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RESEARCH ARTICLE

Comparative Analysis of Lipids and Proteins of *Teleogryllus emma* and *Gryllus bimaculatus* Using Mass Spectrometry

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ABSTRACT: Concerns about food sustainability have increased interest in insects for their substantial nutritional value, economic benefits, and environmental advantages. This study compares the protein and lipid profiles of Teleogryllus emma and Gryllus bimaculatus to evaluate their potential as edible insects and to investigate whether they contain components that may pose consumption-related risks. Lipid profiles were analyzed using an FT-ICR mass spectrometer and identified through cross-referencing with Lipid Maps, while protein sources were determined using Tims TOF Pro MS. FT-ICR results revealed a significant presence of phospholipids in the digestive systems of these insects. Gryllus bimaculatus exhibited a rich concentration of sphingolipids, reinforcing its value as a promising food source. Tims TOF Pro MS analysis identified two key proteins: troponin T, which was highly expressed in Gryllus bimaculatus, and Hexamerin-like protein 2, which showed greater prevalence in Teleogryllus emma. While Hexamerin-like protein 2 is associated with rhinoconjunctivitis and oropharyngeal symptoms, limited research was conducted on troponin T consumption. Overall, these findings highlight the two species as viable nutritional sources, with Gryllus bimaculatus presenting a lower consumption risk due to the absence of Hexamerin-like protein 2. This research underscores the potential of edible insects in addressing food insecurity and promoting sustainable nutrition.

KEYWORDS: Animal Sciences, Nutrition and Growth, Entomophagy, Teleogryllus emma, Gryllus bimaculatus, TOF-MS.

Introduction

Entomophagy (the practice of eating insects) is increasingly considered a sustainable alternative to traditional livestock due to its numerous benefits. Insects' availability, nutritional enrichments, and environmental benefits outperform livestock. Rooting from the growth of attention to entomophagy, this study focuses on determining the edibility of two insect species, *Gryllus bimaculatus* and *Teleogryllus emma*. These two insect species were selected for analysis because crickets are among the most widely consumed insects in entomophagy, and both *Teleogryllus emma* and *Gryllus bimaculatus* are commonly found or cultivated in the Republic of Korea, where the experiment was conducted. The edible value of the subject was assessed through two methods: protein analysis using Tims TOF Pro MS and lipid analysis using FT-ICR. These methods use mass spectroscopy to analyze and compute protein and lipid content on the specimens at a micro level. Each result was compared to discover substances showing significant differences in their content. The following substances were subjected to an in-depth literature review to further determine both positive effects and harms of consumption. This study aims to be a valuable resource for defining the compatibility of both *Gryllus bimaculatus* and *Teleogryllus emma*, not only on entomophagy, but also on insect-induced products. However, further studies should be done on this subject to finalize the compatibility.

Entomophagy - Nutritional benefits:

Food insecurity has been a constant issue in human history. With more than 2086 insect species consumed around the globe, there is no doubt that entomophagy, the practice of humans consuming insects, provides a solution to food insecurity.[1] Entomophagy has great benefits as an alternative protein source from its huge biodiversity, representing 95% of the animal kingdom, and consumption in different stages of growth: eggs, larvae, pupae, and adults.[2] A multitude of insects can be reproduced in the short term in a more compact space than traditional protein sources. *Acheta domesticus L.*, a cricket species, can lay around 1,300-1,500 eggs in 3-4 weeks, making the reproduction cycle rapid and affordable.[3]

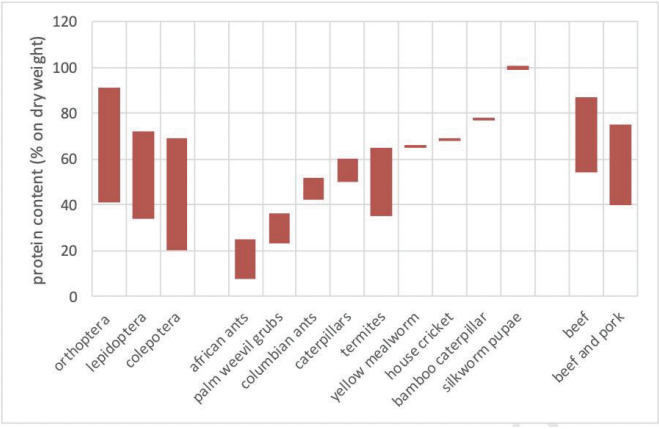

Figure 1: Protein content of edible insects compared to conventional meats (dry weight basis). Silkworm pupae and Orthoptera, including crickets, demonstrate significantly higher protein content on a dry-weight basis compared to beef and pork. This result emphasizes the nutritional value of insects as efficient alternative protein sources for human consumption.

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Figure 1 represents each insect's protein percentage on dry weight compared to that of meat house crickets, showing that insects are a great alternative protein source. Notably, silkworm pupae exhibited an exceptionally high protein content (almost 100%), while beef and pork ranged from 40 to 66%, depending on the cut.[4] In addition to protein, insects are rich in carbohydrates, fats, minerals, and vitamins, and offer a high energy value.[4] Further research on entomophagy is needed to leverage these nutritional benefits more broadly. However, comparative studies on specific insect species, such as crickets and silkworms, remain limited.[5]

Entomophagy - Environmental benefits:

Insects also place a far lower burden on the environment compared to livestock. All five insect species examined emit approximately 0.019 ± 0.002 kg CO_2 per kg per day, whereas livestock emit an average of 2.8 kg CO_2 per kg.[6,7] Livestock are responsible for emitting 7.1 Gt of CO_2 annually, accounting for roughly 11%-17% of total global emissions.[8] This high level of carbon output has made large-scale meat consumption a significant environmental concern. Emission levels skyrocketed to 37.4 billion tons, and the global average temperature increased by 1.18 degrees Celsius in 2023 alone.[9] Yellow mealworms, a common edible insect, demonstrate a lower Global Warming Potential (GWP) per unit of edible protein compared to milk, chicken, pork, and beef.[10] Additionally, entomophagy also outperforms traditional livestock in terms of land, water, and energy efficiency.[4] For example, land use for milk, chicken, pork, and beef is approximately 1.81–3.23, 2.30–2.85, 2.57–3.49, and 7.89–14.12 times higher, respectively, than that required for yellow mealworms.[10] Additionally, Oonincx found that mealworms are more drought-resistant than cattle and approximately 40% more efficient at digesting biomass.[11] Given these environmental advantages, further research on entomophagy is essential to support its broader adoption.

Methods

Cricket Sample Preparation:

10 of each species, *Teleogryllus emma* and *Gryllus bimaculatus*, were used in this study. *Teleogryllus emma* originated from a farm in Ilsan, Goyang-si, and *Gryllus bimaculatus* from Hangan-myun, Buan-gun. Upon arrival, both species were fed lettuce and water for two weeks in the process of adapting to the lab environment. The sizes of *Teleogryllus emma* ranged from 20 to 26 millimeters and *Gryllus bimaculatus* from 24 to 29 millimeters.

Lipid analysis:

Samples were prepared from 30 µm sections of Optimal Cutting Temperature (OCT) compound-embedded tissue from two cricket species: *Gryllus bimaculatus* and *Teleogryllus emma*. Tissue sectioning was performed using a Leica UV cryostat. Each sample was then sprayed with 2,5-dihydroxybenzoic (DHB) by the HTX M5 sprayer (HTX Technologies, Chapel Hill, NC). Fourier Transform Ion Cyclotron Resonance (FT-ICR): Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging was performed by the FT-ICR MS by Bruker Daltonics.

After the samples were put in the FT-ICR MS, the laser was maintained constant at an optimal setting to preserve a spatial resolution of 100 µm. Data acquisition was conducted in positive ion mode, covering a mass range of mass-to-charge (m/z) 150 to 1,500. Using the SCiLS Lab software, regions of interest (ROIs) were manually defined based on the optical images and MS data with a focus on the lipid-abundant areas of the crickets' digestive systems.

Protein Analysis:

100µg cricket samples of each species (*Gryllus bimaculatus* and *Teleogryllus emma*) were disintegrated with buffer (5% Sodium Dodecyl Sulfate (SDS), 50 mM Triethylammonium bicarbonate buffer (TEAB), pH 8.5) for complete solubilization. The samples were reduced with 5 mM tris(2-carboxyethyl) phosphine (TCEP) at 55°C for 15 minutes and alkylated with 20 mM methyl methanethiosulfonate (MMTS) at room temperature for 10 minutes (pH was adjusted to ≤1 with phosphoric acid). The protein solutions were mixed with 350µL of 100 mM TEAB in 90% methanol, then loaded into the S-Trap™ mini spin columns (ProtiFi, LLC). The columns were placed into a centrifugation machine and washed three times with the 5% buffer solution. On-column digestion was performed with trypsin (1:10 enzyme-to-protein ratio) in 50 mM TEAB at 47°C for 1–2 hours. Peptides were sequentially eluted with 50 mM TEAB, 0.2% formic acid, and 50% acetonitrile. Only the supernatant was collected from the solution. The separated peptide eluates were dried using a SpeedVac and reconstructed in an appropriate buffer solution for mass spectroscopy analysis.

The Tims TOF Pro 2 mass spectrometer was operated in the Parallel Accumulation-Serial Fragmentation (PASEF) mode using Compass Hystar. Using the Peaks Studio, the MS/MS spectra were analyzed, and the corresponding protein sequence was presented from the database.

Figure 2 below shows the simplified flowchart of the described methodology.

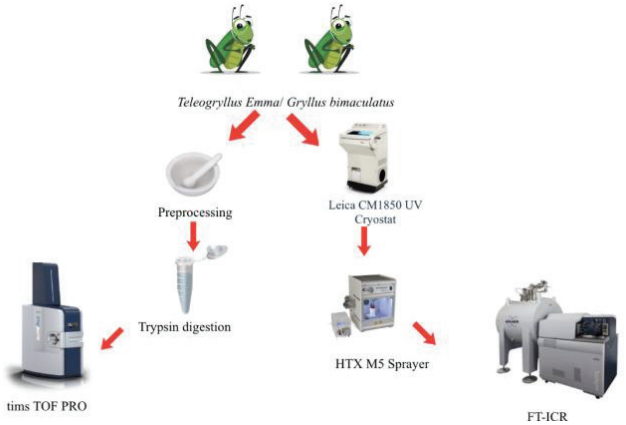
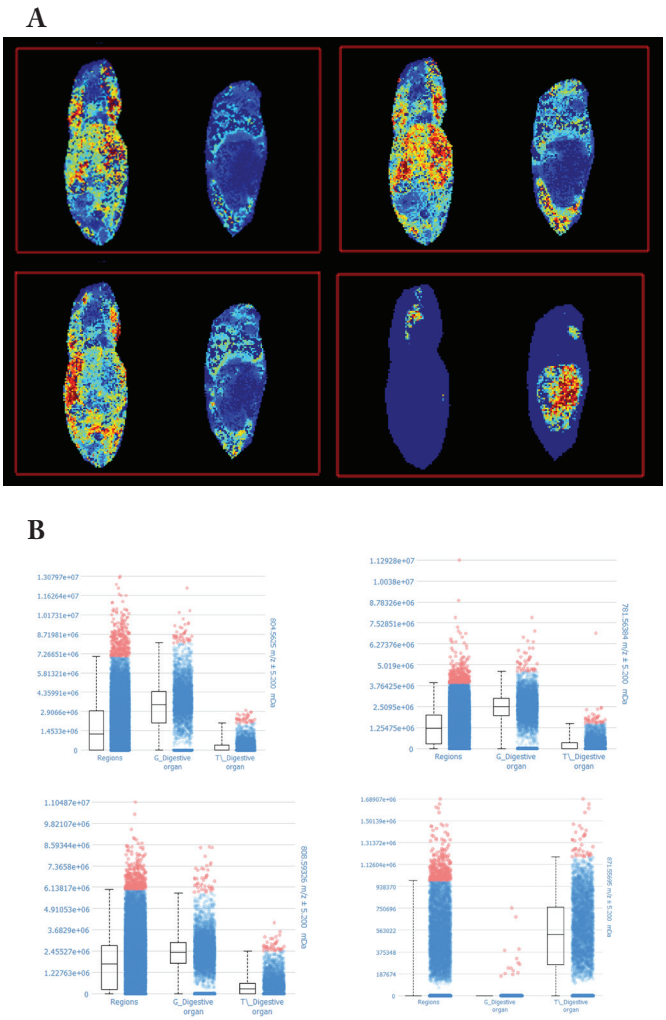


Figure 2: Experimental workflow for protein and lipid profiling of Gryllus bimaculatus and Teleogryllus emma. The diagram outlines the sample preparation procedures and analytical platforms employed in the study. Lipids were visualized using MALDI imaging coupled with FT-ICR MS, while proteins were extracted, digested, and identified via PASEF-enabled timsTOF Pro mass spectrometry.

Results and Discussion

Lipid Analysis:

Cross-sectional samples of each cricket species were prepared to observe the distribution of lipid molecules throughout the crickets' whole bodies. Four different lipid molecules of m/z values (804.5625, 781.56384, 808.59326, 871.55695) were identified and located in both species through FT-ICR mass spectrometry, enabling comparison between the two cricket species' lipid composition. The selected m/z values were entered in Lipid Maps' MS Data Bulk Search, which generated a bulk of lipid species from a list of commonly occurring acyl/alkyl chains. Identifying the adduct ions used in the ionisation process was also crucial, as it aids in differentiating and accurately annotating lipid structures. Three such ions were identified in this process: [M+H]+, [M+Na]+, [M+K]+. This analysis generated a list of 24 possible matching lipids. MALDI imaging was then performed to see the distribution of these molecules in both cricket species. Figure 3 shows that both species contained phospholipids in their digestive system, with *Gryllus bimaculatus* additionally expressing abundant sphingolipids. Common phospholipid examples include phosphatidylcholine and phosphatidylethanolamine, while sphingolipids include inositol phosphorylceramide and hexosyl ceramide.



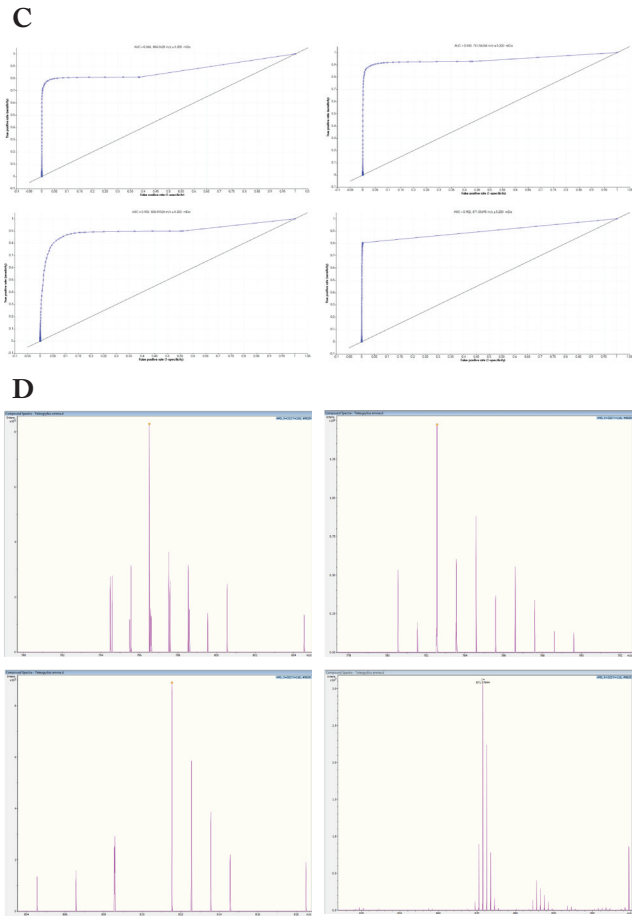


Figure 3: MALDI imaging-based lipidomic comparison between Gryllus bimaculatus and Teleogryllus emma. (A) Ion distribution maps for four selected m/z values reveal a marked enrichment of sphingolipids in the digestive tissues of G. bimaculatus, while both species exhibited phospholipid localization. (B) Box plots demonstrate significantly higher ion intensities of specific lipid-related m/z signals in G. bimaculatus than in T. emma. (C) Area Under the Curve (AUC) analysis confirms the statistical relevance of the observed spectral differences. (D) Peak annotation supports the identification of key lipid species, including phosphatidylcholine, phosphatidylethanolamine, inositol phosphorylceramide, and hexosyl ceramide.

Figure 3A shows significantly higher concentrations of molecules with m/z values of 796.53214, 782.57741, and 811.55813 in the digestive organs of *Gryllus bimaculatus*, marked by warm colors (yellow and red). In contrast, *Teleogryllus emma* shows minimal presence of these molecules, predominantly represented by blue. This distribution suggests a species-specific difference in lipid localization within the digestive system. However, the m/z 871.57944 molecules are observed in high concentrations, not in *Gryllus bimaculatus*, but in *Teleogryllus emma*. This also suggests possible species-specific metabolic or physiological differences that favor a higher concentration of m/z 871.57944 molecules in *Teleogryllus emma*.

Based on this observation, Figure 3B focuses on the digestive organs to enable a numerical comparison. The second and third bars represent the concentration of m/z values in the digestive organs of each cricket species. Figures 1-3 show a notable abundance of m/z values 804.5625, 781.56384, and 808.59326 in *Gryllus bimaculatus*' digestive organs. *Gryllus bimaculatus*, with over 75% statistical significance compared to

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that of *Teleogryllus emma*, demonstrates higher lipid content in the digestive organs. In comparison, *Teleogryllus emma* has a compressed range in Figures 1-3, reflecting its scarce amount of m/z 804.5625, 781.56384, and 808.5932 molecules. Again, Figure 3 shows the abundance of m/z 871.55695 molecules in the digestive organs of *Teleogryllus emma*.

Figure 3C shows the AUC (Area Under the Curve) graph generated through MALDI Imaging, which evaluates the mass distribution of ionized substances. The curve helps distinguish significant m/z data points from less meaningful ones after the ionized molecules are processed through the mass spectrometer. Data points where the curve approaches 1 indicate greater significance in the mass distribution.

In Figure 3D, these significant m/z values are plotted as peaks. The height of each peak corresponds to the relative abundance of specific masses: the tallest peaks represent the most abundant ionized substances. Four distinctive lipids were chosen from corresponding peaks: phosphatidylcholine, phosphatidylethanolamine, inositol phosphorylceramide, and hexosyl ceramide. Among these, the first two are phospholipids common to both cricket species, and the latter are sphingolipids absent in *Teleogryllus emma*.

Protein Analysis:

The extracted solution from the crickets' ground whole bodies was analyzed using Tims-TOF Pro to see abundant proteins.

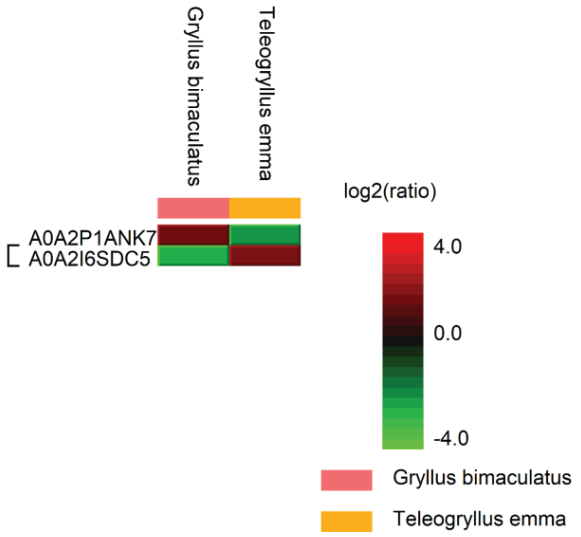

Figure 4: Comparative protein abundance profiles in Gryllus bimaculatus and Teleogryllus emma. Heat map visualization of MS-based proteomic data shows differential expression of key proteins between the two species. Notably, troponin T is highly expressed in G. bimaculatus, whereas Hexamerin-like protein 2 is predominantly found in T. emma, indicating potential species-specific differences relevant to nutritional and allergenic assessments.

Figure 4 compares the expression levels of specific genes or proteins between the two species using log2 ratio values. The heatmap highlights the differential expression patterns of each gene depending on the species. Among the analyzed proteins, troponin T (A0A2P1ANK7) and Hexamerin-like protein 2 (A0A2I6SDC5) displayed the most pronounced difference in their log2 ratio values between the species, with a log2 ratio close to +4 and -4, respectively.

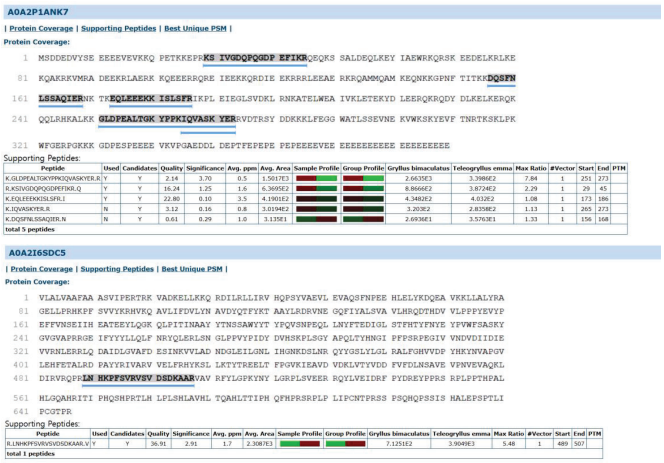

Figure 5: Peptide sequence confirmation of species-specific proteins identified by timsTOF Pro MS. (A) In Gryllus bimaculatus, five unique peptides matching troponin T were identified with high mass accuracy (e.g., 'EQLEEKK ISLSFR', 'GLDPEALTGK YPPKIQVASK YER'), supporting robust identification. (B) In Teleogryllus emma, the peptide 'LN HKPFSVRVSV DSDKAAR' was uniquely assigned to Hexamerin-like protein 2 with high confidence (mass deviation: 36.91 ppm), suggesting its role as a potential allergenic marker.

The possible peptide sequences obtained from Tims TOF Pro were compared to an existing genomics database. According to the protein.html report, approximately 295 proteins were found to have similar peptide sequences. Among these, troponin T (A0A2P1ANK7) was distinctive in *Gryllus bimaculatus*, and Hexamerin-like protein 2 (A0A2I6SDC5) was distinctive in *Teleogryllus emma*. In Figure 5(A), representative peptide sequences of troponin T (A0A2P1ANK7) include 'GLDPEALTGK YPPKIQVASK YER', among others, with a total of 5 matched peptides. Notably, the peptide 'EQLEEKK ISLSFR' showed a relatively high matching quality of 22.8 ppm, while the other peptides exhibited excellent matching quality, ranging between 0.6 and 3.7 ppm. In the case of Hexamerin-like protein 2 (A0A2I6SDC5), one matching peptide sequence, 'LN HKPFSVRVSV DSDKAAR', was identified with a strong matching quality of 36.91 ppm, as shown in Figure 5(B).

Though troponin T is well-known as a serum biomarker for cardiac examinations, little research has been done regarding troponin T consumption. Further experimentation involving animal models would be helpful to assess whether the consumption of *Gryllus Bimaculatus* is physiologically and toxicologically safe. This could include long-term feeding studies to evaluate physiological changes in cardiac and muscle tissues. Hexamerin-like protein 2, on the other hand, can lead to allergic reactions varying from rhinoconjunctivitis to oropharyngeal among consumption. Due to its allergenic potential, Hexamerin-like protein 2 appears less promising than troponin T for industrial purposes.

These factors combined make troponin T and Hexamerin-like protein 2 noteworthy for further analysis or studies.

Conclusion

The lipid analysis revealed that both *Teleogryllus emma* and *Gryllus bimaculatus* contain abundant phospholipids. However, sphingolipids were notably prevalent only in *Gryllus bimaculatus*. Sphingolipids play essential roles in signal transduction, membrane stability, and cell recognition.

Protein analysis identified two distinct proteins: troponin T, highly expressed in *Gryllus bimaculatus*, and Hexamerin-like protein 2, predominantly found in *Teleogryllus emma*. Troponin T is well known for its role in human cardiac function evaluation, yet research on its consumption as food remains limited. In contrast, De Las Marinas *et al.* reported that Hexamerin-like protein 2 may trigger allergic reactions, ranging from rhinoconjunctivitis to oropharyngeal symptoms.

In conclusion, comparative analysis of the two cricket species indicates that *Teleogryllus emma* presents lower consumption risks due to the absence of Hexamerin-like protein 2 in *Gryllus bimaculatus*. Further research, particularly into the dietary effects of troponin T, would provide a more comprehensive understanding of the nutritional viability of these species as sustainable food sources.

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