

## Article

# Development and Characterization of Pasta Enriched with Giant Mealworm (*Zophobas morio*) Powder: An Innovative Nutritional Alternative

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## Simple Summary

The use of insect powders in food products is a recognized strategy for enhancing the nutritional profile of conventional foods, particularly with respect to protein content. Nevertheless, it is important to identify the specific interactions that each powder, derived from different insect species, establishes with various types of food and their preparation methods. Therefore, the objective of this study was to develop and technologically characterize pasta enriched with giant mealworm (*Zophobas morio*) powder as an alternative and sustainable product.

## Abstract

The growing global population has intensified the search for sustainable and nutritious food sources. This study evaluated the effects of incorporating giant mealworm (*Zophobas morio*) flour at levels of 0%, 7.5%, 15%, and 17.5% on the nutritional, physicochemical, and technological properties of fresh tagliatelle pasta. The formulations were characterized in terms of proximate composition, caloric value, amino acid profile, cooking quality, texture, color, microstructure, and structural organization. Protein content increased from 8.16% in the control to 16.29% at the highest enrichment level, while crude fiber rose from 0.25% to 1.30%. The amino acid profile revealed seven essential amino acids, with branched-chain amino acids representing approximately 50% of the essential fraction. Enriched pasta showed a significant reduction in cooking solid loss, decreasing from 0.61 g to 0.21 g, while volume increase remained unchanged. Texture analysis indicated reduced hardness (12.59 to 10.05 gf) and chewiness (5.29 to 2.93 gf), resulting in a softer and less adhesive product, without significant changes in elasticity. Although lightness decreased, the visual appearance was comparable to whole-grain pasta products. Overall, *Zophobas morio* flour improved the nutritional profile and technological performance of fresh pasta, supporting its use as a sustainable and functional protein ingredient.

**Keywords:** alternative protein; sustainability; microstructure; physicochemical characterization



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## 1. Introduction

Since 2013, following the announcement by the United Nations (UN) and the Food and Agriculture Organization (FAO) and the publication of the document *Edible Insects: Future Prospects for Food and Feed Security*, the world has become increasingly aware of the opportunities that insect proteins offer for our future. It is estimated that by 2050, the global population will reach 10 billion people, resulting in a considerable increase in the demand for protein [1], with an average growth of about 75% in consumption of this nutrient [2]. It is essential to emphasize that with the significant increase in consumption, the corresponding environmental impacts may also be greatly amplified.

The consumption of insects is gaining popularity in Western markets. Some continents have already begun the production and commercialization of edible insects, with projections to reach over 7.6 billion USD by 2028 [3], creating a completely innovative market. Protein consumption is a cosmopolitan habit that dates to the origins of civilization and remains present in traditional foods and specific cultures to this day. Despite the global movement toward popularizing insect consumption, countries such as Brazil, which has a privileged geographic position for such production, are still far from entering this sector, as they have not yet given sufficient attention to this promising market. Investments are needed in legislation to regulate this type of production and in incentives for potential producers.

Insect protein is a highly flexible food source since it can be produced in small spaces and vertically, enabling production in urban areas and eliminating the need to open new land [4]. Furthermore, insect protein is considered sustainable as it can reduce environmental impacts by up to 97% when compared with other conventional protein sources [5]. Nevertheless, there are still cultural barriers to insect consumption, mainly related to neophobia and negative perceptions regarding its appearance [6,7]. One way to overcome these barriers is by highlighting the benefits of insect protein intake [8], which can be promoted through initiatives that demonstrate its socioeconomic value and increase consumer interest [9], such as tasting events and educational workshops [10]. Another effective way to reduce neophobia is by incorporating insect-based ingredients into foods that are already widely accepted worldwide [10].

Insect consumption can provide a solution to some of the current climate challenges [5], offering a nutritionally rich food with a more sustainable production chain. However, there remains a cultural stigma associated with insect protein, particularly due to its appearance [11], which hinders the perception of its nutritional and environmental benefits. Therefore, it is crucial that insect protein be introduced into the human diet in a flexible and integrated manner to facilitate its acceptance among more sensitive consumers.

One way to incorporate this protein is through foods that use wheat flour in their composition [12], as it can be supplemented with insect flour without significantly altering the final product, as observed in the study by Looy et al. [13], which used protein from mealworms (*Tenebrio molitor*) and crickets (*Acheta domesticus*).

Pasta products are well-established in the global market, with an average per capita consumption of 7.2 kg/year in 2024 [14]. Although they are rich in carbohydrates, pasta products present limited nutritional diversity, which has stimulated interest in strategies to improve their nutritional balance. In this context, the technological quality of pasta plays a central role in consumer acceptance and is strongly influenced by the integrity of the gluten network, cooking performance, and textural properties.

The technological quality of pasta is primarily determined by the integrity of the gluten network, cooking performance, and textural properties, as the gluten matrix entraps starch granules, limits their swelling, and reduces solid loss during cooking. Partial replacement of wheat flour with alternative protein sources can modify starch–protein interactions,

affecting cooking behavior and texture, which highlights the importance of preserving technological quality when developing nutritionally enriched pasta products [15].

For contemporary societies facing nutritional challenges such as obesity and food insecurity [16], the development of nutritionally balanced foods that preserve desirable technological attributes is essential. Therefore, enriching pasta with insect flour represents an innovative strategy to improve nutritional quality while maintaining technological performance, sustainability, and social acceptance, facilitating its incorporation into the human diet. In this context, the present study aimed to develop pasta enriched with giant mealworm (*Zophobas morio*) powder and to evaluate its technological characteristics as a sustainable alternative product.

## 2. Materials and Methods

### 2.1. Materials

The *Zophobas morio* larvae were obtained from the company Protômega, located in Fortaleza, CE, Brazil. The larvae were housed in plastic containers measuring  $30 \times 50 \times 15$  cm (height) and stocked at a density until reaching a total biomass of 2 kg. They were fed, during their development, with a cereal-based diet and a moisture source obtained from assorted fruits, until they reached the ideal processing stage, between 2 and 3 months after hatching. The pre-processing step consisted of withholding solid feed from the larvae for two days, while providing access only to a moisture source to allow cleansing of the digestive system.

The materials used in this study consisted of analytical-grade reagents, and the ingredients (egg, salt, and wheat flour) for pasta preparation were purchased from local markets in Fortaleza, CE, Brazil.

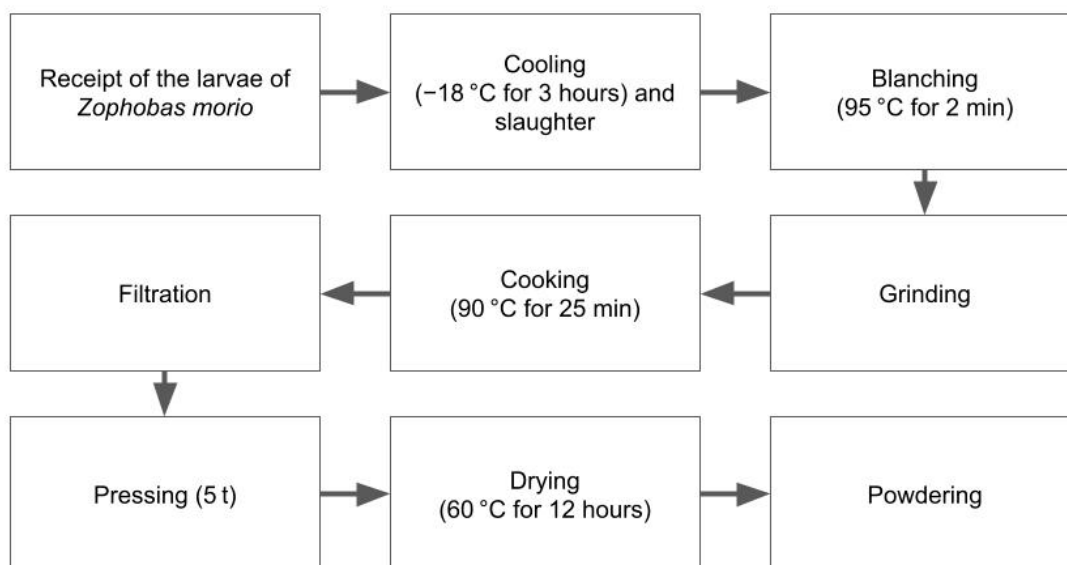
### 2.2. Methods

#### 2.2.1. Insect Powder Preparation

The preparation of the powder followed the flowchart shown in Figure 1. Initially, the larvae of the giant mealworm (*Zophobas morio*) were subjected to low temperatures ( $-18\text{ }^{\circ}\text{C}$ ) for one hour to induce diapause, characterized by a reduction in metabolic activity, and were euthanized through thermal shock by immersion in boiling water [17]. After euthanasia, a blanching process was applied using the time–temperature combination of 2 min at  $95\text{ }^{\circ}\text{C}$ , followed by an ice bath ( $5\text{--}10\text{ }^{\circ}\text{C}$ ) to stop cooking. The blanched larvae were ground in a food processor (MPN-01-BE, Mondial, Barueri, Brazil) until a homogeneous, pasty consistency was obtained. The insect paste was then transferred to a stainless steel pot containing distilled water in a 1:2 (water/paste) ratio and heated at  $90\text{ }^{\circ}\text{C}$  for 25 min.

The cooked insect paste was wrapped in a cotton cloth to remove excess liquid, first by manual twisting and then using a 15 t hydraulic press, reaching a maximum pressure of 5 t. The resulting cake was transferred to stainless steel trays lined with aluminum foil and spread evenly to form a thin layer of sample over the metallic surface. The trays were then placed in a forced-air circulation oven (SSDc-30L, Solidsteel, Piracicaba, Brazil) maintained at  $60\text{ }^{\circ}\text{C}$  for 10 h for drying.

After drying, the heated sample was allowed to rest for 2 h to reach room temperature ( $25\text{ }^{\circ}\text{C}$ ). Subsequently, the cooled sample was processed again in a food processor equipped with a filter to ensure that the resulting powder had a uniform and fine granulometry.



**Figure 1.** Flowchart of insect powder preparation.

### 2.2.2. Proximate Composition of *Zophobas morio* Powder and Caloric Value

The proximate composition of the giant mealworm powder was determined in triplicate using the methodology described by AOAC [18].

#### Moisture Content

Moisture content was determined by gravimetric analysis. Porcelain capsules were dried at 105 °C for 4 h (119 Fabbe Ltda., São Paulo, Brazil), cooled in a desiccator (25 °C), and weighed. Approximately  $5.0 \pm 0.001$  g of sample was added, and the capsules were dried at 105 °C for 15 h, cooled to room temperature (25 °C), and reweighed. Moisture content was calculated using the equation below.

$$\% \text{Moisture} = \frac{(UW - DW) \times 100}{UW - CW} \quad (1)$$

where

CW = weight of the capsule (g);

UW = weight of the capsule + wet sample (g);

DW = weight of the capsule + dry sample (g).

#### Protein Content

Protein content was determined using the micro-Kjeldahl method. Samples ( $0.1 \pm 0.001$  g) were digested (Q-216.21, Quimis, São Paulo, Brazil) with  $\text{H}_2\text{SO}_4$  in the presence of a  $\text{CuSO}_4/\text{Na}_2\text{SO}_4$  catalyst at 350 °C until complete digestion. After cooling, the digest was alkalized with 50% NaOH and the released ammonia was distilled (SL-74, Solab, São José do Rio Preto, Brazil) into 4% boric acid containing mixed indicators. The distillate was titrated with 0.1 M HCl, and protein content was calculated using a 6.25 conversion factor, according to the equation below.

$$\% \text{Protein} = \frac{V \times M \times 1.4 \times Cf}{SW} \quad (2)$$

where

V = volume of hydrochloric acid used in titration (mL);

M = molarity of standardized hydrochloric acid;

$Cf$  = conversion factor;  
 $SW$  = sample weight (g).

#### Lipid Content

Lipid content was determined by Soxhlet extraction using a TE-044 apparatus, (Tecnal Ltda., Piracicaba, Brazil). Previously dehydrated samples were wrapped in anhydrous cotton, placed in filter paper cartridges, and extracted with hexane for 8 h. After solvent removal by distillation, the extraction tubes were dried in a forced-air oven (SSDc-30L, SolidSteel Ltda., São Paulo, Brazil) at  $105 \pm 5$  °C for 1 h, cooled in a desiccator, and weighed. Lipid content was calculated using the equation below.

$$\%Lipids = \frac{(FW - TW) \times 100}{SW} \quad (3)$$

where

$FW$  = weight of the tube + fat (g);  
 $TW$  = weight of the empty tube (g);  
 $SW$  = sample weight (g).

#### Ash Content

Ash content was determined by incineration. Samples ( $5.0 \pm 0.001$  g) were placed in pre-weighed porcelain crucibles and heated in a muffle furnace (F1-DM, Quimis, Diadema, Brazil) at  $550 \pm 5$  °C until complete combustion of organic matter. After cooling in a desiccator to room temperature, the crucibles were reweighed, and ash content was calculated using the equation below.

$$\%Ash = \frac{(AW - CW) \times 100}{SW} \quad (4)$$

where

$AW$  = weight of crucible + ash (g);  
 $CW$  = weight of crucible (g);  
 $SW$  = weight of sample (g).

#### Carbohydrates Content

The carbohydrate content was determined by subtracting from 100% the sum of the percentages of moisture, protein, lipids, and ash, as shown in the following equation:

$$\%Carbohydrates = 100 - (\%M + \%P + \%L + \%A) \quad (5)$$

where

$\%M$  = Moisture percentage.  
 $\%P$  = Protein percentage.  
 $\%L$  = Lipid percentage.  
 $\%A$  = Ash percentage.

#### Caloric Value

The amount of caloric value (CV) was determined using the Atwater conversion factors, following the method established by Osborne and Voogt [19], according to the specified relationship. The obtained values were expressed in kilocalories per gram ( $Kcal \cdot 100 g^{-1}$ ), as shown in the following equation:

$$CV = (\%P \times 4.0) + (\%L \times 9.0) + (\%C \times 4.0) \quad (6)$$

where

%P = Protein percentage.

%L = Lipid percentage.

%C = Carbohydrate percentage.

### 2.2.3. Giant Mealworm (*Zophobas morio*) Powder Amino Acid Profile Analysis

For the determination of the amino acid profile of the giant mealworm powder, the samples required prior preparation. The samples were frozen and ground in liquid nitrogen until a fine, homogeneous powder was obtained. The extraction of polar metabolites was carried out according to Lisec et al. [20], with some modifications. Approximately 10 mg of the ground samples were shaken in a thermoshaker for 15 min at 350 rpm and 70 °C in the presence of pure methanol containing ribitol as an internal analytical standard. After 10 min of centrifugation (5424 R, Eppendorf, Hamburg, Germany) at 11,000 RCF (relative centrifugal force), methanol was added to the collected supernatant, followed by the addition of Milli-Q water. After 15 min of centrifugation at 10,000 × g and 4 °C, the polar phase was collected and dried in a vacuum concentrator.

To the extracted polar metabolites, a solution of 20 mg of methoxyamine hydrochloride per mL of pyridine was added, followed by shaking in a thermoshaker for 2 h at 37 °C and 500 rpm. Then, the derivatizing agent MSTFA (N-trimethylsilyl-N-methyl trifluoroacetamide) was added, followed by shaking in a thermoshaker for 30 min at 37 °C and 500 rpm.

The samples were subsequently used for metabolic profiling using a gas chromatograph coupled to a mass spectrometer (QP-PLUS 2010, Shimadzu, Kyoto, Japan), equipped with a capillary column (SPB™-5, Supelco, Bellefonte, PA, USA), Fused Silica Capillary Column, 30 m length × 0.25 mm internal diameter × 0.25 µm film thickness) Programmed with an initial temperature of 80 °C, maintained for 5 min, followed by a heating rate of 10 °C·min<sup>-1</sup> up to 310 °C. Helium was used as the carrier gas, with a flow rate of 1.0 mL/min.

The metabolic profile was obtained from the analysis of chromatograms and mass spectra using Xcalibur® 2.1 software (Thermo Fisher Scientific, Waltham, MA, USA) and the Golm Metabolome Database (<http://gmd.mpimp-golm.mpg.de/> accessed on 15 January 2026). The results are presented in terms of Relative Metabolic Content, where the intensity of each identified metabolite is normalized by the intensity of the internal analytical standard and by the exact amount of sample used in the metabolic extraction.

### 2.2.4. Preparation of the Control and Enriched Pasta Formulations Samples with *Zophobas morio* Powder

For the preparation of the control pasta formulation (C), the ingredients and proportions described in the manual for the production of fresh pasta were used [21], as shown in Table 1. All pasta formulations were produced in three distinct batches to ensure experimental replication and reliability of the analyses.

For the development of pasta formulations enriched with *Zophobas morio* powder, three different treatments were used. Formulation 1 (F1) was prepared by partially replacing wheat flour with 7.5% insect powder, and this proportion was gradually increased to 15% in Formulation 2 (F2) and to 17.5% in Formulation 3 (F3).

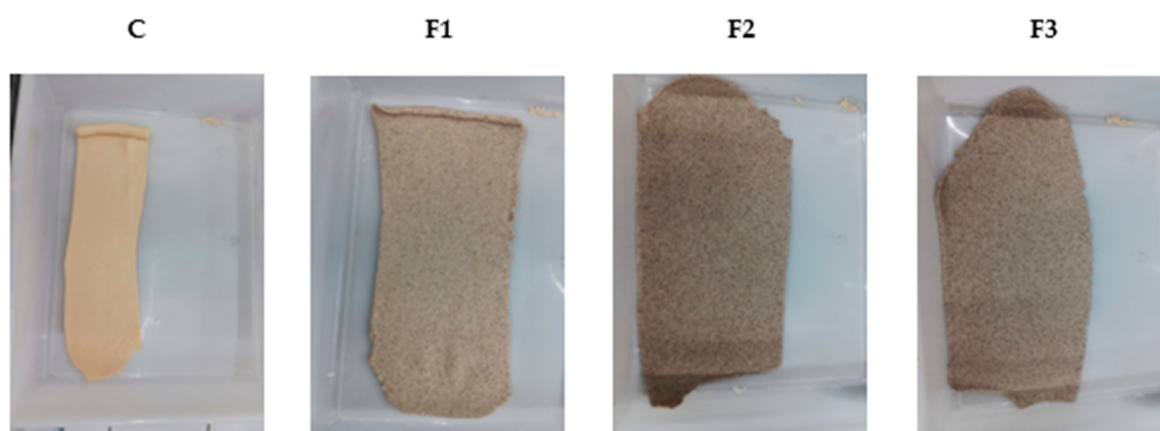
After incorporating the insect powder into the wheat flour in the three aforementioned proportions, the remaining ingredients were accurately weighed, mixed in a dough mixer (Artisan Empire Red, KitchenAid, Benton Harbor, MI, USA), and homogenized for 5 min at speed 3. The homogeneous dough (Figure 2) was then laminated using a roller-type pasta machine (MMX 5X1, Multimix, São Paulo, Brazil) until reaching a thickness of 2 mm,

presenting a smooth, uniform, and non-brittle appearance. To obtain pasta sheets suitable for tagliatelle-type noodles, the dough was cut into strips with a width of 7.5 mm.

**Table 1.** Composition of the control pasta formulation and pasta formulations enriched with *Zophobas morio* powder.

| Ingredients (%) <sup>1</sup> | C   | F1  | F2  | F3   |
|------------------------------|-----|-----|-----|------|
| Wheat flour                  | 100 | 100 | 100 | 100  |
| Insect powder                | 0   | 7.5 | 15  | 17.5 |
| Eggs                         | 15  | 15  | 15  | 15   |
| Salt                         | 3   | 3   | 3   | 3    |
| Water                        | 25  | 25  | 25  | 25   |

C, F1, F2, and F3 represent pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively. <sup>1</sup> The amounts of the ingredients used were calculated based on the quantity of wheat flour in the formulation.



**Figure 2.** Homogenous doughs of pasta formulations with and without the addition of giant mealworm (*Zophobas morio*) powder. C, F1, F2, and F3 represent pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively.

#### 2.2.5. Crude Fiber Analysis of Giant Mealworm Powder (*Zophobas morio*)

The methodology was based on the method described by IAL for crude fiber analysis [22]. Initially, all fat was extracted from the sample using a Soxhlet apparatus (TE 044, Tecnal Ltda., Piracicaba, Brazil) with hexane as the solvent. The defatted sample was heated to remove excess solvent and then transferred to an Erlenmeyer flask, to which sulfuric acid solution and glass wool (as a filtering agent) were added. The mixture was stirred under heating for 40 min, and at the end of this period, it was filtered using diatomaceous earth. Filtration initially involved the use of boiling water until no acidic reaction was detected, after which the residue was washed with alcohol and ether. Finally, the dried sample was incinerated in a muffle furnace and repeatedly weighed until a constant weight was obtained. The loss in weight corresponded to the crude fiber content, which was calculated as follows:

$$\% \text{Crude fiber} = \frac{100 \times FW}{SW} \quad (7)$$

where

FW = g of fiber.

SW = g of sample.

### 2.2.6. Quality Analysis of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

#### Cooking Analysis

The cooking process was carried out according to the guidelines of the AACC [23]. Approximately 10 g of each sample were cooked in 140 mL of boiling distilled water in a 500 mL beaker. The optimal cooking time was determined by observing the gradual disappearance of the opaque center of the pasta during the process, at 10 s intervals, by pressing the pasta strands between two transparent glass plates.

#### Volume Variation

The volume variation was measured before and after the optimal cooking time using the AACC methodology [23]. The samples were immersed in 140 mL of hexane in a 200 mL graduated cylinder before and after cooking, and the displaced hexane volume was measured.

#### Solids Loss

The solids loss in the cooked pasta was evaluated according to AACC [23]. A 25 mL aliquot of the cooking water was drained and evaporated in an oven at 100 °C until a constant weight was reached. After cooling in a desiccator, the residue was weighed to determine the amount of soluble solids lost during the cooking process.

### 2.2.7. Physical Analyses of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

#### Color Analysis

Color analyses were performed using a colorimeter (NR60CP, 3NH, Shenzhen, China) based on the CIE Lab\* color system. A pasta sample was placed in a specific container for analysis, and the device performed readings covering the lightness coordinate (L\*), which ranges from black (0) to white (100). The a\* coordinate was used to determine the intensity of red and green tones, where +a indicates red (0 to 100) and −a indicates green (−80 to 0), while the b\* coordinate was used to evaluate the intensity of yellow and blue tones, where +b indicates yellow (0 to 70) and −b indicates blue (−100 to 0). The ΔE value was calculated using the following equation:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (8)$$

where

$$\Delta L^* = L^* \text{ sample} - L^* \text{ control.}$$

$$\Delta a^* = a^* \text{ sample} - a^* \text{ control.}$$

$$\Delta b^* = b^* \text{ sample} - b^* \text{ control.}$$

### Water Activity Analysis of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

Water activity was measured according to the manufacturer's instructions using a water activity meter (4TE, AquaLab, Pullman, WA, USA) at a temperature of 25 °C. The samples were placed in the containers designated for automatic reading by the device, filling approximately half of the container volume. The containers were then sequentially attached to the instrument, which was activated to perform the readings, and the results were properly recorded. For this measurement, the reference point adopted was the relative humidity of water vapor equal to 1.0.

### 2.2.8. Physicochemical Analyses of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

#### pH

The pH of the pasta was measured according to the methodology described by IAL [22]. A 5 g portion of the ground sample was weighed and transferred to a beaker, followed by the addition of 50 mL of distilled water. The mixture was stirred until the particles were uniformly suspended. Finally, the pH was measured using a potentiometer (TEC-5, Tecnal, Piracicaba, Brazil) previously calibrated.

#### Titrateable Acidity

The methodology used followed the procedures described by IAL [22]. A 4 g portion of the sample was weighed and transferred to a 125 mL Erlenmeyer flask with the aid of 50 mL of water. Then, 2 to 4 drops of phenolphthalein solution were added, and the mixture was titrated with 0.1 M sodium hydroxide solution until a pink coloration was observed. The volume of NaOH used to neutralize the solution was recorded and used to calculate the total acidity percentage. The results were expressed in mL of NaOH per 100 g of dry matter. The titrateable acidity in mL of NaOH solution per 100 g of pasta was calculated using the following equation:

$$\text{Acidity (mL of NaOH solution per 100 g of pasta)} = \frac{V \times F \times 100}{W \times C} \quad (9)$$

where

$V$  = volume (mL) of 0.1 or 0.01 M sodium hydroxide solution used in the titration.

$F$  = correction factor of the 0.1 or 0.01 M sodium hydroxide solution.

$W$  = weight (g) of the sample used in the titration.

$C$  = correction factor for 1 M NaOH solution, 10 for 0.1 M NaOH solution, and 100 for 0.01 M NaOH solute/on.

### 2.2.9. Scanning Electron Microscopy (SEM) of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

The microstructural analysis of the pasta samples was carried out using scanning electron microscopy (SEM). Samples from the different treatments were dried in a forced-air circulation oven at 60 °C for 10 h, then transferred to a desiccator until reaching room temperature. After complete water removal, the samples were cut to obtain areas of approximately 1 cm<sup>2</sup>.

Subsequently, they were gold-coated to improve electron conductivity, and the readings were performed. The microstructures were observed using a QUANTA FEG (FEI, Hillsboro, OR, USA), with digital images captured at magnifications of ×500, ×1000, and ×2000, according to the methodology described by Sung et al. [24].

### 2.2.10. Texture Profile of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

The texture profile analysis of the pasta samples was performed using a TA.XTi Texture Analyser<sup>®</sup> (Stable Micro Systems, Godalming, UK). The tests were conducted with an aluminum cylindrical probe (35 mm in diameter). Two compression cycles were carried out at a speed of 2 mm/s and 85% compression.

Data were collected and processed using Exponent Lite software, version 6.1.16.0, and the following parameters were calculated: hardness (g), adhesiveness (g·s), springiness, and chewiness (g).

### 2.2.11. X-Ray Diffraction (XRD) Analysis of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

The X-ray patterns were obtained using an X-ray diffractometer (X'Pert PRO, PANalytical, Almelo, Netherlands). The samples, previously oven-dried (105 °C for 12 h) and ground with a mortar and pestle, were scanned over an angular range of 10–100° 2 $\theta$  at a rate of 2° / min at 25 °C. The diffractograms were processed and plotted using Origin 2025 software. To facilitate comparative visualization between formulations and improve graphical interpretation, the X-axis of the diffractograms was adjusted from 10–100° to a range of 0–100° for better visualization.

### 2.2.12. Statistical Analysis

The experimental data were subjected to one-way analysis of variance (ANOVA) to evaluate the effect of different formulation levels. When significant differences were detected ( $p < 0.05$ ), mean values were compared using Tukey's pairwise. All statistical analyses were performed using PAST software (version 2023). Results are expressed as mean  $\pm$  standard deviation, and different letters on the same column indicate statistically significant differences among samples.

## 3. Results and Discussion

### 3.1. Results of the Proximate Composition of *Zophobas morio* Powder and Caloric Value

Moisture content (Table 2), although there is no specific reference value for insect powder, complies with IN n° 8 of 2 June 2005, issued by the Brazilian Ministry of Agriculture and Livestock (MAPA), which establishes a maximum moisture content of 15.0% for wheat flour. Borges et al. found a moisture value of 3.39% for the same type of flour [17]. This difference may be associated with the equipment used as well as the drying time and temperature conditions.

**Table 2.** Proximate composition and caloric value of giant mealworm (*Zophobas morio*) powder.

| Moisture (%)    | Ash (%)         | Lipid (%)        | Protein (%)      | Carbohydrate (%) | Caloric Value (Kcal.100 g <sup>-1</sup> ) |
|-----------------|-----------------|------------------|------------------|------------------|-------------------------------------------|
| 6.15 $\pm$ 0.19 | 1.65 $\pm$ 0.05 | 28.79 $\pm$ 1.11 | 60.95 $\pm$ 0.04 | 2.46 $\pm$ 1.29  | 512.75 $\pm$ 5.00                         |

Values are expressed as mean  $\pm$  standard deviation ( $n = 3$ ).

Regarding ash content, the value of 1.65% (Table 2) is lower than the 2.49% and 2.52% reported by Borges et al. and Silva et al. [17,25], respectively, who worked with the same insect species. This variation may be related to the diet provided to the insects during development, which is generally composed only of cereals and a source of moisture.

Among the macronutrients, lipids (Table 2) stood out, accounting for nearly 30% of the powder composition, which directly affects its caloric value of 512.75 Kcal.100 g<sup>-1</sup>. This high fat content can be considered either a positive or negative factor, since the demand for protein-rich foods has been growing considerably and, in many cases, is associated with weight management efforts [26]. Therefore, the high caloric value of *Zophobas morio* powder may pose an obstacle for its use in products aimed at this consumer profile. However, this limitation can be mitigated through the application of more efficient lipid extraction methods. On the positive side, this high lipid fraction may provide health benefits due to the increased intake of important compounds known to be abundant in giant mealworms, such as oleic acid (omega-9) and linoleic acid (omega-6) [27].

The *Zophobas morio* powder used in this study presented lipid content values similar to those reported by González et al. for *Tenebrio molitor* (30.69%) [28], *Hermetia illucens* (35.82%), and *Acheta domestica* (27.08%), indicating that variables such as species or phylogenetic

proximity, larval age or developmental stage, diet, rearing conditions (temperature, humidity, and population density), as well as drying, milling, powder preparation methods, and storage conditions prior to analysis, can influence the variability of nutritional composition.

As shown in Table 2, the proximate composition of the insect powder reveals that protein is the most abundant macronutrient, representing approximately 60% of the total. However, the high protein content found in this study is lower than that reported by Borges et al. [17] who found values of 70%. This higher result may be related to the processing method used, which allowed for more efficient lipid extraction through pressing, as well as differences in diet and insect maturity. When compared with other insect powders that underwent similar processing, the results obtained for *Zophobas morio* powder position it as a promising protein source. Kowalski et al. found lower protein levels (47.64%) in *Tenebrio molitor* powder [29], a species that is phylogenetically close to *Zophobas morio*. Meanwhile, Vanqa et al. investigated three different insect powders and reported that the one with the highest protein content [30], derived from *Macrotermes subhyllanus* (a termite species), contained only 50% protein.

As an animal-derived raw material, the carbohydrate content (Table 2) was significantly lower (2.46%) compared to the other macronutrients. Borges et al. reported 9.45% carbohydrates for *Zophobas morio* powder [17], which may be attributed to factors influencing the final value, such as larval maturity, diet, and the efficiency of lipid extraction during processing.

### 3.2. Giant Mealworm (*Zophobas morio*) Powder Aminoacid Profile

According to Table 3, seven of the nine essential amino acids were identified in the analyzed profile: leucine, valine, isoleucine, phenylalanine, lysine, threonine, and methionine. An interesting point to highlight regarding the essential amino acids found in the sample is the high concentration of branched-chain amino acids (BCAAs) present in the protein profile of *Zophobas morio*. As shown in Table 3, the three most abundant amino acids were valine, leucine, and isoleucine, which together account for approximately 50% of all essential amino acids found in muscle proteins [31]. These amino acids also play a key role in protein synthesis, particularly in the post-exercise period [32], suggesting the potential of this insect protein as a sports protein supplement.

**Table 3.** Amino acid profile of giant mealworm (*Zophobas morio*) powder.

| Essential AAs     | RMC    |
|-------------------|--------|
| Valine            | 63.45  |
| Leucine           | 33.10  |
| Isoleucine        | 31.06  |
| Phenylalanine     | 25.84  |
| Lysine            | 20.04  |
| Methionine        | 4.23   |
| Threonine         | 4.14   |
| Non-essential AAs |        |
| Alanine           | 168.83 |
| Glycine           | 119.31 |
| Proline           | 75.12  |
| Glutamate         | 70.57  |
| Aspartic acid     | 5.77   |
| Serine            | 7.30   |

AAs = Amino acids; RMC = Relative Metabolite Content. Amino acid values are expressed as Relative Metabolite Content (RMC, in normalized arbitrary units), obtained by GC-MS after normalization to the internal standard (ribitol) and to the sample mass used for extraction.

Perez-Santaescolastica et al. found similar results for the concentrations of these compounds when analyzing the amino acid profiles of *Tenebrio molitor* and *Zophobas morio* [33], except for histidine and tryptophan, which were not identified in the present study, possibly due to their low concentrations, insufficient to be detected by the analytical method or equipment used.

Finke reported a different amino acid profile for *Zophobas morio*, in which alanine and glutamate were the most abundant non-essential amino acids [34]. Alanine is important for gluconeogenesis, whereas glutamate participates in the synthesis of  $\gamma$ -aminobutyric acid (GABA), the main inhibitory neurotransmitter in the central nervous system [34]. Although the predominance of these amino acids is also evident in the results presented in Table 3, in the present study glutamate appears only as the fourth most concentrated non-essential amino acid. This discrepancy may be related to differences in the diet provided to the larvae.

### 3.3. Results of the Crude Fiber Analysis of Giant Mealworm Powder (*Zophobas morio*)

According to the data presented in Table 4, a positive effect was observed between the increasing proportion of *Zophobas morio* powder in the formulations and the increase in crude fiber content, a behavior previously documented by Acosta-Estrada et al. in other foods enriched with insect powder [35]. In the control sample, the presence of fiber was already detected, which is possibly due to the use of refined wheat flour, an ingredient present in all formulations that, even in small amounts, still allows for the detection of this component [36].

**Table 4.** Crude fiber content of pasta formulations with and without the addition of giant mealworm (*Zophobas morio*) powder.

| Pasta Formulations | Crude Fiber (%)              |
|--------------------|------------------------------|
| C                  | 0.25 <sup>a</sup> $\pm$ 0.01 |
| F1                 | 0.74 <sup>b</sup> $\pm$ 0.04 |
| F2                 | 0.98 <sup>c</sup> $\pm$ 0.04 |
| F3                 | 1.30 <sup>d</sup> $\pm$ 0.08 |

C, F1, F2, and F3 represent pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively. Mean values  $\pm$  standard deviation ( $n = 3$ ) followed by different letters on the same column differ significantly according to Tukey's test ( $p < 0.05$ ).

### 3.4. Results of the Quality Analysis of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

The results for solids loss and volume increase are presented in Table 5. It can be observed that formulations F2 and F3 do not show significant differences between them, which may be related to the fact that the added powder concentrations in both are not as distinct compared to formulations C and F1. The lower loss of soluble solids in F2 and F3 pasta can be explained by the higher concentrations of insect protein, whose composition includes hydrophilic fatty acids capable of forming a protein network that results in a gel, thereby improving the physical structure of the product [13].

Similar results were observed in a study that used *Tenebrio molitor* and *Acheta domesticus* to enrich pasta, where the samples containing insect protein showed a reduction in soluble solids loss compared to the control sample [13].

Regarding the cooking time, it was not possible to determine the ideal cooking time of the pasta using the AACC method [23], since the opaque center did not disappear in the enriched samples when pressed between the glass slides, even after more than 20 min of heating. Therefore, the ideal cooking time of the pasta was determined sensorially, from

the moment when the pasta was no longer “al dente,” with all samples reaching this point after 10 min.

All formulations showed an increase in volume due to water absorption during cooking, with no significant differences among them. The fact that the proportional increase in *Zophobas morio* protein did not significantly affect the increase in the cooked pasta volume may be attributed to the high content of globulins in its protein composition, which are water-insoluble, as also observed in *Tenebrio molitor* [37]. A different result was reported in pasta enriched with other animal-derived proteins, such as whey, whose addition was shown to reduce water absorption [38].

**Table 5.** Loss of solids and volume increase in pasta formulations with and without the addition of giant mealworm (*Zophobas morio*) powder.

| Pasta Formulations | Solids Loss (g)           | Volume Increase (mL)     |
|--------------------|---------------------------|--------------------------|
| C                  | 0.61 <sup>a</sup> ± 0.09  | 5.67 <sup>a</sup> ± 1.52 |
| F1                 | 0.53 <sup>a</sup> ± 0.05  | 5.33 <sup>a</sup> ± 1.15 |
| F2                 | 0.21 <sup>bc</sup> ± 0.07 | 5.16 <sup>a</sup> ± 1.15 |
| F3                 | 0.28 <sup>c</sup> ± 0.08  | 5.50 <sup>a</sup> ± 1.32 |

C, F1, F2, and F3 represent pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively. Mean values ± standard deviation ( $n = 3$ ) followed by different letters on the same column differ significantly according to Tukey's test ( $p < 0.05$ ).

### 3.5. Results of the Physical Analyses of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

#### 3.5.1. Color Analyses

Table 6 presents the values of the color coordinates ( $L^*$ ,  $a^*$ , and  $b^*$ ) of pasta samples with and without the addition of insect powder. The  $L^*$  coordinate (lightness) showed the expected behavior, since *Zophobas morio* powder is relatively darker than wheat flour, and its inclusion resulted in proportionally darker pasta samples. For the  $L^*$  coordinate, no significant difference ( $p > 0.05$ ) was observed between samples F2 and F3. This result may be attributed to the fact that these two formulations exhibited only a small difference in their enrichment levels compared to the others. This behavior was corroborated by the  $\Delta E$  value  $< 1$ , which indicates that the color differences are visually imperceptible, a result also observed in the images shown in Figure 3.

**Table 6.** Values of color coordinates  $L^*$ ,  $a^*$ , and  $b^*$  and  $\Delta E$  of pasta formulations with and without the addition of giant mealworm (*Zophobas morio*) powder.

| Coordinates        | C                         | F1                         | F2                         | F3                        |
|--------------------|---------------------------|----------------------------|----------------------------|---------------------------|
| $L^*$              | 74.81 <sup>a</sup> ± 0.50 | 64.43 <sup>b</sup> ± 0.53  | 57.37 <sup>c</sup> ± 0.25  | 57.04 <sup>c</sup> ± 1.49 |
| $a^*$              | 3.97 <sup>a</sup> ± 0.19  | 3.27 <sup>bcd</sup> ± 0.29 | 3.28 <sup>cd</sup> ± 0.09  | 3.36 <sup>d</sup> ± 0.23  |
| $b^*$              | 27.53 <sup>a</sup> ± 0.58 | 22.06 <sup>bc</sup> ± 0.94 | 21.08 <sup>cd</sup> ± 0.07 | 20.56 <sup>d</sup> ± 0.29 |
| $\Delta E$ control | -                         | 11.75                      | 18.61                      | 19.10                     |

C, F1, F2, and F3 represent pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively. Mean values ± standard deviation ( $n = 3$ ) followed by different letters on the same line differ significantly according to Tukey's test ( $p < 0.05$ ).  $\Delta E$ : Color variation in the formulations compared with the control.

Regarding the  $a^*$  coordinate, a significant difference was observed between the control formulation and the others ( $p < 0.05$ ) (Table 6). These effects were similar to those described by Kowalski et al. [39], who supplemented cakes with *Tenebrio molitor* powder. On the other hand, Kowalski et al. obtained different results [29], as increasing the addition of insect powder also increased the  $a^*$  values. In that specific case, the author pointed out that the replacement of chicken eggs in the cake formulation with a plant-based alternative was a possible factor contributing to the unexpected outcome.

A behavior similar to that of the  $a^*$  parameter was observed for the  $b^*$  parameter, except for the mean values between samples F1 and F3, which showed a significant difference from each other. There was a tendency for the  $b^*$  value to decrease with the addition of insect flour, making the samples less yellow. This result is consistent with the findings reported by Çabuk [40], who investigated the influence of *Locusta migratoria* and *Tenebrio molitor* in protein muffins, where the crumb color tended to be less yellow compared to the control sample. Duda et al. also obtained results similar to those of the present study regarding the  $b^*$  coordinate when analyzing the color of pasta enriched with cricket flour [41], which decreased from 22.04 to 9.00 with the gradual addition of the insect flour. It is important to note that differences may occur between the results of different studies, as the final color of the samples also depends on the preparation method adopted for the product, which directly influences Maillard reactions.

The  $\Delta E$  values, as well as the  $L^*$ ,  $a^*$ , and  $b^*$  coordinates, were also consistent with those reported in other studies [29,42]. As more *Zophobas morio* powder was added, a darkening trend was observed in the formulations. These formulations presented  $\Delta E$  values higher than 6 compared to the control formulation, a value that, according to the CIE Lab\* system, indicates the presence of visually distinct colors. Arp and Pasini stated that the marked difference observed between the samples occurs largely due to the use of refined wheat powder in the control formulations of studies of this nature [43]. Thus, if this flour were replaced by a whole-wheat version, the color variations would tend to be less pronounced and could even reach levels of imperceptible difference. Furthermore, according to the authors, the darker coloration observed in the enriched formulations may present a positive commercial appeal, as it visually resembles whole-grain products widely accepted in the market and often associated with healthier eating habits.



**Figure 3.** Doughs of pasta formulations with and without the addition of giant mealworm (*Zophobas morio*) powder. C, F1, F2, and F3 represent pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively.

### 3.5.2. Water Activity Analysis

Through Table 7, it was observed that there was no significant difference among the samples evaluated. Water activity directly depends on the amount of free water available, and since all the pasta samples were hydrated in a similar way during processing, water activity tends to be equivalent. Despite the addition of *Zophobas morio*, the incorporation of proteins and lipids was not sufficient to significantly modify, at the 5% significance level, the binding or retention of water in the food matrix. It is important to emphasize that raw/fresh pasta generally presents high water activity. Within this range, small differences in formulation do not result in large changes in water activity.

**Table 7.** Water activity of fresh and raw pasta formulations with and without the addition of giant mealworm (*Zophobas morio*) powder.

| Pasta Formulations | Water Activity               |
|--------------------|------------------------------|
| C                  | 0.9662 <sup>a</sup> ± 0.0010 |
| F1                 | 0.9614 <sup>a</sup> ± 0.0007 |
| F2                 | 0.9636 <sup>a</sup> ± 0.0009 |
| F3                 | 0.9641 <sup>a</sup> ± 0.0004 |

C, F1, F2, and F3 represent pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively. Mean values ± standard deviation ( $n = 3$ ) followed by different letters on the same column differ significantly according to Tukey's test ( $p < 0.05$ ).

This behavior, showing no change in water activity, differs from the results reported in other studies with enriched fresh pasta, such as that of Bianchi et al. [44], in which a slight reduction in water activity was observed between the control sample and that enriched with *Aristotelia chilensis*. It is also observed that the values obtained in this study ranged from 0.961 to 0.966, which falls within the expected range for fresh pasta products, whose water activity values usually range between 0.92 and 0.97 [45], and therefore should be kept refrigerated to prevent the proliferation of microorganisms.

### 3.6. Proximate Composition of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

Table 8 presents the proximate composition (moisture, ash, lipids, proteins, and carbohydrates), as well as the caloric value, of pasta samples with and without the addition of giant mealworm (*Zophobas morio*) powder. A significant difference ( $p < 0.05$ ) was observed between formulation C and the other formulations, resulting from the partial replacement of wheat flour with *Zophobas morio* powder. Among the enriched formulations, sample F3 showed an increase in moisture compared to the others, although it still remained lower than that of the control sample.

**Table 8.** Proximate composition and caloric value of pasta formulations with and without the addition of giant mealworm (*Zophobas morio*) powder.

| Samples | Moisture (%)              | Ash (%)                  | Lipid (%)                | Protein (%)               | Carbohydrate (%)          | Caloric Value (Kcal.100 g <sup>-1</sup> ) |
|---------|---------------------------|--------------------------|--------------------------|---------------------------|---------------------------|-------------------------------------------|
| C       | 39.39 <sup>a</sup> ± 0.17 | 2.19 <sup>a</sup> ± 0.02 | 0.84 <sup>a</sup> ± 0.07 | 8.16 <sup>a</sup> ± 0.03  | 49.41 <sup>a</sup> ± 0.15 | 237.88 <sup>a</sup> ± 0.89                |
| F1      | 36.55 <sup>b</sup> ± 0.16 | 2.23 <sup>a</sup> ± 0.01 | 1.01 <sup>b</sup> ± 0.01 | 10.98 <sup>b</sup> ± 0.08 | 49.24 <sup>a</sup> ± 0.15 | 249.94 <sup>b</sup> ± 0.68                |
| F2      | 36.16 <sup>b</sup> ± 0.18 | 2.03 <sup>b</sup> ± 0.04 | 1.35 <sup>c</sup> ± 0.00 | 13.30 <sup>c</sup> ± 0.07 | 47.16 <sup>b</sup> ± 0.23 | 253.10 <sup>cd</sup> ± 0.67               |
| F3      | 36.94 <sup>c</sup> ± 0.13 | 2.10 <sup>c</sup> ± 0.01 | 1.56 <sup>d</sup> ± 0.02 | 16.29 <sup>d</sup> ± 0.12 | 43.11 <sup>c</sup> ± 0.19 | 251.61 <sup>d</sup> ± 0.56                |

C, F1, F2, and F3 represent pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively. Mean values ± standard deviation ( $n = 3$ ) followed by different letters on the same column differ significantly according to Tukey's test ( $p < 0.05$ ).

This behavior, characterized by a marked reduction in moisture when comparing C with F1 and a slight increase as the proportion of *Zophobas morio* powder rises, was also described by Zielińska and Pankiewicz when analyzing cookies enriched with *Tenebrio molitor* [46]. The authors attributed this decrease in water content to the lower water-holding capacity of insect proteins compared to wheat flour proteins, due to their less hydrophilic molecular structure.

Regarding ash content, there was no significant difference between the control formulation and F1 (Table 8). However, formulations F2 and F3 showed differences between each other and in relation to formulations C and F1 (Table 8). In the present study, a significant reduction in ash content was observed as the proportion of insect powder increased in formulations. This result differs from that reported in the literature, where an overall increase

in ash content was observed in pasta enriched with proteins from different sources [46]. Such divergence may be explained by the fact that, in this study, the proximate composition analysis was performed on raw samples. The absence of the cooking process may have influenced the final ash content.

One of the characteristics observed with the addition of *Zophobas morio* powder was the increase in lipid concentration in the enriched formulations, which showed significant differences among all samples. This increase is attributed to the processing of insect powder, which resulted in a material containing approximately 30% fat. The rise in the lipid fraction contributed to the higher caloric value of the pasta, even with a reduction in carbohydrate content. However, in a study conducted by Hidalgo et al. [47], which analyzed the proximate composition of pasta enriched with proteins from silkworm (*Bombyx mori*) and black soldier fly (*Hermetia illucens*), this effect was not observed. This may have been due to the proteins used underwent an isolation process involving saline extraction and subsequent freeze-drying, which removed most of the lipid fraction.

Studies such as those by Musika et al. and Kowalski et al. [29,48], which used whole powders of *Acheta domesticus* and *Tenebrio molitor* without purification steps, reported results similar to those obtained in the present work. In these studies, an increase in lipid content and, consequently, in the energy value of insect-enriched food products was also observed.

Furthermore, a progressive increase in protein content (Table 8) was observed, proportional to the increase in the concentration of *Zophobas morio* powder in the formulations. The differences among all samples were statistically significant, with formulation F3 showing the highest protein content. These results are consistent with previously reported data [46,48], which indicate that edible insects are relevant protein sources, partly due to the presence of chitin in their exoskeletons. The increase in protein content in foods enriched with insect powder may improve both their nutritional and sensory qualities [41].

On the other hand, regarding the carbohydrate content found in the present study, a reduction was observed as the addition of insect powder increased. Biró et al. also corroborate these results for carbohydrate content [49], as they found values ranging from 50 to 57.1% when analyzing the enrichment of pasta with silkworm powder. The authors observed a proportional decrease in carbohydrate content as protein addition increased, an effect attributed to the relative reduction in the concentration of this macronutrient in the total sample composition.

Considering that products enriched with insect powder would be better positioned in the market as “fit” or “healthy” foods [43], the increase in caloric value (Table 8) may not be desirable, but it can potentially be addressed by employing protein isolation and purification techniques for insect proteins [47], generating a product with lower lipid content.

### 3.7. Results of the Physicochemical Analyses of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

#### pH and Titratable Acidity

In the present study, significant differences were observed in titratable acidity values between the control sample and the formulations enriched with insect powder. However, among the enriched formulations, F1 showed a statistical difference compared to F2 and F3 (Table 9). The lack of a significant difference between F2 and F3 may be related to the smaller difference in the amount of powder added to these two formulations when compared to the others. A similar trend was reported by Biró et al. [50], who analyzed the enrichment of oatmeal cookies with cricket powder and observed that the titratable acidity of the cookies increased proportionally to the addition of insect powder, with values ranging from 9.95 to 17.6 (NaOH/100 g of sample).

**Table 9.** Titratable acidity and pH of pasta formulations with and without the addition of giant mealworm (*Zophobas morio*) powder.

| Pasta Formulations | * Titratable Acidity      | pH                       |
|--------------------|---------------------------|--------------------------|
| C                  | 2.06 <sup>a</sup> ± 0.01  | 6.50 <sup>a</sup> ± 0.01 |
| F1                 | 2.53 <sup>b</sup> ± 0.01  | 6.47 <sup>a</sup> ± 0.06 |
| F2                 | 2.75 <sup>cd</sup> ± 0.01 | 6.43 <sup>a</sup> ± 0.06 |
| F3                 | 2.75 <sup>d</sup> ± 0.01  | 6.43 <sup>a</sup> ± 0.02 |

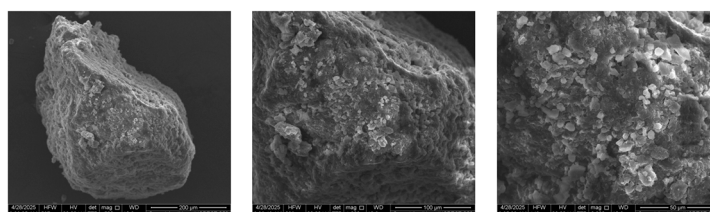
C, F1, F2, and F3 represent pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively. Mean values ± standard deviation ( $n = 3$ ) followed by different letters on the same column differ significantly according to Tukey's test ( $p < 0.05$ ). \* Values expressed in mL of NaOH/100 g of sample.

The pH results of the pasta samples with and without the addition of insect powder are presented in Table 9 which ranged from 6.43 to 6.50 without any significant differences.

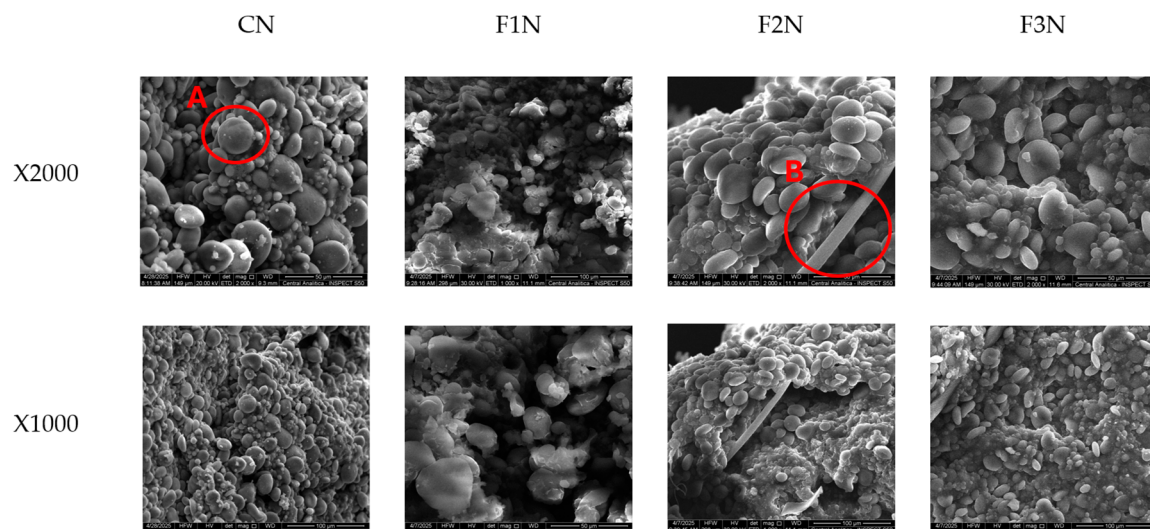
In a study conducted by Bartkiene et al. [51] who evaluated the influence of cricket flour on the quality of wheat bread, pH values lower than those found in the present work were observed. Such pH variations reported across different studies may be related to the distinct amino acid compositions present in insect flours, which are rich in lysine, for example, an amino acid that contains amine groups capable of absorbing protons, thereby increasing the pH of the medium.

### 3.8. Results of the Scanning Electron Microscopy (SEM) of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

The images of *Zophobas morio* powder allowed, by comparison, the identification of the insect powder (Figure 4) added to the formulations within the composition of both cooked and raw pasta, as well as its behavior when incorporated into the food matrix. Mshayisa et al. conducted a scanning electron microscopy analysis on defatted and freeze-dried *Hermetia illucens* powder [52]. The micrographs obtained from the freeze-dried samples exhibited morphological characteristics more similar to those observed in the present study, particularly a relatively smooth surface with fractured areas and the presence of irregularly shaped particles. The similarity between these samples and those subjected only to the freeze-drying process may be associated with the presence of lipids in the matrix, which possibly contribute to the formation of a more cohesive and smoother surface.

**Figure 4.** Giant mealworm (*Zophobas morio*) powder magnified at ×500, ×1000, and ×2000 from left to right.

The electron micrographs from Figure 5 revealed differences in the microstructure of raw pasta as a result of the partial addition of wheat flour with *Zophobas morio* powder. In the uncooked control sample (CN), well-defined wheat starch granules (Letter A, Figure 5) were observed, arranged in a relatively uniform matrix partially surrounded by the gluten protein network. This structure is typical of wheat-based pasta and provides cohesion, elasticity, and water retention to the product. With the addition of *Zophobas morio* powder (F1N), a greater disorganization of the matrix and less evident compaction of starch granules were noted, indicating that the insect powder interferes with the formation of the protein network. The gluten becomes partially diluted by insect proteins, which do not exhibit the same rheological properties, thereby compromising the continuity of the matrix.



**Figure 5.** Photomicrographs of raw pasta formulations with and without the addition of giant mealworm (*Zophobas morio*) powder, magnified at  $\times 1000$  and  $\times 2000$ . CC, F1C, F2C, and F3C represent cooked pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively. Letter A indicates wheat starch granules in the control sample (CN), while letter B highlights elongated filamentous structures in sample F2N.

An aspect observed in the micrographs of sample F2N is the presence of a straight and elongated structure (Letter B, Figure 5), integrated into the composition of both insect and wheat flour. Pornsuwan et al. [53], through scanning electron microscopy analyses aimed at investigating the microstructure of *Hermetia illucens* larval powder subjected to different drying methods, also identified similar structures with a morphology resembling capillary filaments. According to Rebora et al. [54], such structures correspond to chitinous bristles. In the present study, even after the grinding process of *Zophobas morio* powder, it is possible that these bristles resisted processing and therefore remain visible in the images.

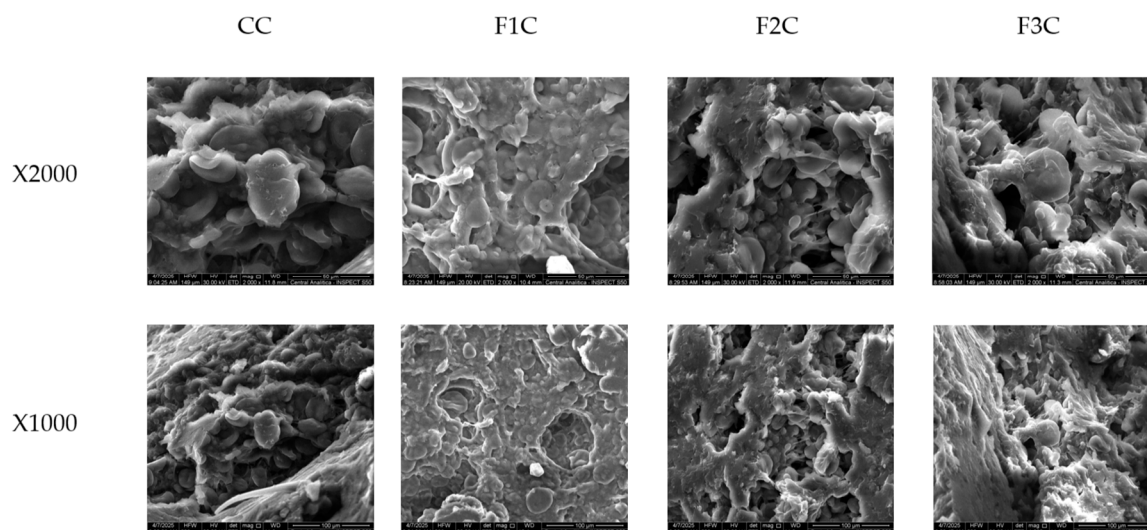
The images captured at magnifications of  $\times 1000$  and  $\times 2000$  allowed precise identification of wheat starch granules and *Zophobas morio* powder particles, making it possible to observe in the cooked samples (CC, F1C, F2C, and F3C) (Figure 6) that the thermal cooking process resulted in the formation of a surface matrix that compacted the starch granules and the *Zophobas morio* powder.

This behavior was similar to that observed by Renoldi et al. when cooking *durum* wheat pasta enriched with psyllium [55], a soluble fiber obtained from the husk of *Plantago ovata* seeds. It is believed that this surface matrix may have prevented the action of heated water on the innermost layers of the pasta, since even after cooking, the center of the samples did not appear completely vitreous and gelatinized, as evidenced by the “honeycomb” structures seen in images F1, F2, and F3 of Figure 6 [24,56].

In the F1C, F2C, and F3C formulations (Figure 6), the incorporation of *Zophobas morio* powder led to visible changes in the organization of the matrix. With the addition of insect powder, greater structural heterogeneity was noted, with less cohesive regions and the presence of irregular fragments. This effect may be associated with the reduction in the gluten network, as *Zophobas morio* proteins do not have the same ability to form the tridimensional bonds that confer elasticity and integrity to the matrix.

In samples F2C and F3C (Figure 6), the increase in the proportion of *Zophobas morio* powder accentuated structural discontinuity, evidenced by void spaces and lower compaction among starch granules. This behavior indicates that higher substitution levels hinder the formation of a continuous network. Another relevant aspect is the presence of insect powder particles integrated into the matrix, which appear not to fully interact with

starch and gluten. This suggests that these components act more as fillers than as active structural elements, directly affecting the final texture and water absorption capacity of the pasta.



**Figure 6.** Photomicrographs of cooked pasta formulations with and without the addition of giant mealworm (*Zophobas morio*) powder, magnified at  $\times 1000$  and  $\times 2000$ . CC, F1C, F2C, and F3C represent cooked pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively.

It is possible to observe, when comparing the wheat starch granules between raw and cooked samples, a slight reduction in their volume after cooking. However, Renoldi et al. reported a different behavior when enriching pasta with psyllium [55], as the raw samples presented smaller granules compared to the cooked ones. This difference can be explained by the fact that, in the mentioned study, the raw samples underwent a drying process, which considerably reduced their moisture content, thus affecting the structure and volume of the starch granules.

As observed in the present work, Li et al. reported that in rice noodles enriched with silkworm powder [57], the microstructure of the control pasta matrix became progressively more complex with sample enrichment. This additional microstructural complexity can interfere with the starch gelatinization process, an essential mechanism for water absorption, which may result in a firmer and softer texture in the cooked product, as well as an increase in soluble solid loss during cooking [57].

### 3.9. Results of the Texture Profile of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

Table 10 shows the texture profile (hardness, chewiness, adhesiveness, and elasticity) of pasta samples with and without the addition of *Tenebrio* powder.

Based on the data presented in Table 10, it is possible to observe that only formulation F3 showed a significant difference in hardness compared to the control sample. Nevertheless, the other enriched formulations (F1 and F2) did not present mean values significantly different from F3, which indicates a gradual and slight decrease in hardness, suggesting that the pasta structure became less rigid. This implies that the addition of insect protein and its relevant lipid content favors the softness of the pasta.

The hardness obtained (Table 10) were relatively high when compared with those reported the literature [58], those studies observed values between 1527 and 2011 gf for enriched pasta. Such a difference may be strongly influenced by factors such as the type of equipment, probe used, test speed, as well as the shape and thickness of the samples.

**Table 10.** Texture profile of pasta formulations with and without the addition of *Zophobas morio* powder.

| Pasta Formulations | Hardness (gf)                     | Chewiness (gf)                 | Adhesiveness (gs)               | Elasticity (Dimensionless) |
|--------------------|-----------------------------------|--------------------------------|---------------------------------|----------------------------|
| C                  | 12,591.04 <sup>a</sup> ± 2331.99  | 5290.86 <sup>a</sup> ± 2698.21 | −1082.41 <sup>a</sup> ± 668.72  | 0.73 <sup>a</sup> ± 0.27   |
| F1                 | 11,326.81 <sup>ab</sup> ± 1582.56 | 3542.96 <sup>ab</sup> ± 778.35 | −351.55 <sup>bcd</sup> ± 179.10 | 0.59 <sup>a</sup> ± 0.13   |
| F2                 | 11,003.01 <sup>ab</sup> ± 1023.60 | 3666.77 <sup>ab</sup> ± 743.64 | −539.92 <sup>cd</sup> ± 189.77  | 0.68 <sup>a</sup> ± 0.13   |
| F3                 | 10,058.25 <sup>b</sup> ± 2139.11  | 2934.38 <sup>b</sup> ± 1293.30 | −511.54 <sup>d</sup> ± 436.27   | 0.57 <sup>a</sup> ± 0.18   |

C, F1, F2, and F3 represent pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively. Mean values ± standard deviation ( $n = 3$ ) followed by different letters on the same column differ significantly according to Tukey's test ( $p < 0.05$ ). gf: gram-force. gs: gram-force second.

However, higher values for the same parameter were also reported by Hussein et al. [59], ranging from 25.03 to 93.80 N (2552 to 9566 gf), with the increase in pea flour, while Kumalasari et al. found values above 25,000 gf for cooked noodles produced in different extruders [60].

An increase in pasta hardness is an important attribute since it contributes to a desirable texture, as well as to the reduction in stickiness which is a characteristic preferred by consumers [61].

Chewiness is expressed as the amount of force required to break down solid foods during the mastication process [60]. This measurement is determined through a mathematical calculation based on the hardness, adhesiveness, and elasticity values.

Regarding Table 10, it can also be observed that there was a significant difference between samples C and F3. The chewiness and hardness of the samples showed similar behavior, with both values decreasing with the addition of *Zophobas morio* powder to the formulations. This behavior has been previously reported in other studies and can be explained by the positive correlation between these two parameters [62], which may be related to an increase in fiber [63] or protein derived from the enrichment of the formulations. This suggests that the enriched pasta may require less effort to chew by consumers, being perceived as easier to consume but less firm or elastic during mastication.

The adhesiveness value is measured by the work required to overcome the attractive forces between the food and the probe surface, and due to this nature, its values are expressed as negative.

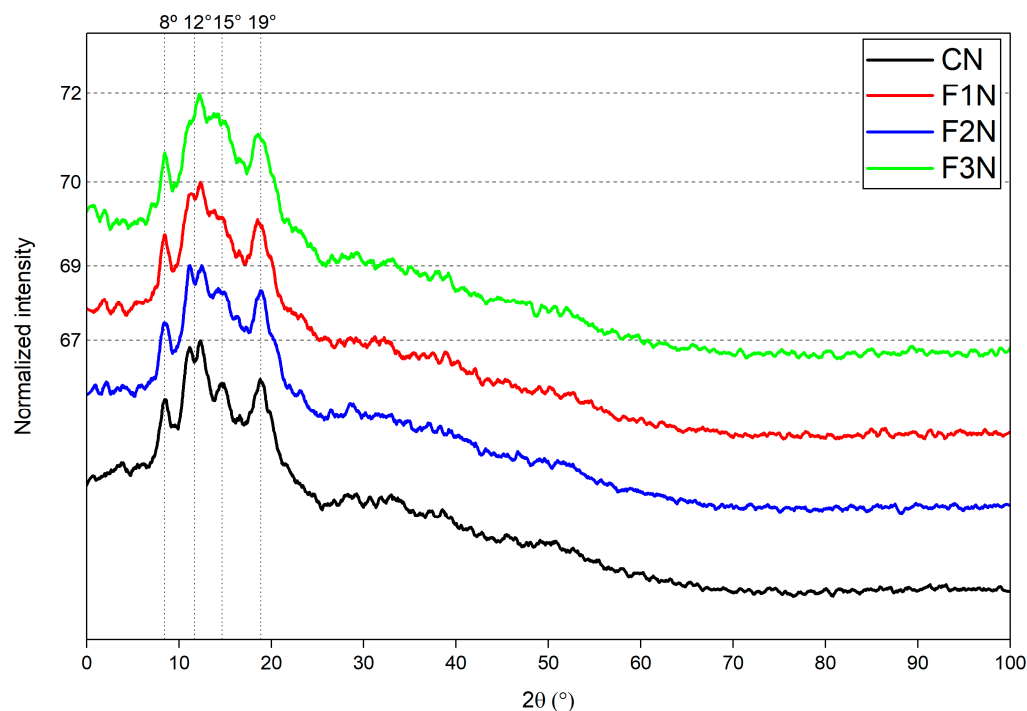
Table 10 presents the adhesiveness values of pasta samples with and without the addition of *Zophobas morio* powder. The values obtained ranged from −1082.41 to −511.54 gs.

According to Table 10, formulation C was the only one that showed adhesiveness significantly different from the other samples. Furthermore, an increase in adhesiveness was observed in the samples enriched with *Zophobas morio* powder, possibly caused by the increase in fiber content resulting from the enrichment of the samples [64], as well as by the improvement in gluten network formation through interaction with insect protein [13].

Elasticity is an attribute that provides the sensation of chewiness when the food is bitten [60]. This parameter can be measured by the ratio between the recovery times of the material after the first and second compression of the probe on the samples. According to Table 10, there were no significant differences among the formulations regarding elasticity values. This result is interesting from a marketing perspective, since, sensorially, an elasticity value similar to that of the control pasta may facilitate consumer acceptance, as they are accustomed to this standard. Additionally, the control sample showed a slightly higher value, while the formulations with insect powder showed a slight reduction. This indicates that the enriched pasta tends to be less elastic, that is, less malleable or resistant to stretching, reflecting the interference of insect protein in diluting the gluten content present in the dough.

### 3.10. Results of the X-Ray Diffraction (XRD) Analysis of Control and Enriched Pasta Formulations with *Zophobas morio* Powder

All raw pasta samples (CN, F1N, F2N, and F3N) exhibited a similar type A starch crystalline diffraction pattern [65], with the main diffraction doublet (Figure 7) at approximately  $12^\circ$  ( $2\theta$ ). An increase in the intensity of the crystalline peaks of the samples enriched with insect protein can be observed when comparing sample CN with F3N (Figure 7). This behavior was also reported by Kumar et al. when adding whey protein to oat starch [66]. One possible explanation for the increase in peak intensity may be related to the formation of hydrogen bonds between protein and starch molecules, which promote the rearrangement and crystallization of structures in a positive manner [67].

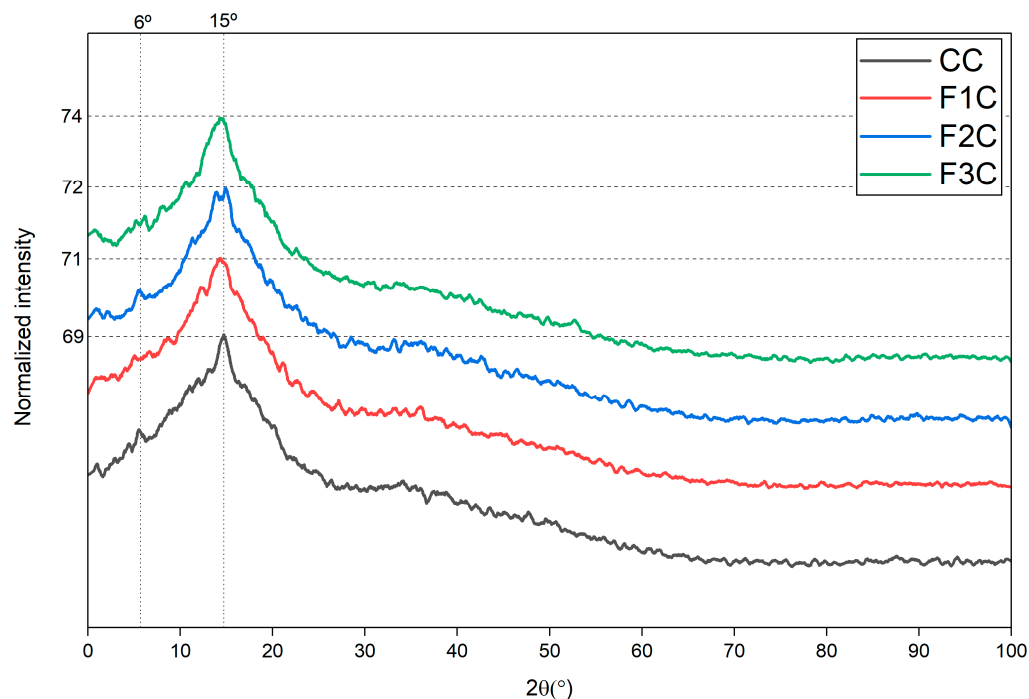


**Figure 7.** Normalized diffractogram of raw pasta formulations with and without the addition of giant mealworm (*Zophobas morio*) powder. CN, F1N, F2N, and F3N represent raw pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively.

With the addition of giant mealworm powder, a slight shift in the peaks can be observed, which may suggest small changes in the organization of starch molecules due to the presence of insect protein and lipids. It is noticeable that, as the amount of insect powder increases, the diffractogram shows a less defined peak profile, typical of a more amorphous material. This indicates that the insect powder interferes with starch crystal formation, probably due to its protein and fat content, which compete with starch for water during mixing and hinder crystalline organization.

Figure 8 shows the diffractograms of the cooked samples, and visually, it is possible to identify that the cooked control sample (CC) displays a peak with lower absolute intensity in the main region ( $\sim 15\text{--}25^\circ$   $2\theta$ ), while the formulations with *Zophobas morio* powder show progressively higher intensities ( $F3C > F2C > F1C > CC$ ). Cooking causes starch gelatinization, characterized by the disruption of the crystalline organization of the granules and the incorporation of water molecules. This change in the crystallinity of pasta dough is also associated with the exposure to high temperatures, which induces the formation of a gel-like structure composed of denatured proteins and starches that recrystallize upon cooling [68]. Therefore, it is expected that cooked samples exhibit more

attenuated signs of crystalline structure compared to raw samples, a behavior that can be observed through the transformation of the standard type A starch profile into a more amorphous profile, with a more pronounced peak at  $15^\circ$  [69]. The presence of a broad peak after cooking is consistent with the increase in the amorphous fraction resulting from gelatinization.



**Figure 8.** Normalized diffractogram of cooked pasta formulations with and without the addition of giant mealworm (*Zophobas morio*) powder. CC, F1C, F2C, and F3C represent cooked pasta dough supplemented with *Zophobas morio* powder at 0%, 7.5%, 15%, and 17.5%, respectively.

It was also possible to observe the disappearance of most of the crystallization peaks (Figure 8) that were present and well-defined in the raw pasta (Figure 7). This result was also reported by Tian et al. when subjecting pasta to different processing methods involving high temperatures [70], including cooking, where the diffractogram showed the same peak pattern as the raw sample, with subsequent preservation of the peak in the same region observed in this study.

Another aspect to highlight is the increase in peak intensity of the cooked samples (Figure 8), which, in addition to the factors previously discussed, can be attributed to the formation of starch–lipid complexes resulting from the fat introduced by the insect powder. These interactions intensify during prolonged heating and are associated with the development of V-type crystalline structures [70]. The extent of this phenomenon is strongly influenced by the defatting method used for obtaining the insect powder. Solvent-based extraction markedly reduces the lipid fraction but may also decrease the antioxidant capacity of the material, whereas mechanical pressing is less efficient at removing lipids yet preserves the natural antioxidant profile of the powder [71]. Since mechanical extraction was employed in the present study, the incorporated *Zophobas morio* powder retained a higher lipid content, which likely favored a greater formation of V-type structures, as reflected in the diffractogram of Figure 8.

From Figure 8, it is also possible to observe that the cooked samples partially followed the pattern of intensity increase already observed in the raw samples of Figure 7.

When performing X-ray diffraction on different samples of isolated *Tenebrio molitor* protein, Huang et al. concluded that the resulting diffractograms exhibited a classical

pattern typically associated with proteins that possess  $\beta$ -sheet structures [56], which are characterized by the presence of two peaks similar to those obtained in Figure 8. An interesting point to note is that, even though the control sample did not contain any addition of giant mealworm powder, its wave profile was similar to that of the enriched samples. This suggests that, after cooking and gelatinization of the starch present in the wheat flour in all samples, the heat treatment may not have been sufficient to denature either the insect or wheat proteins, thereby preserving their peaks in a similar manner.

The presence of  $\beta$ -sheet structures may be related to the difficulty encountered in determining the cooking time of the samples, since such conformations are more resistant and stable, remaining intact even after intense heating processes [72], as shown in Figure 8. This structural stability may hinder the complete gelatinization of the starch in the core of the pasta strips, compromising the reading of the expected cooking endpoint.

#### 4. Conclusions

The results of this study demonstrate that the partial replacement of wheat flour with *Zophobas morio* powder is a viable strategy to improve the nutritional and technological quality of fresh pasta. Insect powder enrichment led to a substantial increase in protein, lipid, and fiber contents, while maintaining key quality attributes such as cooking performance, elasticity, and structural integrity. The reduction in cooking loss and the formation of a more compact microstructure contributed to a softer and less adhesive texture, without compromising consumer-relevant characteristics. Although enrichment resulted in darker coloration, the visual appearance remained comparable to whole-grain pasta products widely accepted in the market. Overall, these findings highlight the potential of *Zophobas morio* powder as a sustainable and functional ingredient for pasta fortification. Future studies should focus on lipid reduction strategies, sensory evaluation, and consumer acceptance to further support the industrial application of insect-based ingredients in food products.

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#### Abbreviations

The following abbreviations are used in this manuscript:

|      |                                             |
|------|---------------------------------------------|
| UN   | United Nations                              |
| USD  | United States Dollar                        |
| CE   | Ceará                                       |
| AOAC | Association of Official Analytical Chemists |
| CV   | Caloric Value                               |
| RCF  | Relative Centrifugal Force                  |

|       |                                                     |
|-------|-----------------------------------------------------|
| RPM   | Rotations Per Minute                                |
| USA   | United States of America                            |
| IAL   | Instituto Adolf Lutz                                |
| AACC  | American Association of Cereal Chemists             |
| SEM   | Scanning Electron Microscopy                        |
| XRD   | X-Ray Diffraction                                   |
| MAPA  | Ministério da Agricultura, Pecuária e Abastecimento |
| ANOVA | Analysis of Variance                                |
| BCAA  | Brached-Chain Amino Acid                            |
| RMC   | Relative Metabolite Content                         |
| GABA  | Gamma-Aminobutyric Acid                             |
| AA    | Amino Acid                                          |

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