumer habits, trade standards, production practices, and even specific regions. According to European Union data, approximately 58 million tons of food waste are generated per year, encompassing not only potatoes but also various other food products [35]. These potatoes can pose risks to human health due to the presence of toxic glycoalkaloids [34]. Commercially available potatoes contain about 2 mg/100 g of glycoalkaloids, while in sprouted and green, their amount increases to about 50–100 mg/100 g. One of the main glycoalkaloids is solanine, according to the Food and Drug Administration, it should not exceed 20 mg/100 g of the product [36]. Utilizing potatoes, deemed unfit for human consumption, as substrates for mealworm cultivation we propose a sustainable solution to reduce agricultural waste and potentially substitute for other feeding diets [37]. There are no detailed studies on the accumulation of glycoalkaloids in edible insects. Only a single study reveals the effect of glycoalkaloids on insect survival, cardiac seriousness, etc. However, potatoes, unsuitable for human consumption, can also pose risks to human health due to the presence of toxic glycoalkaloids [38,39].

Understanding the dietary impact on *T. molitor* is crucial for its safe utilization in food and feed products. Therefore, the primary aim of our research was to enhance the sustainability of mealworm cultivation, with a specific focus on the chemical safety of the larvae. Choosing to rear the larvae on different substrates was made seeking to perform a chemical safety investigation and research the accumulation of biogenic amines, polycyclic aromatic hydrocarbons, heavy metals, and glycoalkaloids, comparing their concentrations in the substrate and lyophilized larvae. We have hypothesized that by cultivating mealworm larvae on by-products obtained from brewing and grain processing, as well as agricultural products, the accumulated level of toxic compounds would not be significant concerning the safety of the final product—mealworm larvae. The results of our research indicated that using by-products from food industry breweries, grain processing, and farm waste for mealworm cultivation does not exceed established limits for regulated chemical and toxic substances, thereby supporting the safety of the final product—mealworm larvae. However, there is apprehension regarding heightened levels of specific chemical toxins, which are merely advisory in quantity but can indeed have tangible health effects.

2. Materials and Methods

2.1. Preparation of Research Material

The study consisted of several stages: substrate selection, larval rearing, lyophilization, milling, coding, and research (Figure 1).

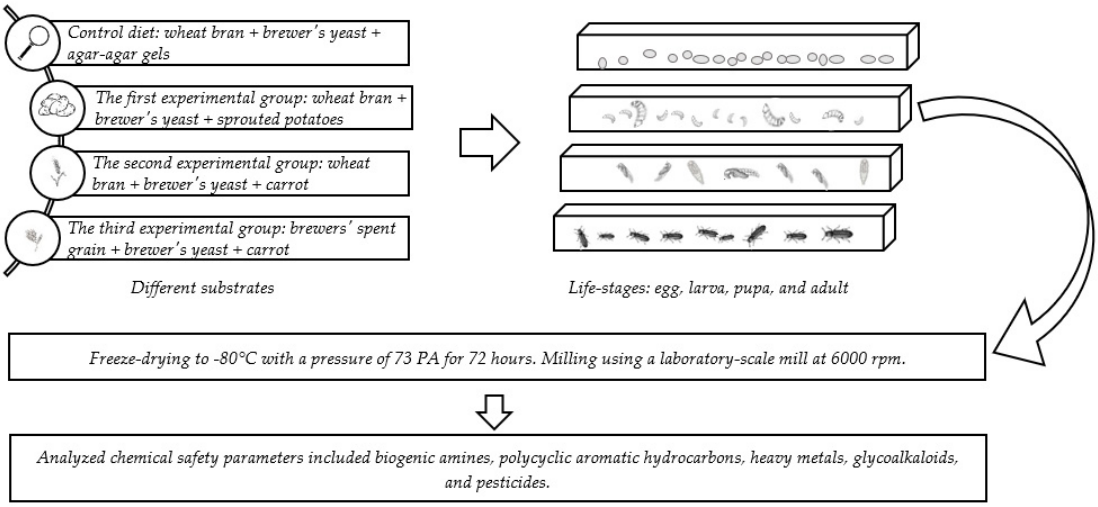

Figure 1. A scheme that depicts the entire research process.

Insect Rearing

Mealworms are also known as the larvae of the yellow mealworm beetle *T. molitor.* This species of dark beetle goes through four life stages: egg, larva, pupa, and adult. It was the larval stages, 56 days, that were used for the studies. In this research, we have analyzed the possibility of replacing conventional plant products with sustainable alternatives that remain as a by-product from farms or breweries' production (Table 1). When using carrots, which are mostly used in industrial cultivation, it is difficult to maintain the same moisture content due to the effect of the season on carrots and repeat the experiment under the same conditions. For this reason, the agar-agar gels were selected due to their particularly excellent reproducibility conditions. Agar-agar gels can be prepared, and the assay reproduced under exactly analogous conditions.

Table 1. Abbreviations of lyophilized ground larvae and substrates were used for further coding.

	Control Diet	The First Experiment	The Second Experiment	The Third Experiment
Proportions used for rearing larvae	wheat bran 3600 g yeast 400 g agar-agar gels 2750 g	wheat bran 3600 g yeast 400 g sprouted potatoes 2750 g	wheat bran 3600 g yeast 400 g carrot 3450 g	brewers' spent grain 3600 g yeast 400 g carrot 3450 g
Proportions used for an experiment	wheat bran 100 g yeast 11.11 g agar-agar gels 76.39 g	wheat bran 100 g yeast 11.11 g sprouted potatoes 76.39 g	wheat bran 100 g yeast 11.11 g carrot 95.83 g	brewers' spent grain 100 g yeast 11.11 g carrot 95.83 g
Proportions used for an experiment after lyophilization	wheat bran 100 g yeast 11.11 g agar-agar gels 0.76 g (lost 99% of total weight)	wheat bran 100 g yeast 11.11 g sprouted potatoes 18.33 g (lost 76% total weight)	wheat bran 100 g yeast 11.11 g carrot 11.50 g (lost 88% total weight)	brewers' spent grain 100 g yeast 11.11 g carrot 11.50 g (lost 88% total weight)
Substrate abbreviation Larvae abbreviation	SWYG LWYG	SWYP LWYP	SWYC LWYC	SBYC LBYC

Experimental rearing of mealworm larvae on various diets was conducted at the Divaks company's (Vilnius, Lithuania) insect-rearing research and development site [40].

During the rearing period, the insects were kept in a climate chamber with a controlled air temperature of 27 ± 2 °C and relative humidity of 60 ± 5%. Lighting in the chamber was only turned-on during insect maintenance and analysis, which lasted up to one hour each day. At the beginning of the experiment, mealworm eggs were collected from various ages adult beetles. 40 × 60 cm insect rearing boxes with beetle inlay trays (Beekenkamp, Maasdijk, The Netherlands) were used for this purpose. Wheat flour (Kauno grūdai, Kaunas, Lithuania) [41], was used as the substrate for egg laying. Carrots were provided as a moisture source for adult beetles ad libitum.

Eggs were collected every 3–4 days using a 0.5 mm sieve. After measuring 17 g of eggs, equivalent to approximately 30,000 individuals, they were placed in one of the aforementioned 40 × 60 cm boxes with 1.5 kg of dry feed. During larval growth, dry feed was supplemented with ad libitum, totaling 4 kg during this period. Two dry feeds were tested in these experiments. One was composed of wheat bran (Fasma, Radviliškis, Lithuania) [42] and dry brewer's yeast (Ekoproduktas, Panevėžys, Lithuania) [43] mixture (9:1). In the other formulation, the bran was replaced with dry brewer grain (Eurokorma, Vilnius, Lithuania) [44]. Wet feed for the larvae was provided three times a week. Carrots (Sanitex, Kaunas, Lithuania) (a total of 3.45 kg per box), potatoes (from local farms suppliers) [45] (2.75 kg), and agar-agar gels (Carl Roth, Karlsruhe, Germany) [46] (10 g/L) (2.75 kg) were placed in separate rearing boxes with the larvae. The larvae were raised for 56 days according to this method.

After this period, the larvae were separated from frass and feed residues using a 2 mm sieve. Subsequently, they were left in the climate chamber for a 24 h fasting period. Starved larvae were then sieved once again following freezing at −18 °C and transferred for further analysis.

2.2. Lyophilization of Larvae and Preparation of Samples

Lyophilization was performed at Alytus College, Lithuania [47]. Mealworms and perishable substrate ingredients (green potatoes and carrots) were fast frozen using a Liebherr fast freezer (LGv 5010 MediLine, Liebherr, Bulle, Switzerland) for 8 h at −35 °C. Freeze drying was performed in a lyophilizer (Harvest Right, Solon Springs, WI, USA) till 80 °C, a pressure of 73 PA, and the whole process lasted 72 h.

Subsequently, lyophilized larvae and substrate were subjected to milling using a laboratory-scale mill (Fritsch Mill Pulverisette 14, Indar-Oberstein, Germany) at 6000 rpm and sieved through a 200 µm sieve.

2.3. Biogenic Amines

The entire study can easily be repeated since the tests were carried out in accredited laboratories. Biogenic amines in substrate and larvae were determined in an accredited laboratory in Lithuania: the Kaunas University of Technology, Food Institute, and Chemical Science Laboratory [48].

All reagents were of analytical grade, acetonitrile, and water—HPLC grade. HPLC water was prepared freshly by the HPLC water purification system Adrona, model CB-1703 (Riga, Latvia). The reagents: (1) perchloric acid solution, 0.4 mol/L, 70%, (Chempur, Mumbai, India); (2) sodium hydroxide solution, 2 mol/L, (VWR chemical, Lachine, QC, Canada); (3) saturated sodium bicarbonate solution, (Chempur); (4) dansyl chloride solution: 200 mg of dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride) (Alfa Aeser, Haverhill, MA, USA) were weighed and dissolved in 20 mL of acetone (Chempur); (5) ammonia solution, NH_3 (25%); (6) mobile phase for liquid chromatography A: ammonium acetate, 0.1 mol/L., was degassed before use and was filtered through a membrane filter during analysis; (7) mobile phase for liquid chromatography B: Acetonitrile (CH_3CN) (Macron Fine Chemicals, Center Valley, PA, USA), was degassed before use and filtered through a membrane filter during analysis; (8) ammonium acetate-acetonitrile mixture (1:1 *v/v*): mixed equal ratio of 0.1 mol/L ammonium acetate and acetonitrile) (Chempur); (9) histamine, cadaverine, pustrecine, tyramine, spermidine (Sigma–Aldrich, St. Louis, MO, USA),

and spermine (Fluka, version 2018–2019), standard solution of concentration 1 mg/mL were prepared in HPLC water for peak identification; (10) internal standard solution, 1,7-diaminoheptane ($C_7H_{18}N_2$), 1 mg/mL in water. Equipment: (1) high-performance liquid chromatography system with UV/VIS detector SPD-20A (Shimadzu Corp., Kyoto, Japan); (2) reversed-phase column: YMC-Pack ODS-A (YMC Co, Ltd., Kyoto Japan), 150 × 4.0 mm, I.D, 12 nm, s—5 µm; (3) working conditions: flow rate of mobile phase—0.9 mL/min; injection volume—20 µL; column temperature—40 °C; detector measurement wavelength—254 nm; gradient: 0 min.—50% B; 19 min—10% B; 20 min—50% B; 28 min.—50% B. Sample preparation and storage: the lyophilized larvae were ground with a grinder and extracted. 5.00 ± 0.01 g of substrate/larvae samples were weighed, mixed with 250 µL of the internal standard solution, diluted with 10 mL of the perchloric acid solution, and stirred with a glass rod for 5 min. Then the samples were centrifuged for 10 min. at 3000 rpm, and the supernatants were filtered into 25 mL flasks. The extraction with a perchloric acid solution was repeated, and the supernatants were mixed and brought up to the 25 mL mark with a perchloric acid solution. Derivatization of sample extract: 100 µL of sodium hydroxide (3.2), 150 µL of saturated sodium bicarbonate, and 1 mL of dansyl chloride solution were added to 0.5 mL of sample extract and mixed well. The reaction mixture was heated in an electric oven for 45 min. at a temperature of (40 ± 2) °C and left to cool a room temperature for 10 min. Residues of dansyl chloride were removed by adding 50 µL of ammonia and mixing well. After 30 min. the reaction mixture was diluted to 5 mL with ammonium acetate: acetonitrile solution (1:1 *v/v*), mixed well, filtered through a membrane filter, and analyzed by high-performance liquid chromatography. Quantitative analysis was performed by the internal standard method using peak areas. Calculation of the results was done using Formula (1):

$$\text{Biogenic amine content (mg/kg)} = \frac{S_H}{S_{Vst}} \cdot 50 \quad (1)$$

where S_H—biogenic amine peak area in the test solution and S_{Vst}—area of the internal standard in the test solution. The coefficient 50 was obtained by extracting 5 g of sample/25 mL of perchloric acid solution → 0.5 mL of extract was diluted to 5 mL; (5 mL·25 mL)/(5 g 0.5 mL).

2.4. Polycyclic Aromatic Hydrocarbons

The content of all Polycyclic aromatic hydrocarbons (PAH) in substrate and larvae was determined in the accredited laboratory: the Lithuanian Research Centre for Agriculture and Forestry, Agrochemical Research Laboratory, Analytical department, Lithuania (LRC for AF) [49]. The examination was performed in larvae and substrate according to the standard ISO 13859:2014 [50]. This method pacifies the quantitative determination of 16 PAH. Soil quality—Determination of polycyclic aromatic hydrocarbons by gas chromatography and high-performance liquid chromatography. A lower limit of application of 0.01 mg/kg (expressed as dry matter) was ensured for each individual PAH [50]. Analytical chromatography products used for PAH determination: QTM PAH Mix CRM47930 certified reference material, Sigma–Aldrich [51].

2.5. Heavy Metals

Heavy metals (cadmium, chrome, nickel, lead, and manganese) results, were also obtained in an accredited laboratory: LRC for AF [49].

2.5.1. Determination of Copper, Manganese, Zinc

The official BS EN 16170:2016 [52] method—Sludge, treated biowaste and soil. Determination of elements using inductively coupled plasma optical emission spectrometry (ICP-OES) was used for the determination of nickel, cadmium, chromium, and lead in samples [52].

2.5.2. Determination of Copper, Manganese, Zinc

The results were obtained in an accredited laboratory: LRC for AF [49]. The content of copper, manganese, and zinc was determined according to the standard BS EN 15621:2017 [53] Animal feeding stuff: Methods of sampling and analysis. Determination of calcium, sodium, phosphorus, magnesium, potassium, sulfur, iron, zinc, copper, manganese, and cobalt after pressure digestion by ICP-AES [53].The standard used for research: ICP multi-element Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Tl, Zn standards, Certipur, Supelco 1.11355.0100 [54]. Reagents used by Honeywell manufacturer: HCl (30721) [55] and HNO3 (30709) [56].

2.6. Glycoalkaloids

Glycoalkaloid (tomatidine, tomatine, α-solanine, α-chaconine, and solanidin) content was determined in a laboratory: LUFA Nord-West is the accredited service laboratory of the Lower Saxony Chamber of Agriculture (Germany) [57].

2.6.1. Determination of α-Solanine, α-Chaconine, and Solanidin

Determination of contaminants in freeze-dried larvae which were reared on a substrate with green potatoes (LWYP) and substrate with lyophilized potatoes (SWYP) and control samples (LWYG), using liquid chromatography with mass selective detectors (MS/MS). The method used was: Quick Method for Analysis of Numerous Highly Polar Pesticides Food Involving Extraction with Acidified Methanol and LC-MS/MS.

Measurement—l. Food of Plant Origin (QuPPe-PO-Method), Version 12 [58].

α-Solanine, α-chaconine, and solanidin were also found in the main ingredient of the substrate-green potatoes. The mentioned alkaloids were examined according to the following document: BVL L 00.00-115:2018-10 [59]. Examination of foodstuffs-multi-method for the determination of pesticide residues with GC and LC after acetonitrile extraction/distribution and cleaning with dispersive SPE in plant-based foods-modular QuEChERS method (adoption of the standard of the same name DIN EN 15662, July 2018) [59].

2.6.2. Determination of Tomatidin and Tomatin

Tomatidin and tomatin were investigated not only in the substrate with green potatoes but also in the potatoes themselves. The possible accumulation in the larvae and control was also analyzed. The alkaloids were tested according to the method, described in BVL L 00.00-115:2018-10. Examination of foodstuffs-multi-method for the determination of pesticide residues with GC and LC after acetonitrile extraction/distribution and cleaning with dispersive SPE in plant-based foods-modular QuEChERS method (adoption of the standard of the same name DIN EN 15662, July 2018) [59].

2.7. Pesticides

The pesticide content was determined in an accredited laboratory: LRC for AF [49]. Two methodologies were used to determine the amount of pesticides in the substrate and larvae. For sample preparation: EN 12393-2 [60] Foods of plant origin-Multiresidue methods for the determination of pesticide residues by GC or LC-MS/MS—Part 2: Methods for extraction and clean-up were used. According to this test, the amount of Acephate was determined [60]. The remaining 95 pesticides were determined according to the following methodology: EN 12393-2:2013 (MAIN) Foods of plant origin—multi-residue methods for the determination of pesticide residues by GC or LC-MS/MS—Part 3: Determination and confirmatory tests [60].

The following solvents were used for pesticides according to the methodologies: Sigma–Aldrich 208752-1L n-Hexane ≥95% [61]. Acetonitrile 34851, Honeywell for HPLC, ≥99.9% [62].

2.8. Statistical Analysis

Data analysis was conducted using IBM SPSS Statistics 29.0.0.0 (241). Means and standard deviations of the studied characteristics of the compared groups were calculated. Differences between study groups were evaluated by ANOVA with the Bonferroni test. Differences are considered statistically significant when $p < 0.05$.

3. Results and Discussion

3.1. Biogenic Amines

The main reasons for the formation of biogenic amines are endogenous biosynthesis, uptake from the feed source, and bacteria in the gut microbiota of insects [63]. Biogenic amines often form during food fermentation and aging processes and through biological interactions with microorganisms and other food components [64]. Biogenic amines, such as histamine, tyramine, phenylethylamine, etc., are frequently found in certain food products (e.g., fermented cheese, and fish) [65–67]. It is important to mention that biogenic amines are naturally found in animal-derived products (meat, fish, and eggs) [68,69].

Mealworms, like many other organisms, can produce biogenic amines through their cellular metabolism [70]. It is also worth mentioning that larvae can have symbiotic relationships with bacteria or other microorganisms that can assist them in producing or acquiring biogenic amines [71,72]. The synthesis of biogenic amines in larvae involves the conversion of amino acids through a series of chemical reactions, the participation of enzymes, and other cellular components, allowing the organism to meet its requirements for proteins and other essential substances [73,74].

High histamine content in food can trigger an immune system response causing food intolerance/malabsorption or allergies for consumers [75]. In some cases, it can cause diarrhea, shortness of breath, headaches, or skin problems [76]. According to the results of our research (Table 2), histamine does not accumulate in the larvae, the determined amounts were below the detection limit. The highest content of histamine was detected in the substrate with wheat bran, brewer's yeast, and agar-agar gels. According to Regulation (EU) 2015/2283, which describes the safety of dried larvae, the range of reported values in larvae was 2.7–6.56 mg/kg histamine, therefore the preferred quantity is similar [2,77]. Legislation does not specify the exact amount of histamine that can be detected in edible insects, however, based on the limits allowed for fishery products, the amount of histamine must not exceed 200 mg/kg according to Regulation (EC) No. 2073/20057 [78].

Table 2. The amounts of biogenic amines in lyophilized larvae and substrate (mg/kg of dry matter), average ± standard deviation, n = 3, (Average ± S.D).

	Material	Histamine	Cadaverine	Putrescine	Tyramine	Spermine	Spermidine
Mealworms	LWYG (control)	1.72 ± 0.24 a	56.57 ± 2.41 b	2042.47 ± 37.37 d	10.51 ± 0.67 a	235.65 ± 43.25 ab	203.85 ± 7.24 a
	LWYP	1.48 ±0.20 a	14.72 ± 1.19 a	1475.29 ± 20.14 a	7.44 ± 1.73 a	98.79 ± 20.57 a	210.44 ± 22.3 a
	LWYC	1.53 ±0.16 a	15.65 ± 0.60 a	1260.06 ± 13.28 b	10.54 ± 0.66 a	163.57 ± 97.98 a	193.72 ± 3.34 a
	LBYC	3.20 ± 0.19 b	13.39 ± 0.30 a	1070.40 ± 37.80 c	16.69 ± 1.41 b	370.54 ± 42.63 b	205.62 ± 3.70 a
Substrate	SWYG (control)	17.23 ± 1.09 a	22.52 ± 3.93 a	153.47 ± 8.93 a	8.33 ± 0.66 a	20.16 ± 1.02 a	119.68 ± 8.53 b
	SWYP	15.97 ± 1.21 a	29.41 ± 14.38 a	160.03 ± 6.76 a	7.92 ± 0.83 a	19.19 ± 0.70 a	131.08 ± 4.31 ab
	SWYC	15.96 ± 0.47 a	24.60 ± 9.04 a	150.04 ± 5.17 a	7.38 ± 0.87 a	19.61 ± 1.07 a	141.16 ± 5.58 a
	SBYC	16.27 ± 0.37 a	42.84 ± 6.31 a	193.01 ± 5.78 b	15.68 ± 0.63 b	41.47 ± 1.11 b	133.67 ± 6.86 ab
Mealworms		1.98 ± 0.76	25.03 ± 18.95 a	1462.05 ± 381.45 a	11.3 ± 3.66 a	217.14 ± 116.65 a	203.41 ± 12.04 a
Substrate		16.36 ± 0.91	25.03 ± 18.95 a	164.16 ± 18.72 b	9.83 ± 3.60 a	25.11 ± 9.91 b	131.4 ± 9.78 b

a,b,c,d—means marked with different letters in the column (in the groups Larvae and Substrate separately) differed statistically significantly ($p < 0.05$, Bonferroni criterion); SWYG—substrate, control (wheat bran + brewer's yeast + agar-agar gels). SWYP—substrate (wheat bran + brewer's yeast + sprouted potatoes). SWYC—substrate (wheat bran + brewer's yeast + carrot). SBYC—substrate (brewers' spent grain + brewer's yeast + carrot). LWYG—larvae, control (wheat bran + brewer's yeast + agar-agar gels). LWYP—larvae (wheat bran + brewer's yeast + green potatoes). LWYC—larvae (wheat bran + brewer's yeast + carrot). LBYC—larvae (brewers' spent grain + brewer's yeast + carrot).

The European Food Safety Authority (EFSA) states that of the biogenic amines, putrescine and cadaverine are among the most common ones formed in food products [79]. Comparing the pharmacological activity of histamine and cadaverine, the latter has much weaker activity [63]. However, there were a few cases where cadaverine has been linked to vascular and heart problems. Regardless, based on the results of our research, it can be inferred that cadaverine did not accumulate in all tested larvae samples [80]. In one sample of LWYG, the results showed the highest cadaverine amount (56.57 ± 2.41 mg/kg). Meanwhile, the amount of cadaverine in larvae, according to the application submitted by EFSA scientists, is found to be in the range of 6.66–8.01 mg/kg [81]. In any case, the amounts detected are low, compared to the amount of cadaverine found in other products. In comparison, cadaverine accumulates in high concentrations in cheeses (up to 3170 mg/kg), fish and fish products (up to 1690 mg/kg), and other products such as fermented sausages [82–84].

The amount of putrescine in the larvae increased by 5.5–13 times compared to the substrate, which indicates an obvious accumulation. The lowest concentration—1070.40 ± 37.80 mg/kg was found in LBYC samples. The amounts of putrescine in our experiment were two to three times higher than the amounts in the application submitted by EFSA [81]. However, there is no legal regulation regarding putrescine. Compared to other products, high concentrations can accumulate in cheese (up to 1560 mg/kg) and fermented sausages (up to 1550 mg/kg) [85]. Besides it should be noted that the diamines putrescine and cadaverine can enhance the effect of histamine when used simultaneously [86].

Tyramine is associated with stimulating the release of the hormone norepinephrine in the human body, which increases blood pressure (headache is one of the symptoms) and heart rate [87,88]. The results of our research have shown that the lowest amount (8 mg/kg) of tyramine was detected in LWYP samples, and a substrate of these larvae. Although the accumulation is noticeable, the amount of tyramine found in larvae is really low compared to the amount found in other products, especially meat and cheese products [81,89].

Spermine can cause significant toxicity to cellular components, for example, DNA [90]. In our research, the lowest amount (98.79 ± 20.57 mg/kg) of spermine was found in the LWYP sample. Samples of beef, pork, and chicken have been reported by Nelly C. Muñoz-Esparza et al., to exhibit spermine values exceeding 148 nmol/g, with no notable variations between fresh meats and their processed forms [91]. The amount of spermine in the larvae increased 5–12 times compared to the amount found in the substrate. The permissible amount of spermidine and spermine in food products is also not limited by law. According to EFSA, the concentrations of spermine in powder and dried larvae are 168–178 mg/kg and 22.3–28 mg/kg respectively [92]. Linares, D.M. et al. in their study examined the factors that can influence the formation of histamin in edible insects, specifically crickets. The study found that 95% of farmed crickets from thirty-two farms in Thailand contained histamine levels of less than 50 mg/kg $^{-1}$. For comparison, the highest amount we have found (in the LBYC sample) was 3.20 ± 0.19 mg/kg [83].

Recent epidemiologic data suggest that increased dietary spermidine intake reduces overall mortality associated with cardiovascular disease and cancer [93]. Spermidine, even if it does not tend to accumulate as much in larvae as compared to putrescine and spermine, but a large amount of it is also found in substrates. The highest amount of spermidine detected in the LWYP sample was 210.44 ± 22.3 mg/kg. Compared to dried larvae grown on a balanced substrate, the spermidine content was determined in the range of 154–197 mg/kg [92]. Elena Bartkiene et al. have studied the formation of biogenic amines in baked bread with cricket flour and have found that the amount of spermine was also formed in cricket flour, as much as 307.2 ± 21.84 mg/kg. While in our study the highest amount was in the LBYC sample (370.5 ± 42.63 mg/kg) [94].

The amount of total putrescine, tyramine, spermine, and spermidine in mealworm larvae was statistically higher compared to the amount detected in the substrate ($p < 0.001$). The total amount of histamine detected in mealworms was statistically significantly lower compared to the amount detected in the substrate ($p < 0.001$), so it can be concluded that

histamine does not accumulate in mealworms. No statistically significant difference was observed between mealworms and substrate when comparing cadaverine accumulation.

In summary, it can be asserted that biogenic amines do not surpass the regulated thresholds, and in certain instances, comparable quantities are observed as in fish or cheese and other products. However, it is crucial to conduct additional research to elucidate biogenic amines, such as putrescine origin, and explore methods for their mitigation.

3.2. Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAH) are compounds widely distributed in the environment, many of which have carcinogenic effects [95] (Figure 2). Cooking processes (e.g., grilling, smoking, toasting, roasting, and frying) have been identified as the main source of PAH in food [96,97]. Not all PAH is considered dangerous to health, the content of benzo(a)pyrene in products and the total sum of dangerous PAH (benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene) are separately distinguished [98].

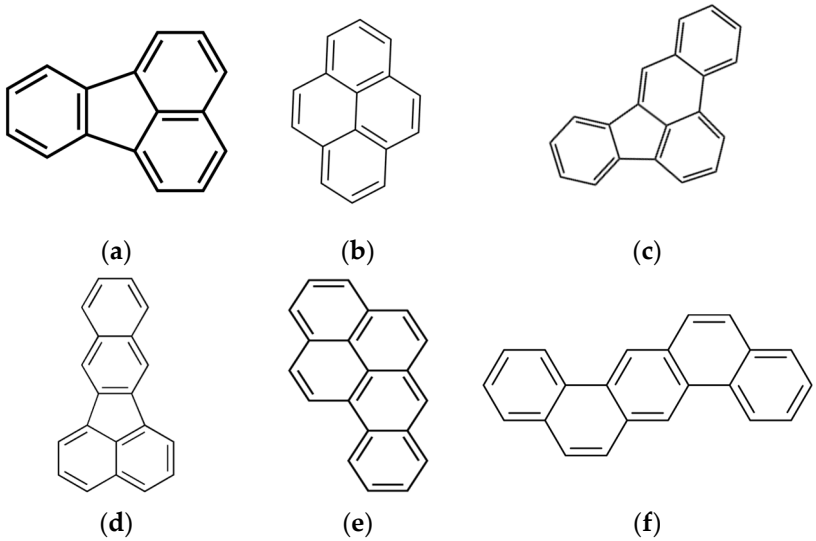

Figure 2. The chemical structure of Polycyclic aromatic hydrocarbons (**a**) Fluoranthene; (**b**) Pyrene; (**c**) Benzo(b)fluoranthene; (**d**) Benzo(k)fluoranthene; (**e**) Benzo(a)pyrene; (**f**) dibenzo(a,h)anthracene.

In our research, no PAH was detected in larvae: LWYP, LWYC, and LWYG samples (Table 3). The highest amount (0.00070 ± 0001 mg/kg) of PAH was found in a larva that was fed brewers' spent grain, carrots, and brewer's yeast (LBYC). Depending on the substrate used, a statistically significantly higher amount of PAH was detected in the SBYC sample ($p < 0.01$) and, respectively, in the larvae reared on this substrate LBYC ($p < 0.01$). The lyophilization process itself has no data that can affect the formation of PAH, since the amount of PAH detected is exceptionally higher only when using brewery by-products. Ewa Mackiewicz-Walec et al. study has shown that weather conditions and microbial activity can cause large seasonal variations in PAH levels during barley cultivation [99]. Therefore, we can cautiously put forward the following hypothesis that in barley used for beer production and in its biowaste-barley malting used as a substrate for the larvae-higher amounts of PAH were formed due to the growing conditions, compared to LWYG, LWYP, and LWYC.

Table 3. The amounts of Polycyclic aromatic hydrocarbons in lyophilized larvae and in substrate, (mg/kg of dry matter), average ± standard deviation, n = 3, (Average ± S.D).

	LWYG (Control)	LWYP	LWYC	LBYC	SWYG	SWYP	SWYC	SBYC
Naphthalene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Acenaphthene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Fluorene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Phenanthrene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Anthracene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Fluoranthene	N.D.	N.D.	N.D.	0.026 ± 0.001	N.D.	N.D.	0.019 ± 0.002	0.034 ± 0.002
Pyrene	N.D.	N.D.	N.D.	0.032	N.D.	N.D.	N.D.	0.044 ± 0.005
Benz(a)anthracene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Chrysene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Benzo(b)fluoranthene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.004 ± 0.001
Benzo(k)fluoranthene	N.D.	N.D.	N.D.	0.0007 ± 0.0002	N.D.	N.D.	N.D.	0.002 ± 0.001
Benzo(a)pyrene	N.D.	N.D.	N.D.	0.0007 ± 0.0001	N.D.	N.D.	N.D.	0.006 ± 0.002
Benzo(g. h,i)perylene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
dibenzo(a,h)anthracene	N.D.	N.D.	N.D.	0.003 ± 0.0004	N.D.	N.D.	N.D.	0.012 ± 0.001
Indene(1,2,3-cd)pyrene	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene	N.D.	N.D.	N.D.	0.0007 ± 0.0003	N.D.	N.D.	N.D.	0.01 ± 0.003

N.D.—not detected SWYG—substrate, control (wheat bran + brewer's yeast + agar-agar gels). SWYP—substrate (wheat bran + brewer's yeast + sprouted potatoes). SWYC—substrate (wheat bran + brewer's yeast + carrot). SBYC—substrate (brewers' spent grain + brewer's yeast + carrot). LWYG—larvae, control (wheat bran + brewer's yeast + agar-agar gels). LWYP—larvae (wheat bran + brewer's yeast + green potatoes). LWYC—larvae (wheat bran + brewer's yeast + carrot). LBYC—larvae (brewers' spent grain + brewer's yeast + carrot).

Khanittha Chinarak et al. pubished a novel strategy for the production of edible insects, emphasizing the lack of research not only on biogenic amines but also on PAH [100]. The only study for the comparative analysis of PAH is the research done by Fègbawè Badanaro and Edmond A. Dué [101]. In this study, the fluoranthe (0.31 µg) was detected in processed *Cirina forda*, while in our study it was found, in much higher amounts, in the LBYC sample (0.026 ± 0.001 mg/kg, corresponding to 26 µg).

Commission's regulation (EU) No 835/2011 regulates maximum levels of polycyclic aromatic hydrocarbons in foodstuffs [102]. Compared to the regulatory amount, our detected amount was extremely low, as low as could be a suitable product for use in young children and infants. As an example, in bivalve molluscs (smoked), the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene can be as high as up to 35.0 µg/kg.

Therefore, it can be concluded that when choosing raw materials, it should be taken into account whether PAH is not formed in the substrate, as these pollutants can also enter the larvae, but no significant accumulation was observed [100].

3.3. Heavy Metals

The uptake of heavy metals by plants and their subsequent accumulation in the food chain can pose a threat to animal and human health [103,104]. In the human body, heavy metals can cause disorders of the digestive tract, kidney function, nervous system, skin and vascular damage, immune system dysfunction, teratogenic effects, and even cancer [82,83].

EFSA issued a scientific opinion on chromium and suggested a tolerable daily intake of 0.3 mg/kg body weight per day. Our results have shown that chromium was found only in the substrate SBYC and the amount found was 1.45 ± 0.02 mg/kg, however, no accumulation was observed in larvae (Table 4) [105]. The highest concentration of nickel was found in substrate SBYC (0.65 ± 0.06 mg/kg) as well. It can be inferred that, under various growing conditions, none of the tested heavy metals pose a risk and they do not accumulate in the larvae of yellow mealworm. Comparing the different rearing conditions, the highest amount of heavy metals was detected in the substrate SBYC and the LBYC larvae (except Manganese). However, the determined total amount of manganese in mealworm larvae was significantly lower compared to the substrate. Therefore, it can be concluded that manganese does not accumulate in the studied larvae ($p < 0.001$), in a few cases it was found seven to nine times less in all larvae compared to the substrate. When comparing the total amount of cadmium detected in the substrate and mealworms, no statistically significant difference was observed ($p > 0.05$). H.J. van der Fels-Klerx et al. in the study analyzed uptake of cadmium and another heavy metal by *T. molitor* from contaminated substrates [106]. We can juxtapose the results of the mealworm control sample from this research, where the detected cadmium content was 5.8 ± 1.0, with our study, where its content ranged from 0.03 ± 0.004 to 0.08 ± 0.003 mg/kg of dry matter, indicating similar results. In the same corresponding study, lead was identified in the control group within the range of 1.1 ± 0.05 to 1.8 ± 0.8. However, in our investigation, lead levels remained below the detection limits (<0.1), resulting in a dose that was 11–18 times lower [106]. In all samples, analogically, as reported by EFSA, the concentration of cadmium was below 0.1 mg/kg [81]. The amount of heavy metals can be compared with the limits set in Regulation (EC) No. 1881/2006 of 19 December, as the EU legislation does not set maximum levels of heavy metals for insects and their products as food [107].

Table 4. The content of heavy metals in lyophilized larvae and substrate (mg/kg of dry matter), average ± standard deviation, n = 3, (Average ± S.D).

	LWYP	LWYC	LBYC	LWYG	SWYP	SWYC	SBYC	SWYG
Cadmium	0.06 ± 0.005 a	0.05 ± 0.001 a	0.08 ± 0.003 b	0.03 ± 0.004 c	0.04 ± 0.002 a	0.04 ± 0.05 a	0.09 ± 0.003 b	0.04 ± 0.34 ab
Chrome	<1	<1	<1	<1	<1	<1	1.45 ± 0.02	<1
Nickel	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	0.65 ± 0.06	<0.5
Lead	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Manganese	8.91 ± 0.04 a	9.43 ± 0.09 b	5.62 ± 0.08 c	7.95 ± 0.88 ab	72.34 ± 0.79 a	69.34 ± 0.28 b	52.49 ± 0.18 c	71.76 ± 0.77 a

a,b,c—means marked with different letters in the column (in the groups Larvae and Substrate separately) differed statistically significantly ($p < 0.05$, Bonferroni criterion). SWYG—substrate, control (wheat bran + brewer's yeast + agar-agar gels). SWYP—substrate (wheat bran + brewer's yeast + sprouted potatoes). SWYC—substrate (wheat bran + brewer's yeast + carrot). SBYC—substrate (brewers' spent grain + brewer's yeast + carrot). LWYG—larvae, control (wheat bran + brewer's yeast + agar-agar gels). LWYP—larvae (wheat bran + brewer's yeast + green potatoes). LWYC—larvae (wheat bran + brewer's yeast + carrot). LBYC—larvae (brewers' spent grain + brewer's yeast + carrot).

It can be concluded that concentrations of heavy metals (lead, cadmium, mercury) do not exceed the maximum concentrations determined for other food products and are within the limits provided by EFSA [81]. However, in the presence of contaminated substrate (e.g., cadmium) the larvae would be able to accumulate higher amounts of heavy metals.

3.4. Glycoalkaloids

Glycoalkaloids are a group of nitrogen-containing compounds produced naturally in *Solanaceae* plant species (potatoes, tomatoes, eggplants, and peppers) (Figure 3). Glycoalkaloids can be toxic in amounts that occur naturally in potatoes [108]. Higher levels of glycoalkaloids are usually caused by environmental factors: harvesting and storage, such as light and temperature [109].

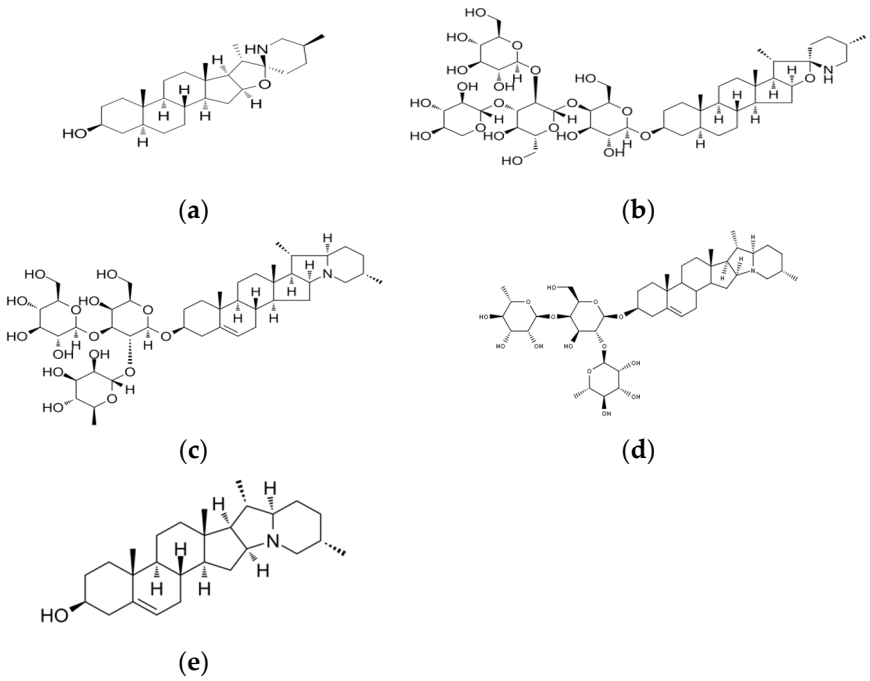

Figure 3. The chemical structure of the studied glycoalkaloids. (**a**) tomatidin; (**b**) tomatin; (**c**) α-solanine; (**d**) α-chaconine; (**e**) solanidin.

Potatoes are deliberately selected to contain higher than normal levels of glycoalcoloids—green, sprouted, and suitable only for disposal; therefore, the cumulative amount of glycoalkaloids detected is 225 ± 0.32 mg/kg (Table 5).

Table 5. The content of glycoalkaloids in lyophilized larvae, potatoes, and substrate, (mg/kg of dry matter), average ± standard deviation, n = 3, (Average± S.D).

Material	Tomatidin	Tomatin	α-Solanine	α-Chaconine	Solanidin	Sum
Potatoes	<0.0100	<0.0100	62.54 ± 0.19 a	145.01 ± 0.01 a	15.52 ± 0.12 a	225 ± 0.32 a
LWYP	<0.0100	<0.0100	175.12 ± 0.21 b	139.32 ± 0.32 b	3.59 ± 0.02 b	317.87 ± 0.55 b
SWYP	<0.0100	<0.0100	58.41 ± 0.22 c	116.44 ± 0.11 c	0.39 ± 0.01 c	175.24 ± 0.34 c
LWYG	<0.0100	<0.0100	-	-	-	-

a,b,c—means marked with different letters in the column differed statistically significantly ($p < 0.05$, Bonferroni criterion). SWYG—substrate, control (wheat bran + brewer's yeast + agar-agar gels). SWYP—substrate (wheat bran + brewer's yeast + sprouted potatoes). SWYC—substrate (wheat bran + brewer's yeast + carrot). SBYC—substrate (brewers' spent grain + brewer's yeast + carrot). LWYG—larvae, control (wheat bran + brewer's yeast + agar-agar gels). LWYP—larvae (wheat bran + brewer's yeast + green potatoes). LWYC—larvae (wheat bran + brewer's yeast + carrot). LBYC—larvae (brewers' spent grain + brewer's yeast + carrot).

To our knowledge, our study was the first study related to the analysis of the accumulation of glycoalkaloids in the substrate and *T. molitor* larvae using sprouted potatoes and providing some insights on this topic. Marta Spochacz et al. have studied the effect of glycoalkaloids on the physiology of *T. molitor*, although this was not the subject of our study. It should be taken into account that its large amount, according to the authors, affects mealworms in impaired development, food intake, and reproduction [39]. Comparing the control sample (LWYG) with larvae grown on green potatoes (LWYP) in the latter, the total amount of all glycoalkaloids was 317.87 times higher. Meanwhile, some studies state that choosing the wrong potato varieties can lead to high levels of glycoalkaloids in potatoes alone. The study of Knuthsen et al. on glycoalkaloids in potatoes showed that more than 200 mg total glycoalkaloids/kg (3 out of 386 samples) were found in a small number of samples [108]. No food safety standards have been established for the amount of glycoalkaloids in food, but according to EFSA scientific opinion, the generally accepted

safe upper limit is considered to be 200 mg of glycoalkaloids per 1 kg of product [110,111]. Nordic evaluations recommend that the content of total glycoalkaloids in potato varieties should not exceed 100 mg/kg [108].

Thus, it can be concluded that α-solanine (accumulated three times more than in the substrate), solanidin (accumulated nine times more than in the substrate), and **α**-chaconin, accumulate in the larvae. Concentrations of tomatidin and tomatin were detected below the detection limit.

3.5. Pesticides

Michael Houbraken et al. research found that fresh, low-value agricultural waste contaminated with pesticides can be used as a substrate for edible insects. The findings of this study show that using the method of starving mealworms significantly reduces pesticide residues in insects after just 24 h [112]. Pesticides break down over time, but they do not disappear, they are converted into less toxic substances: carbon dioxide, water minerals, etc [113]. Kathrine Eggers Pedersen et al. studied the influence of mealworm stages on insecticide sensitivity. The findings have shown that larvae detoxify faster than pupae [114].

The substrate used for growing mealworms was not intentionally contaminated with pesticides. As our study involved starving the mealworms, we suspect that this is the reason why none of the 96 pesticides exceeded the detection limit (Appendix A).

4. Conclusions

The results of the concentrations of toxic compounds, produced in larvae grown on a special diet, were different. The regulated amount of biogenic amines (histamine) was below the detection limit. The highest amount of PAH detected in larvae was 0.0007 mg/kg. In lyophilized larvae, neither the total amount of PAH nor benzo(a)pyrene exceeded the regulated limits. Although an increased number of heavy metals (cadmium, chrome, nickel, lead) was detected on the substrate SBYC, no accumulation was observed in the larvae and compared to the regulated amount for solid products-it is not significantly high. Accumulation of glycoalkaloids (α-solanine, α-chaconine, and solanidin) was observed in larvae grown on green and germinated potatoes compared to the control samples. However, it is emphasized that the amount of glycoalkaloids is recommendatory and compared to potatoes—it is not consumed in such large quantities. As the pesticides were not detected in the samples, it was assumed that they degraded into simpler compounds over time. Therefore, we can suggest that our larval samples: LWYP, LWYC, LBYC, and LWYG, grown in different conditions on specific by-products from the farm or industry, do not accumulate significant amounts of regulated toxic substances. However, in some cases, they exceeded the recommended amounts (in the case of biogenic amines or glycoalkaloids). We see the need for more detailed studies and conditions for the formation of biogenic amine, also nutritional studies (amino acids, fatty acids, etc.) were not performed yet, therefore, for detailed results and to submit recommendations for mealworms larvae growers, it should be investigated thoroughly. We see the importance of more extensive studies, considering the geographical area from which the substrate is taken, and even the season, to determine the wider influence of circumstances on the substrate and subsequently on the accumulation of toxic substances in larvae. It would also be useful to compare the substrate with the larvae not only when a naturally small amount of chemicals is found in the substrate (as in our study), but after specifically infecting the group larvae with toxins, to check the tendency of the larvae to accumulate a specific chemical substance and amount.

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Conflicts of Interest: Author Dominykas Aleknavičius was employed by the company Divaks, UAB. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A

Table A1. The content of pesticides in lyophilized larvae and substrate, mg/kg.

Analytical Pesticide	Detection Threshold	LWYP	LWYC	LBYC	LWYG	SWYP	SWYC	SBYC	SWYG
Acephate	<0.05	-	-	-	-	-	-	-	-
Alachlor	<0.01	-	-	-	-	-	-	-	-
Aldrin + dieldrin (sum)	<0.01	-	-	-	-	-	-	-	-
Azinphos ethyl	<0.05	-	-	-	-	-	-	-	-
Azinphos methyl	<0.05	-	-	-	-	-	-	-	-
Alpha-cypermethrin	<0.05	-	-	-	-	-	-	-	-
Atrazine	<0.05	-	-	-	-	-	-	-	-
Bromophos ethyl	<0.05	-	-	-	-	-	-	-	-
Bromophos methyl	<0.05	-	-	-	-	-	-	-	-
Bromopropylate	<0.01	-	-	-	-	-	-	-	-
Beta-cyfluthrin	<0.05	-	-	-	-	-	-	-	-
Chlordane (sum of cis, trans isomers, oxychlordane)	<0.02	-	-	-	-	-	-	-	-
Chlorpyrifos ethyl	<0.01	-	-	-	-	-	-	-	-
Chlorpyrifos methyl	<0.01	-	-	-	-	-	-	-	-
Chlorthalodimethyl	<0.01	-	-	-	-	-	-	-	-
Cyfluthrin (sum of isomers)	<0.01	-	-	-	-	-	-	-	-
λ–cyhalothrin	<0.01	-	-	-	-	-	-	-	-
Cypermethrin (sum of α, β and z- isomers)	<0.05	-	-	-	-	-	-	-	-
DDT (sum of p,p'-DDT, o,p'-DDT, o,p'- DDE, p,p'-DDE, o,p' -TDE, p,p'-TDE)	<0.01	-	-	-	-	-	-	-	-
Deltamethrin	<0.05	-	-	-	-	-	-	-	-
Diazinon	<0.02	-	-	-	-	-	-	-	-
Dichlorfluanid	<0.05	-	-	-	-	-	-	-	-
Dichlorvos	<0.05	-	-	-	-	-	-	-	-
Dicofol	<0.01	-	-	-	-	-	-	-	-
Dimethoate + omethoate	<0.01	-	-	-	-	-	-	-	-
Dieldrin	<0.01	-	-	-	-	-	-	-	-
Difenoconazole	<0.05	-	-	-	-	-	-	-	-
Dimethomorph	<0.05	-	-	-	-	-	-	-	-
Endosulfan (sum of α, β endosulfan and endosulfan sulfate)	<0.01	-	-	-	-	-	-	-	-
Endrin	<0.005	-	-	-	-	-	-	-	-
Ethion	<0.02	-	-	-	-	-	-	-	-
Etrimphos	<0.05	-	-	-	-	-	-	-	-
Fenchlorophos	<0.05	-	-	-	-	-	-	-	-
Fenitrothion	<0.005	-	-	-	-	-	-	-	-
Fenpropathrin	<0.01	-	-	-	-	-	-	-	-
Fensulfothion (sum)	<0.05	-	-	-	-	-	-	-	-
Fenthion (sum)	<0.05	-	-	-	-	-	-	-	-
Fenvalerate	<0.05	-	-	-	-	-	-	-	-
Flucitrinate	<0.05	-	-	-	-	-	-	-	-
τ-fluvalinate	<0.01	-	-	-	-	-	-	-	-
Fonofos	<0.05	-	-	-	-	-	-	-	-
Fentin acetate	<0.05	-	-	-	-	-	-	-	-

Table A1. *Cont.*

Analytical Pesticide	Detection Threshold	LWYP	LWYC	LBYC	LWYG	SWYP	SWYC	SBYC	SWYG
Fentin Hydroxide	<0.05	-	-	-	-	-	-	-	-
Fluazifop-P-butyl	<0.05	-	-	-	-	-	-	-	-
Flutriafol	<0.01	-	-	-	-	-	-	-	-
Flutricinate	<0.05	-	-	-	-	-	-	-	-
Fozalon	<0.05	-	-	-	-	-	-	-	-
Heptachlor (sum of heptachlor, heptachlor epoxide)	<0.01	-	-	-	-	-	-	-	-
Hexachlorobenzene	<0.01	-	-	-	-	-	-	-	-
Hexachorcyclohexane (sum of α, β, δ, d- isomers)	<0.01	-	-	-	-	-	-	-	-
Imidacloprid	<0.05	-	-	-	-	-	-	-	-
Iprodione	<0.01	-	-	-	-	-	-	-	-
Lambda cyhalothrin	<0.01	-	-	-	-	-	-	-	-
Lindane (γ-hexachorcyclohexane)	<0.01	-	-	-	-	-	-	-	-
Malathion + malaoxon	<0.05	-	-	-	-	-	-	-	-
Mecarbam	<0.05	-	-	-	-	-	-	-	-
Metacryphos	<0.05	-	-	-	-	-	-	-	-
Metalaxyl	<0.05	-	-	-	-	-	-	-	-
Methamidophos	<0.05	-	-	-	-	-	-	-	-
Metamitron	<0.01	-	-	-	-	-	-	-	-
Metazachlor	<0.01								
Metribuzin	<0.05	-	-	-	-	-	-	-	-
Methidathion	<0.05	-	-	-	-	-	-	-	-
Methoxychlor	<0.01	-	-	-	-	-	-	-	-
Mirex	<0.01	-	-	-	-	-	-	-	-
Monocrotophos	<0.05	-	-	-	-	-	-	-	-
Parathion ethyl + paraoxon ethyl	<0.01	-	-	-	-	-	-	-	-
Parathion methyl + Paraoxon methyl	<0.01	-	-	-	-	-	-	-	-
Pendimethalin	<0.01	-	-	-	-	-	-	-	-
Pentachloroanisole	<0.01	-	-	-	-	-	-	-	-
Permethrin (sum of isomers)	<0.01	-	-	-	-	-	-	-	-
Fozolone	<0.05	-	-	-	-	-	-	-	-
Fosmet	<0.05	-	-	-	-	-	-	-	-
Piperonyl butoxide	<0.05	-	-	-	-	-	-	-	-
Pirimifos ethyl	<0.05	-	-	-	-	-	-	-	-
Pirimiphosmethyl (pirimiphosmethyl+N-desethyl-pirimiphosmethyl)	<0.05	-	-	-	-	-	-	-	-
Promethrin	<0.01	-	-	-	-	-	-	-	-
Propiconazole	<0.01	-	-	-	-	-	-	-	-
Procimidone	<0.05	-	-	-	-	-	-	-	-
Profenofos	<0.05	-	-	-	-	-	-	-	-
Prothiophos	<0.05	-	-	-	-	-	-	-	-
Pyrethrins (pyrethrins 1 and 2, cinerins 1 and 2,	<0.01	-	-	-	-	-	-	-	-
the sum of jasmolins 1 and 2)	<0.05	-	-	-	-	-	-	-	-
Quinalphos	<0.05	-	-	-	-	-	-	-	-
Quintocene (sum of quintocene, pentachloroaniline, methyl pentachlorophenyl sulfide)	<0.001	-	-	-	-	-	-	-	-
Kaptan	<0.02	-	-	-	-	-	-	-	-
S-421	<0.05	-	-	-	-	-	-	-	-
Simazine	<0.05	-	-	-	-	-	-	-	-

Table A1. *Cont.*

Analytical Pesticide	Detection Threshold	LWYP	LWYC	LBYC	LWYG	SWYP	SWYC	SBYC	SWYG
Teknazen	<0.05	-	-	-	-	-	-	-	-
Tetradifon	<0.01	-	-	-	-	-	-	-	-
Tau-fluvinate	<0.005	-	-	-	-	-	-	-	-
Thiamethoxam	<0.05	-	-	-	-	-	-	-	-
Triadimefon	<0.005	-	-	-	-	-	-	-	-
Triadimenol	<0.01	-	-	-	-	-	-	-	-
Trifluralin	<0.01	-	-	-	-	-	-	-	-

Below the device detection threshold. SWYG—substrate (wheat bran + brewer's yeast + agar-agar gels). SWYP—substrate (wheat bran + brewer's yeast + green potatoes). SWYC—substrate (wheat bran + brewer's yeast + carrot). SBYC—substrate (brewers' spent grain + brewer's yeast + carrot). LWYG—larvae (wheat bran + brewer's yeast + agar-agar gels). LWYP—larvae (wheat bran + brewer's yeast + green potatoes). LWYC—larvae (wheat bran + brewer's yeast + carrot). LBYC—larvae (brewers' spent grain + brewer's yeast + carrot).

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