Final PhD Research Report:

General Information:

Name: Solomon Nkaka
Address: Unteres Tor 12, 95445 Bayreuth
Email: solomon.nkaka@uni-bayreuth.de

Department/Institution:
Chair of Nutritional Biochemistry,
Faculty of Life Sciences: Food, Nutrition and Health
University of Bayreuth.

Supervisors:
Prof. Dr. Janin Henkel-Oberländer
Dr. Brit Maren-Schjeide
Prof. Dr. Gerald Lackner

PhD Project Title:
Protein for the Future: Assessing the Nutritional, Quality and Safety Aspects of Alternative Protein Sources for Human Consumption.

Total Funding/reporting Period:
October 1, 2023, to September 30, 2025 (24 months)

Funding Organization:
Heinrich-Stockmeyer-Stiftung

Table of Content:

Abstract 2
Background And Introduction 2
Aims and Objectives 3
Materials And Methods 3
Sample Collection and Preparation 3
Insect Samples 3
Reference Food Samples 3
Commercial Insect Products 4
Protein Quantification Methodologies 4
Kjeldahl Nitrogen Analysis 4
Total Amino Acid Analysis 5
Protein Quality Assessment 5
Enzymatic Digestion Methods 5
INFOGEST Static In Vitro Digestion 5
Gel Electrophoresis Analysis 6
DNA-Based Authentication 6
DNA Extraction 6
Genetic Marker Identification 7
Detection Limit Determination 7
Results And Discussion 8
Phase 1: Protein Quantification Studies 8
Crude Nitrogen and Protein Estimation 8
Chitin Interference and Its Impact on Protein Quantification 9
Phase 2: Amino Acid Profiles and Nutritional Quality 9
Comprehensive Amino Acid Composition 9
Protein Digestibility Assessment 10
Phase 3: DNA-Based Authentication Method Development 12
Primer Specificity and Species Discrimination 12
Authentication of Commercial Products 12
General Conclusions 13
Project Successes and Positive Developments 14
Acknowledgement: 14
Appendix 15
References: 20

Abstract

The global demand for sustainable protein sources is increasing amid population growth and environmental pressures from conventional animal agriculture. Edible insects offer a viable alternative, boasting high nutritional value, low resource requirements, and reduced greenhouse gas emissions although their integration into the mainstream diet remains a challenge. This PhD research addresses key barriers to the integration of edible insects into food systems by investigating protein quantification accuracy, nutritional quality, and authentication methods for four major insect species i.e., house cricket (*Acheta domesticus*), migratory locust (*Locusta migratoria*), mealworm (*Tenebrio molitor*), and black soldier fly (*Hermetia illucens*). Analytical methods used in this thesis included Kjeldahl nitrogen analysis, chitin quantification, total amino acid profiling via HPLC and in vitro digestibility assays (including the INFOGEST protocol) among others. Results showed that conventional nitrogen-to-protein conversion factors (e.g., 6.25) overestimate insect protein by 10 – 20% due to non-protein nitrogen from chitin (0.37 – 0.76% of dry weight). Nonetheless, insect contained a considerable amount of crude protein (30 – 65% on dry weight basis) even after accounting for chitin nitrogen. Insect proteins exhibited competitive essential amino acid profiles (e.g., high in lysine and valine) but were limited in methionine (0.8 – 1.2 g/100g protein). In vitro digestibility ranged from 85-95%, comparable to animal proteins like chicken (96%) and superior to plants like peas (61%). Furthermore, the developed DNA based method for the detection of insect samples achieved 100% specificity and detected insect DNA at 0.001% in mixtures, enabling fraud detection in processed products.

These findings provide insights in protein quantification, validation of insect protein quality for human nutrition, and provide robust authentication tools, advancing edible insects toward mainstream adoption.

Keywords: Edible insects, protein quantification, chitin correction, amino acid profiling, in vitro digestibility, qPCR authentication, sustainable proteins.

Background And Introduction

Global food systems face several sustainability challenges. By 2050, the world population is estimated to reach over 9.7 billion, requiring a 70% increase in food production (Michiel van Dijk et al. 2021). Conventional animal protein production contributes 14.5% of greenhouse gas emissions and occupies 77% of agricultural land while providing only 18% of global calorie supply (Bahar et al. 2020). This unsustainable pattern necessitates exploration of alternative protein sources. Edible insects represent a promising sustainable protein source. Insects require significantly less feed (12 times less than cattle for equivalent protein production) and emit 80-100 times fewer greenhouse (A. Van Huis and F. V. Dunkel 2017). Additionally, insects can be reared on organic waste streams, thus contributing to circular economy principles (Anna Bordiean et al. 2022). Despite these advantages, several critical knowledge gaps hinder the integration of edible insects into mainstream food systems.

First, protein quantification in insect-based foods lacks standardization. Current methods rely predominantly on the Kjeldahl technique, which measures total nitrogen and converts it to protein using a universal conversion factor (N-P factor) of 6.25, derived from animal proteins assuming 16% nitrogen content (Hsiaoling Wang et al. 2016). However, insects contain substantial non-protein nitrogen (NPN) from chitin (the major component of their exoskeleton),

nucleic acids, and other nitrogen-containing compounds. Chitin content alone can represent 5-15% of insect dry weight depending on species and developmental stage (Samy Boulos et al. 2020). Consequently, conventional protein quantification methods systematically overestimate true protein content which may be misleading consumers and regulatory authorities.

Second, comprehensive protein quality assessment of edible insects remains under explored. While some studies have reported amino acid profiles, systematic comparative analyses with conventional protein sources using standardized digestibility methods are limited. Protein quality depends not only on amino acid composition but also on digestibility and bioavailability, which can be affected by chitin and other anti-nutritional factors.

Third, the emerging edible insect market faces authenticity and food fraud vulnerabilities. As commercial value increases, economic incentives for adulteration also grow. Unlike established animal protein supply chains with DNA-based traceability systems, edible insect products lack validated authentication methods. This gap creates risks for both economic fraud (substitution with cheaper proteins) and food safety (undeclared allergens, unapproved species). The development of species-specific detection methods is essential for market transparency and consumer protection.

Aims and Objectives

The overarching aim of this PhD research project was to evaluate the potential of edible insects as sustainable protein sources and develop analytical methods for quality assessment and authentication.

Specific objectives:

1. To accurately quantify protein content in edible insects, accounting for NPN interferences, and derive species-specific conversion factors for comparison with conventional foods.
2. To assess protein quality through amino acid profiling and in vitro digestibility, determining nutritional equivalence to traditional proteins.
3. To develop and validate DNA-based methods for species-specific detection in processed products to ensure authenticity and prevent fraud.

Materials And Methods

Sample Collection and Preparation

Insect Samples

Four commercially important edible insect species were selected for investigation based on market availability and regulatory approval status in European markets. These included House cricket (*Acheta domesticus*), Migratory locust (*Locusta migratoria*), mealworm (*Tenebrio molitor*) from 2 different sources (designated BT and PT), and black soldier fly (*Hermetia illucens*) from 2 different sources (illucens and Amazon).

Reference Food Samples

Fifteen common food products representing diverse protein sources were analysed for comparative assessment. These included beef, chicken breast, whey protein isolate, fish, tofu, pea protein, protein bars, protein-enriched cracker, bread, and various marketed high-protein products. All reference samples were obtained from local retail markets in Germany. Samples

were prepared according to their physical state i.e., solid foods were milled, powdered products and fresh samples were analysed/used as received.

Commercial Insect Products

Commercial edible insect products for authentication testing were purchased from Entosus GmbH (Bremen, Germany). All products were labelled as containing house cricket (*Acheta domesticus*) as the insect ingredient. Products included whole dried crickets, cricket powder, and processed formulations containing cricket flour as an ingredient.

Protein Quantification Methodologies

Kjeldahl Nitrogen Analysis

Total nitrogen content was determined using the classical Kjeldahl method following the method established by C. Gerhardt Analytical Solutions with slight modifications. Briefly, approximately 0.25 – 1 g of sample was weighed in Nitrogen-free weighing paper. Samples were transferred in the Kjeldahl digestion glass tube. Depending on the protein concentration in the sample, 10 – 20 ml of Concentrated Sulphuric acid (98%) and Kjeldahl catalyst mixture were added. Digestion was performed using a Kjeldahl digestion system at 420°C for 1 hour with suction set on through the Scrubber Unit, VACUSOG. Following digestion and cooling, samples were diluted with deionized water and transferred to a Kjeldahl distillation apparatus. Sodium hydroxide solution (40%, 50 mL) was added to convert ammonium ions to ammonia gas. Ammonia was steam-distilled into boric acid solution (4%, 50 mL) containing mixed indicator (bromocresol green and methyl red). The absorbed ammonia was quantified by titration with standardized hydrochloric acid (0.1 N) to the endpoint indicated by colour change from green to pink. Blank determinations using reagents without sample were performed in parallel.

Calculation: Total nitrogen percentage was calculated using the formula:

$$\% \text{ Nitrogen} = ((V_{sample} - V_{blank}) \times N_{HCl} \times 14.007) / (M_{sample})$$

Where V represents titration volume (mL), NHCl is hydrochloric acid normality, 14.007 is the atomic weight of nitrogen, and Msample is sample mass (g).

Crude protein content was initially calculated using the conventional factor:

$$\text{Crude Protein (\%)} = \%N \times 6.25$$

All determinations were performed in triplicate (n=3 technical replicates per sample).

Chitin Quantification

Chitin content was determined using an adapted fibre bag methodology based on alkali treatment protocol. Briefly, approximately 1 g of sample was weighed in a fibre bag and placed in glass spacers. Samples were defatted 2 – 3 times in 100 ml Acetone. Defatted samples were treated with 0.25 M sodium hydroxide solution at 90°C for 1 hours to solubilize and remove proteins. Samples were then washed 2 – 3 times in double distilled water. The nitrogen content was then measured using the Kjeldahl method as described above.

Calculation: Total chitin content was calculated using the formula:
Chitin (%) = (V* Ceq, sol * *t* * 20,319)/Msample

Where V represents titration volume (mL), Ceq, sol represents the equivalent concentration of the standard solution, *t* represents titre of the standard solution, 20,319 is the factor for recalculating the chitin content and Msample is the sample weight (g).

Total Amino Acid Analysis

Complete liberation of protein-bound amino acids was achieved through acid hydrolysis following established protocols. Briefly, 10 mg of sample was weighed into hydrolysis tubes to which 10 mL of Hydrochloric acid (6 M) was added, and tubes were purged with nitrogen gas for 30 seconds. The tubes were sealed and heated at 110°C for 18 - 24 hours. Following hydrolysis and cooling, samples were filtered through 0.45 µm membrane filters to remove particulates and prepared for analysis.

HPLC Analysis: Amino acids were quantified by reversed-phase high-performance liquid chromatography (HPLC) with fluorescence detection following pre-column derivatization. The derivatization reagent o-phthalaldehyde (OPA) reacts with primary amino groups in the presence of a thiol (3-mercaptopropionic acid) to form fluorescent isoindole derivatives. The chromatographic conditions are listed in appendix table 5.

Calibration and Quantification: External calibration was performed using amino acid standard solutions (Sigma-Aldrich) containing all proteinogenic amino acids at concentrations ranging from 10-1000 pmol/µL. Calibration curves were constructed by plotting peak area against amino acid concentration. Sample amino acid concentrations were determined by interpolation from calibration curves. Results were expressed as mg amino acid/g dry sample weight. **Note:** Asparagine, Cystine and Tryptophan are destroyed under high temperatures and acidic conditions.

Protein Quality Assessment

Enzymatic Digestion Methods

Pronase Digestion: Pronase (protease from *Streptomyces griseus*, Sigma-Aldrich) provided broad specificity protease activity. Samples (10 mg) were suspended in 1ml of 100 mM ammonium bicarbonate buffer, 20 µL of 4 mg/mL trypsin was added and digested by microwave (10 min, 50 W, temp. 50°C). following digestion, 100 µL of 4 mg/mL Pronase working solution was added and incubated at 37°C for 20 hours. Following digestion, enzymes were inactivated by heating at 95°C for 10 minutes. Samples were centrifuged (10,000 × g, 10 minutes), and supernatants were collected for amino acid analysis using the HPLC method.

INFOGEST Static In Vitro Digestion

The standardized INFOGEST digestion protocol was implemented to simulate human gastrointestinal conditions following (André Brodkorb et al. 2019) with adaptations for insect matrices. Briefly, samples (0.25 g) were mixed with 4 ml of simulated salivary fluid (SSF) containing 0.5 ml α-amylase (1500 U/mL final activity) at pH 7.0. The mixture was incubated at 37°C for 2 minutes with continuous mixing to simulate mastication. The oral bolus was mixed with 4.75 ml simulated gastric fluid (SGF) containing 1 ml of pepsin (20000 U/mL final activity). pH was adjusted to 3.0 using 1 M HCl to simulate gastric conditions. The mixture was incubated at 37°C for 2 hours with continuous orbital shaking (200 rpm) to simulate gastric

motility. The gastric chyme was mixed with 12.5 ml of simulated intestinal fluid (SIF) containing pancreatin (4x USP-Grade, protease activity = 100 U/mg) and 20µL of 0.3M $CaCl_2$. pH was adjusted to 7.0 using 1 M NaOH to simulate duodenal conditions. The mixture was incubated at 37°C for 2 hours with continuous orbital shaking. Following intestinal digestion, samples were heated at 95°C for 10 minutes to inactivate enzymes and halt digestion. Samples were centrifuged (5000 × g, 10 minutes, 4°C), and supernatants containing solubilized digestion products were collected for further analysis.

Digestibility Quantification: Protein digestibility was calculated by comparing amino acid content in digested samples versus total amino acids:

Digestibility (%) = (AAdigested / AAtotal) × 100

Where AAdigested represents amino acids quantified in the supernatant following digestion, and AAtotal represents amino acids from complete acid hydrolysis.

Gel Electrophoresis Analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to visualize protein profiles and assess digestion progression. Samples at different stages of digestion were solubilized in SDS-PAGE sample buffer containing 2-mercaptoethanol while considering the dilution factors and heated at 95°C for 5 minutes. Samples were then loaded onto precast 12% polyacrylamide gels alongside molecular weight markers (10-250 kDa range). Electrophoresis was performed at a voltage of 80V for 30 minutes and then increased to 100V for 40 minutes or until the staining reached the bottom of the gel. Gels were stained with Coomassie Brilliant Blue R-250 for 30 minutes, then destained overnight in methanol/acetic acid/water solution until clear background was obtained. Gel images were captured using a gel documentation system (ChemiDoc, Bio-Rad) and analysed using ImageLab 6.1 software for band intensity quantification.

Digestibility Assessment: Protein hydrolysis was assessed by i) disappearance of high molecular weight bands (>50 kDa), ii) changes in the moderate molecular weight bands (between 50 – 18 kDa) and iii) appearance of low molecular weight bands (<10 kDa)

Hydrolysis (%) = (1 - (Band Intensity digested / Band Intensity undigested)) × 100

DNA-Based Authentication

DNA Extraction

Approximately 20 mg of the sample was mixed 300 µl of solution A (containing 0.1 M Tris-HCl, pH 9.0, 0.1 M EDTA, and 1% SDS) and vortex briefly. The mixture was incubated on a heating block at 70 °C for 20 – 30 minutes before adding 42 µl of 8 M Potassium Acetate (14 µl for each 100 µl of solution A). The mixture was then incubated on ice for 30 minutes and thereafter centrifuged at 4 °C for 15 minutes at 10,000 g. DNA was precipitated by adding 0.5 volumes of Isopropanol at room temperature and centrifuged at room temperature for 5 minutes at 10,000 g. The pellet was carefully washed with 70 % ethanol, re-centrifuged, dried and redissolved in 10 – 100 µl of TE buffer (TE buffer contains Tris-HCl (10mM) pH 8 and EDTA (1mM) pH 8). The gDNA concentration (ng/ µl) and purity (A260/A280 and A260/A230) was measured using NanoDrop (Thermo Fisher Scientific).

Genetic Marker Identification

Sequence Retrieval: Mitochondrial (cytochrome c oxidase subunit I - COI, 16S ribosomal RNA) and nuclear (18S ribosomal RNA, internal transcribed spacer - ITS) gene sequences were retrieved from NCBI GenBank database for all target species and related taxa. Sequences were aligned using UGENE software to identify species-specific sequence variations. Regions showing high intraspecific conservation, but interspecific divergence were targeted for primer design.

Primer Design Strategy: Candidate primers were designed using Primer3Plus tool considering the following criteria: i) Primer length: 18-25 nucleotides, ii) Melting temperature (Tm): 58-62°C (maximum 2°C difference between primer pairs) iii) GC content: 40 – 60%, iv) Amplicon size: 80-150 bp, and v) Avoidance of secondary structures, primer dimers, 3' G/C clamps

Primer Synthesis: All selected primers were synthesized by Metabion International AG (Planegg, Germany). Primers were reconstituted in nuclease free water to 100 µM stock concentration and stored at -20°C. Working solutions (10 µM) were prepared for routine use.

Primer Screening and Validation

Initial Screening: 10 – 20 candidate primer pairs per species were screened systematically. qPCR was performed first to verify amplification: i) Template: 9 ng genomic DNA, ii) Reaction volume: 10 µL, iii) Cycling: 95°C 3 min; 38 cycles (95°C 15 s, 60°C 15 s, 72°C 30 s). PCR products were analysed by agarose gel electrophoresis (2%) with SYBR safe staining to verify product size and absence of non-specific amplification.

Specificity Testing: Each primer pair was tested against i) Target species DNA (positive control, n=3 replicates) ii) All non-target insect species DNA (cross-reactivity testing, n=3 each), ii) DNA from 10 common food sources (beef, chicken, fish, soy, wheat, milk, egg, corn, rice, peanut) iii) No template control (NTC, nuclease-free water)

Primer pairs were considered specific if they showed: i) positive amplification (Ct < 35) for target species only, ii) No amplification (No Ct or Ct > 38) for non-target samples, iii) Correct amplicon size by gel electrophoresis

Detection Limit Determination

Sample Preparation: Mixtures were prepared containing known quantities of insect material mixed in common food matrices (e.g., bread). Serial dilutions represented insect content from 10% down to 0.001% by weight. gDNA was extracted from all dilutions as described earlier and analysed by qPCR using validated primer pairs. Each dilution was analysed in triplicate. The limit of detection (LOD) was defined as the lowest concentration showing positive amplification in all three replicates with Ct values < 35.

Application to Commercial Products

Food products containing edible insects were purchased from Entosus GmbH, Bremen, Germany and analysed to demonstrate method applicability. gDNA extraction was performed in triplicate for each product. qPCR was conducted using species-specific primers alongside: Positive control (pure insect DNA), No template control (NTC, nuclease-free water) and Universal eukaryotic primers (18S rRNA) as internal control to verify DNA quality

Results And Discussion

Phase 1: Protein Quantification Studies

Crude Nitrogen and Protein Estimation

The Kjeldahl nitrogen analysis showed considerable variation in total nitrogen content across insect species and reference food samples (Table 1). House cricket exhibited a high crude nitrogen content (11.02%), followed by migratory locust (9.08%), mealworms (6.78 – 7.30%), and black soldier fly samples (5.04 – 6.11%). When converted to crude protein using the conventional factor of 6.25, these values translated to protein contents ranging from 30% to 70 % across insect species.

Table 1. Total nitrogen and crude protein content of insect and reference food samples.

Samples	Crude Nitrogen (%)	Crude Protein (mg/100mg) *	Declared Protein content (%)	Chitin Nitrogen (%)	Estimated Protein Conversion Factor (Kp)
House Cricket	11.02	68.88 ± 0.36	-	0.76 ± 0.04	6.63
Migratory Locust	9.08	56.77 ± 0.43	-	0.59 ± 0.05	5.95
Black Soldier Fly-illucens	5.04	31.49 ± 0.41	-	0.38 ± 0.01	5.21
Black Soldier Fly-Amazon	6.11	38.18 ± 0.23	-	0.38 ± 0.02	N. D
Mealworm BT	7.30	45.64 ± 0.98	-	0.37 ± 0.07	7.04
Mealworm PT	6.78	42.35 ±0.93	-	0.56 ± 0.01	7.16
Tortilla chips	4.06	25.34 ± 1.18	33.5		
Vegan Kebab	4.24	26.49 ± 0.48	28.0		
Vegan Protein Bar	4.07	25.45 ± 0.37	26.0		
High protein Cracker	3.21	20.07 ± 0.43	21.0		
Tofu	2.68	16.76 ± 0.11	15.4		
Soy Granules	7.83	49.72 ± 0.16	50.0		
Bread (normal)	1.22	7.65 ± 0.47	7.6		
High Protein Bread	3.33	20.83 ± 0.43	23.3		
Vanilla Pudding	0.52	3.27 ± 0.10	3.0		
High Protein Vanilla Pudding	1.64	10.27 ± 0.14	10.0		
Protein bar	4.84	30.24 ± 0.10	30.0		
Whey protein	11.90	74.39 ± 0.35	75.0		
Semolina Pudding	1.34	8.39 ± 0.18	8.0		
Chocolate Pudding (Normal)	0.54	3.35 ± 0.10	3.0		
High protein Chocolate Pudding	1.61	10.09 ± 0.15	10.0		

*Calculated as %N × 6.25; Values are mean ± SD (n=3)

The crude protein content determinations for common food samples showed excellent agreement with declared values on product packaging (mean difference <5%), validating the analytical methodology. This alignment demonstrates that conventional Kjeldahl-based protein

quantification provides accurate results for traditional food matrices where the 6.25 conversion factor is appropriate.

Using the protein conversion factor of 6.25, the crude protein values position insect samples competitively within high protein food categories. House cricket (68.88%) and migratory locust (56.77%) showed protein contents comparable to protein isolates, while mealworms (42 – 46%) and black soldier fly (31 – 38%) showed moderate to high protein levels. These findings align with literature reports indicating orthopterans (crickets, locusts) typically contain 50 – 75% protein, while coleopterans and dipterans range from 40 – 60% (Rumpold and Schlüter 2013). However, the critical question remains whether these crude protein values accurately reflect true nutritional protein content, or rather increased due to non-protein nitrogen (NPN) from chitin and other sources.

Chitin Interference and Its Impact on Protein Quantification

Chitin quantification showed species specific variations with important implications for protein accuracy. Nitrogen derived from chitin ranged from 0.37% to 0.76% across species, with house cricket showing the highest chitin nitrogen content (0.76%), followed by migratory locust (0.59%), mealworms (0.37 – 0.56%), and black soldier fly (0.38%) (table 1). When the chitin nitrogen was subtracted from total nitrogen before applying the conversion factor, adjusted protein estimates would decrease by 4.8 – 7.5 percentage points. For house cricket, this represents a reduction from 68.88% to approximately 64%, while for black soldier fly, the reduction would be from 31.49% to approximately 29% and the migratory locust would reduce from 56.77% to approximately 53%. Interestingly, the two mealworm samples from different sources (BT and PT) showed different chitin nitrogen contents (0.37% vs. 0.56%), a 51% relative difference. This substantial intraspecific variation reflects differences in rearing conditions, diet composition and developmental timing. Such variability challenges the concept of universal species-specific conversion factors and reflects the need for standardized rearing protocols if regulatory frameworks mandate species specific Kp values.

Janssen et al. (Janssen et al. 2017) demonstrated that failing to account for chitin-N leads to protein overestimations of 8 – 15% in *T. molitor* and *H. illucens* consistent with the findings in this research where other insect species were included.

Phase 2: Amino Acid Profiles and Nutritional Quality

Comprehensive Amino Acid Composition

Complete amino acid profiling revealed that all four insect species contain balanced profiles with all essential amino acids present (Table 2, detailed data in Appendix Table 6). Four different extraction methods were compared: complete acid digestion (6M HCl), enzymatic digestion (Pronase), simulated gastrointestinal digestion (INFOGEST), and free amino acids.

Table 2. Summary of Essential Amino Acid Content (mg/g sample) - Acid Digestion Method

Amino Acid	House Cricket	Migratory Locust	Black Soldier Fly	Mealworm BT	Mealworm PT
Essential Amino Acids					
Arginine	54.29	35.67	15.40	32.45	29.15

Amino Acid	House Cricket	Migratory Locust	Black Soldier Fly	Mealworm BT	Mealworm PT
Histidine	15.83	12.61	7.08	15.79	15.43
Isoleucine	32.42	27.15	14.08	27.07	26.70
Leucine	66.38	50.78	20.98	45.60	44.04
Lysine	42.49	27.84	15.94	33.11	28.46
Methionine	12.26	7.10	3.92	5.45	5.16
Phenylalanine	25.40	19.54	11.24	22.11	21.57
Threonine	21.38	16.96	9.48	18.95	18.76
Tryptophan *	0.21	0.16	0.20	0.21	0.18
Valine	39.14	34.75	17.35	32.34	33.32
Total AA**	680.25	504.93	242.75	487.45	444.88

*Tryptophan is a thermolabile amino acid and will be degraded at high temperatures; **Total AA includes both essential and non-essential amino acids measured.

The amino acid data revealed that all insect species contain high lysine levels (27.84 – 42.49 mg/g), representing 5.5 – 6.8 g/100g protein when normalized. This substantially exceeds WHO requirements (4.5 g/100g protein) (WHO 2007) and compares favourably to animal proteins. The high lysine content represents a major advantage over cereal proteins, which are characteristically lysine deficient (Appendix figure 4). This complementarity suggests strategic applications in cereal-based food systems where insects could address the limiting amino acid. Secondly, Branched Chain Amino Acids (BCAAs) such as Leucine, isoleucine, and valine collectively represented 18 – 22% of total amino acids across insect species. House cricket showed particularly high BCAA content (leucine 66.38 mg/g, isoleucine 32.42 mg/g and valine 39.14 mg/g). These values position insect proteins favourably for sports nutrition applications, as BCAAs are critical for muscle protein synthesis with leucine known to activates mTOR signalling pathways (Xu et al. 2025). Thirdly, all insect species showed low methionine content (3.92 – 12.26 mg/g), representing only 0.8 – 1.8 g/100g protein. This falls below or marginally meets WHO recommendations (2.3 g/100g protein for methionine + cysteine combined) (WHO 2007). Methionine represents the clear limiting amino acid in insect proteins implying strategic blending with sources rich in methionine such as eggs, dairy and fish to enhance nutrient wholeness. Interestingly, the methionine limitation in insects and parallel patterns in many plant proteins, suggests that insects occupy an intermediate nutritional position between plant and animal proteins, a finding that could facilitate their positioning as "bridging" proteins in dietary transitions (appendix figure 4).

Protein Digestibility Assessment

Amino Acid Recovery Comparison:

Four methods assessed in this study provided complementary perspectives on protein accessibility and digestibility; Acid hydrolysis (6M HCl) represented a complete amino acid recovery assuming 100% reference point, Pronase digestion achieved 65 – 92% of acid hydrolysis values, while INFOGEST Simulated Digestion achieved 45 – 68% of acid hydrolysis

values and free amino acids representing between 4 – 10% of total amino acids. (appendix table 6). These results show that insect proteins have an excellent susceptibility to proteolytic enzymes. Furthermore, free amino acid pools (3 – 11%) suggests active metabolism or high turnover rates across all insect species. It should also be noted that mealworms showed intermediate digestibility with notable variation between the two sources (BT vs PT), again highlighting the impact of rearing conditions on final product characteristics.

Gel Electrophoresis Visualization

SDS-PAGE analysis provided visual confirmation of protein hydrolysis during INFOGEST simulated digestion. Results generally showed a shift in protein bands from high molecular weight band to moderate and lower molecular weight band as digestion processed from oral, gastric and intestinal phases (figure 1).

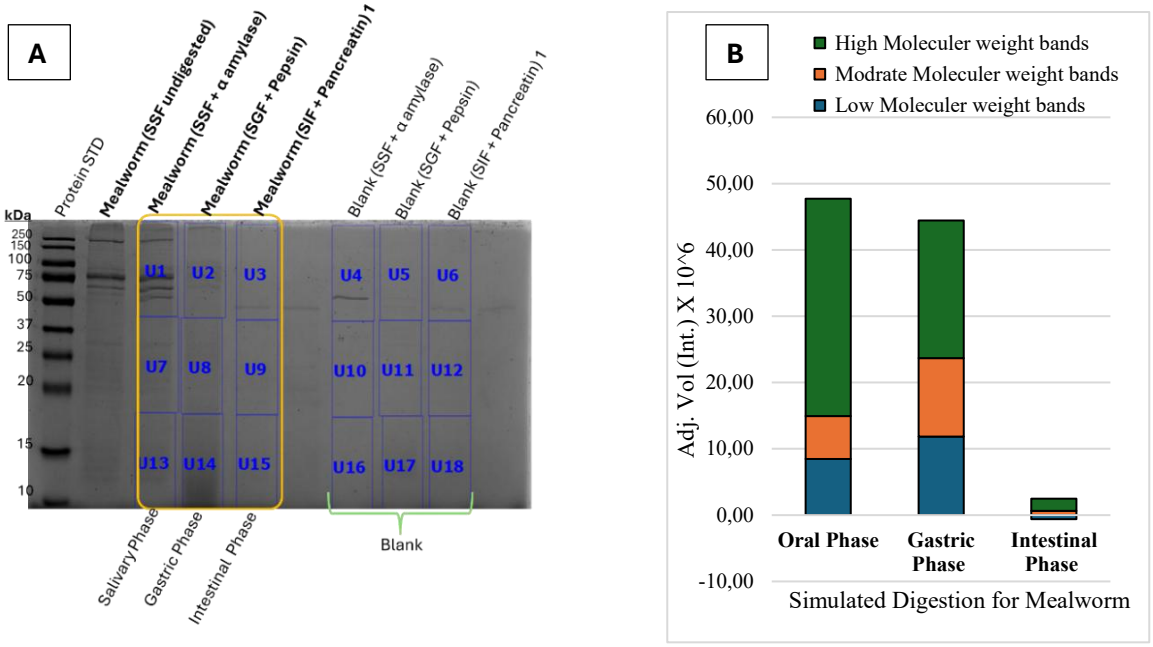


Figure 1. Gel electrophoresis of mealworm sample showing different stages of digestion i.e., oral, gastric and intestinal phases (**A**) and a graph showing the distribution the molecular weight bands at different digestion stages (**B**).

Insect species generally displayed characteristic protein band patterns in the range 250 – 10kDa, High molecular weight bands (250 – 150 kDa) likely myosin heavy chains and hexamerins which are common insect storage proteins. Moderate molecular weight bands (150 – 50kDa) representing various enzymes, structural protein and actin. Low molecular weight bands (50 – 15kDa) representing smaller functional proteins (Barre et al. 2021). Post gastric digestion resulted in reduction of high molecular weight bands (>100 kDa) and accumulation of mostly low molecular weight bands (10 – 50 kDa). Post intestinal digestion resulted in a near complete disappearance of distinct bands into diffuse smearing in <10 kDa region indicating extensive peptide fragmentation (figure 1). Quantitative analysis showed that insect samples had 85 – 96% reduction in band intensity indicating a high digestibility of insect protein in simulated gastrointestinal conditions.

Phase 3: DNA-Based Authentication Method Development

Primer Specificity and Species Discrimination

The species-specific qPCR primers developed for the four target insect species demonstrated high specificity when tested against a comprehensive panel of common food samples and non-target insect species (Table 3).

Table 3. Real time PCR results for species specific primers tested against food and insect samples

Sample	18SE Universal	House Cricket – primer2	Migratory Locust – Primer1	Mealworm-Primer17	Black Soldier Fly-Primer2
Reference Foods (n=10)					
High Protein Semolina Pudding	18.88 ± 0.06	No Ct	No Ct	No Ct	No Ct
Protein Bar	16.33 ± 0.16	No Ct	No Ct	No Ct	No Ct
Soy Granulate	17.44 ± 0.16	No Ct	No Ct	No Ct	No Ct
Tofu	17.21 ± 0.12	No Ct	No Ct	No Ct	No Ct
Whey Protein	19.87 ± 0.16	No Ct	No Ct	No Ct	No Ct
Normal Bread	17.57 ± 0.19	No Ct	No Ct	No Ct	No Ct
High Protein Cracker	15.27 ± 0.21	No Ct	No Ct	No Ct	No Ct
High Protein Bread	16.34 ± 0.15	No Ct	No Ct	No Ct	No Ct
Beef	16.21 ± 0.19	No Ct	No Ct	No Ct	No Ct
High Protein Tortilla chips	17.80 ± 0.21	No Ct	No Ct	No Ct	No Ct
Insect Species (n=4)					
House Cricket	24.10 ± 0.31	25.12 ± 0.40	No Ct	No Ct	No Ct
Migratory Locust	18.95 ± 0.18	No Ct	27.51 ± 0.14	No Ct	No Ct
Mealworm	21.41 ± 0.20	No Ct	No Ct	22.84 ± 0.10	No Ct
Black Soldier Fly	20.93 ± 0.11	No Ct	No Ct	No Ct	23.35 ± 0.26

No Ct = No amplification detected (Ct > 40); Values are mean ± SD (n=3)

The results demonstrate 100% species specificity with zero false positives across all tested samples. Each species-specific primer pair amplified only its intended target species with no cross reactivity among the four insect species tested. This finding is crucial because these four species represent the most commercially important edible insects currently available in European markets. The ability to clearly distinguish among them addresses regulatory requirements under EU Novel Food Regulation 2015/2283, which mandates species-specific authorization (EU-Novel Food 2015).

All ten common food samples showed no amplification with any insect specific primers (No Ct), despite containing diverse protein sources including animal proteins (beef, whey), plant proteins (soy, wheat), and processed formulations. This confirms that the designed primers target insect specific genetic sequences without cross reactivity to conventional food DNA thus demonstrating high specificity. The 18S rRNA universal eukaryotic primer (18 SEU) successfully amplified DNA from all samples, producing Ct values ranging from 15 – 24. This internal control serves as confirmation that the DNA extraction was successful and amplifiable as well as ruling out possibilities of PCR inhibitors that might cause false negative.

Authentication of Commercial Products

The developed primers were applied to commercial products containing insectsamples to demonstrate real-world applicability (Table 4).

Table 4. Authentication of Commercial Insect Products obtained from Entosus GmbH.

Samples Obtained from Entosus*	Primer for 18 SEU	House Cricket P2	Mealworm P17	Migratory Locust P1	Black Soldier Fly P2
Grillen Streichwurst Bauern Art	15.56 ± 0.23	31.22 ± 0.11	No Ct	No Ct	No Ct
Grillen Protein Snack BBQ	13.94 ± 0.26	32.49 ± 0.72	No Ct	No Ct	No Ct
Grillen Protein Snack kakao	16.24 ± 0.68	32.86 ± 0.87	No Ct	No Ct	No Ct
Geröstete Grillen - salz	23.97 ± 0.07	26.56 ± 0.01	No Ct	No Ct	No Ct
Grillen Protein Riegel kakao	18.08 ± 0.30	27.62 ± 0.11	No Ct	No Ct	No Ct
Grillen Protein Riegel beere mandel	17.03 ± 0.33	26.82 ± 0.29	No Ct	No Ct	No Ct
House Cricket – positive control	24.40 ± 0.21	24.79 ± 0.34			

* - All products labelled as containing house cricket; No Ct = No amplification detected (Ct > 40); Values are mean ± SD (n=3)

Commercial food products labelled as containing house cricket (*Acheta domesticus*) showed positive amplification with cricket specific primers (Ct values 26 – 33), confirming species identity. No cross amplification with primers for other species occurred, verifying that products contained only the declared species without contamination or adulteration but further confirming specificity of selected primer. The Ct values for commercial products (26.56 – 32.86) were 2-8 cycles higher than the positive control pure cricket sample (24.79), indicating DNA degradation during processing. This shift is expected and provides insights into processing impact on DNA integrity. Whole roasted crickets (Geröstete Grillen) showed a minimal degradation during processing showing a Ct value of 26.56 which was closer to the Ct value of the pure unprocessed house cricket. Protein bars and snacks represented moderate degradation during processing showing a Ct value between 27 – 33. Protein snacks and Spreads showed more extensive degradation during processing. Processing methods such as heating, grinding, emulsification among others degrade DNA to certain extents. Despite thermal treatment, drying, milling, and formulation with other ingredients, cricket DNA remained detectable.

General Conclusions

As the edible insect market continues to rapidly grow, nutritional analysis as well as validated authentication methods become increasingly critical. This comprehensive doctoral research provides essential analytical foundations for the evidence-based integration of edible insects into sustainable food systems by addressing three critical knowledge gaps: protein quantification accuracy, nutritional quality assessment, and species authentication. Through systematic investigation of four commercially important insect species, this work establishes that insects contain substantial amount of protein (44 – 60% dry weight when accurately measured), exhibit exceptional digestibility (85 – 96% comparable to conventional animal proteins), and can be reliably authenticated using DNA based methods (100% specificity, 0.001% detection limit). By establishing chitin corrected quantification approaches, comprehensive digestibility data using standardized INFOGEST protocols, and validated qPCR authentication methods, this research provides the analytical infrastructure enabling responsible growth of the insect food sector.

Future research will prioritize product development, physiochemical analyses and consumer studies.

Project Successes and Positive Developments

Poster Presentation: Successfully presented a poster entitled "Comparative analysis of protein and lipids in Insects" at the PhD poster presentation session at the University of Bayreuth, campus Kulmbach on the 02.07.2024. Attached in the appendix figure 2 is the poster presented at this event.

Student Training Opportunities: Successfully supervised one master's and one bachelor's students whose theses directly contributed to project. Both students developed and improved laboratory skills in food analysis.

Acknowledgement:

This PhD project was kindly financed by the Heinrich-Stockmeyer-Stiftung.

Appendix

Table 5. Chromatographic conditions for HPLC analysis

System	Agilent 1200 Series HPLC
Column	Reversed-phase C18 column (250 × 4.6 mm, 5 µm particle size)
Mobile phase	Gradient elution using (A) sodium acetate buffer (20 mM, pH 7.2) and (B) acetonitrile/methanol/water (45:45:10, v/v/v)
Flow rate	1.0 mL/min
Detection	Fluorescence (excitation 340 nm, emission 450 nm)
Injection volume	20 µL

Comparative Analysis of Protein and Lipids in Insects.

Solomon Nkaka [a], Brit-Maren Schjeide [a] and Janin Henkel-Oberländer [a]

[a] *University of Bayreuth, Faculty of Life Sciences: Food, Nutrition and Health, Nutritional Biochemistry, Kulmbach, Germany*

UNIVERSITÄT BAYREUTH
LIFE SCIENCES Food · Nutrition · Health

INTRODUCTION

The global population is projected to reach approximately 9.7 billion by 2050, necessitating a profound shift in food production and consumption strategies[1]. Conventional food production systems, such as livestock, face significant ecological, ethical, and efficiency challenges, prompting the exploration of sustainable alternatives [2].

Edible insects emerge as promising sustainable sources of essential nutrients due to their low environmental impact, high resource efficiency, and competitive nutritional profiles[3]. The feed-to-body mass conversion rate for insects is highly efficient, often requiring significantly less feed, water, and land compared to traditional livestock [2].

Despite their potential, the widespread adoption of insects as a mainstream food source is hindered by critical knowledge gaps, such as the lack of standardized methodologies for assessing their nutrient content as well as the distribution of essential micro- and macronutrients.

This study aims to assess the total protein and lipid content, as well as the composition of amino acids and fatty acids in selected insect samples, highlighting how diet affects nutrient yield.

MATERIALS AND METHODS

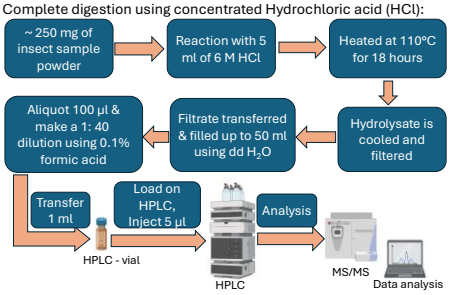
Samples

House cricket, migratory locust, black soldier fly, mealworm larvae fed on oats, muesli, nuts and seeds and mealworm larvae fed on rice.

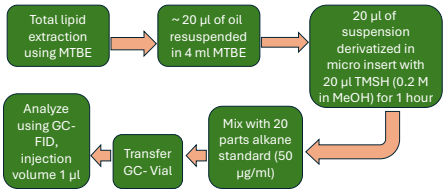
Figure 1. *Edible insect samples that are already approved as novel foods in the European Union (as of 2023), these include House Cricket (**A**), Migratory Locust (**B**), and Mealworm Larvae (**C**) among others.*

Amino Acid Determination.

Complete digestion using concentrated Hydrochloric acid (HCl):



Fatty Acid Determination



GC-FID instrument details and settings

GC	Shimadzu 2025
Column	DB-23, 60 m x 0.25 mm
Film thickness	0.25 µm
Detector	FID
Temperature (injector)	Split/splitless, 250 °C
Temperature (detector)	250 °C
Injection amount	1 µl
Flow	H_2 1.87 ml/min
Temperature Programm	100 °C , 0 min At 5 °C/min to 240 °C, 7 min

RESULTS

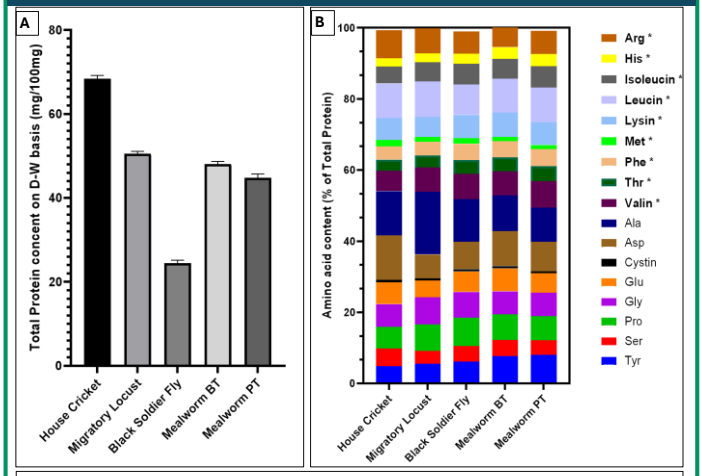


Figure 2. *Total protein concentration of selected insects (a) based on Dry Weight (D-W) and the distribution of amino acid content as a percentage of total protein (b). Amino acids marked with a "*****" and bold are essential amino acids. **NB**: Thermolabile amino acids such as Tryptophan (Trp) and Glutamine (Gln) were not measured in this experiment. **Mealworm BT** are fed on oats, muesli, nuts and seeds and **Mealworm PT** are fed on rice.*

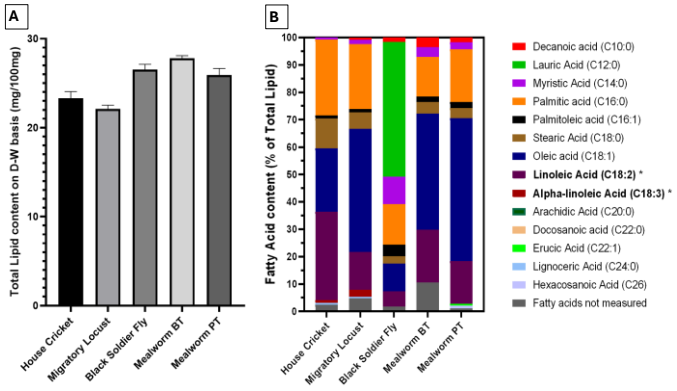


Figure 3. *Total lipid concentration of selected insects (a) and the distribution of fatty acid content as a percentage of total fatty acids (b). Fatty acids marked with a "*****" and bold are essential fatty acids. . **Mealworm BT** are fed on oats, muesli, nuts and seeds and **Mealworm PT** are fed on rice.*

CONCLUSION

This study supports the potential use of insects in addressing global food security and environmental sustainability. Insects could be a source of essential nutrients such as amino acids and fatty acids that enhance the human diet. The findings indicate that the choice of feeding components affects nutrient yield in insect samples, which is an important consideration for optimizing nutrient yield in insect farming.

To build on these findings, further research is necessary to optimize insect diets, standardize nutrient analysis, evaluate health impacts, address allergenic potential, and develop appealing insect-based foods to enhance consumer acceptance. Additionally, economic feasibility studies will support the integration of edible insects into mainstream food systems, promoting a sustainable and nutritious future.

REFERENCES

1. van Dijk, M., Morley, T., Rau, M. L., &Saghai, Y. (2021). A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. https://doi.org/10.1038/s43016-021-00322-9

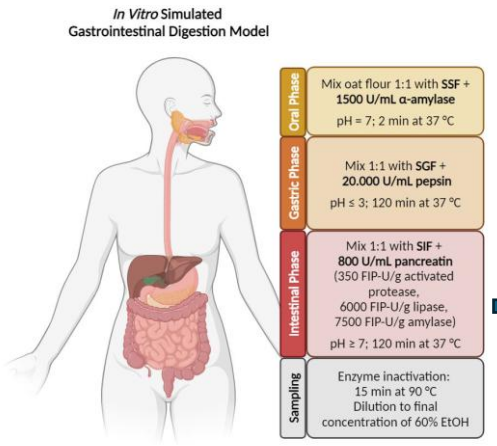
2. van Huis, A., & Oonincx, D. G. A. B. (2017). The environmental sustainability of insects as food and feed. https://doi.org/10.1007/S13593-017-0452-8

3. Lange, K. W., & Nakamura, Y. (2023). Potential contribution of edible insects to sustainable consumption and production. https://doi.org/10.3389/frsus.2023.1112950

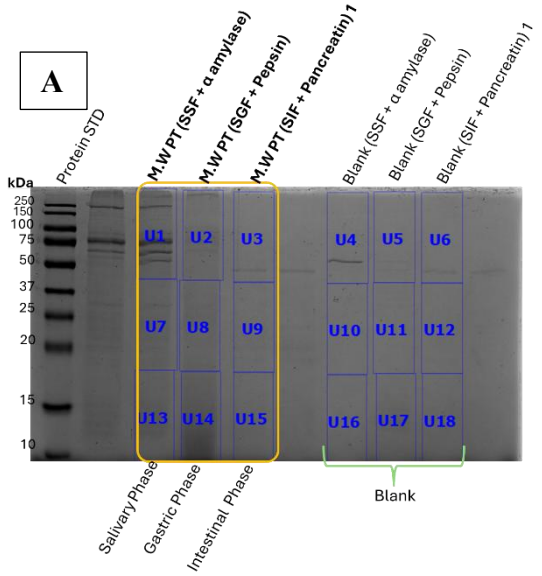
ACKNOWLEDGEMENT

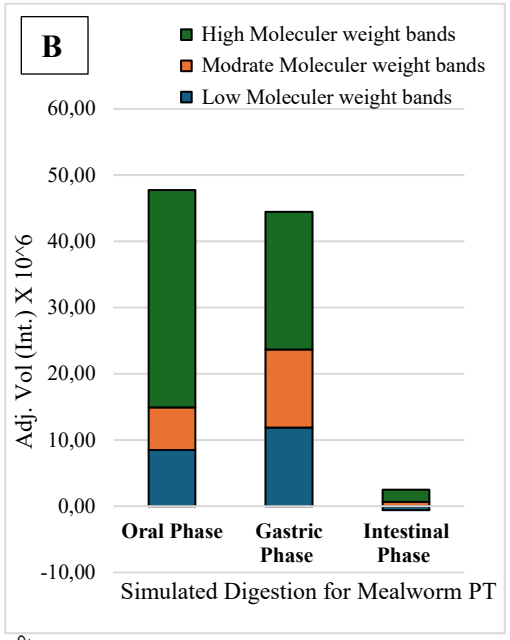
- This doctoral project was supported by the Heinrich-Stockmeyer-Stiftung.
- Special thanks to Prof. Dr. Harshadrai Rawel, University of Potsdam.

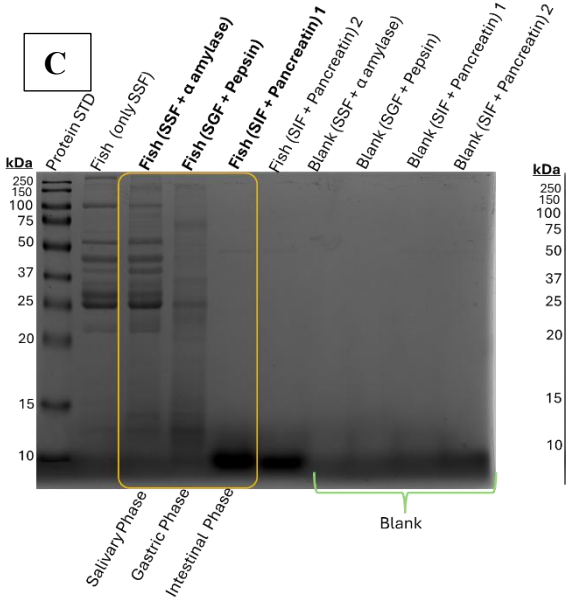
Figure 2. Poster Presented at the PhD poster presentation, university of Bayreuth, campus Kulmbach on 02.07.2024.

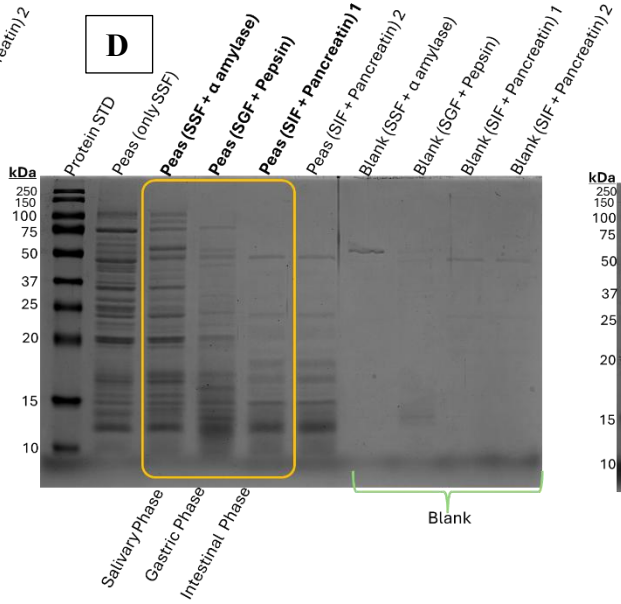


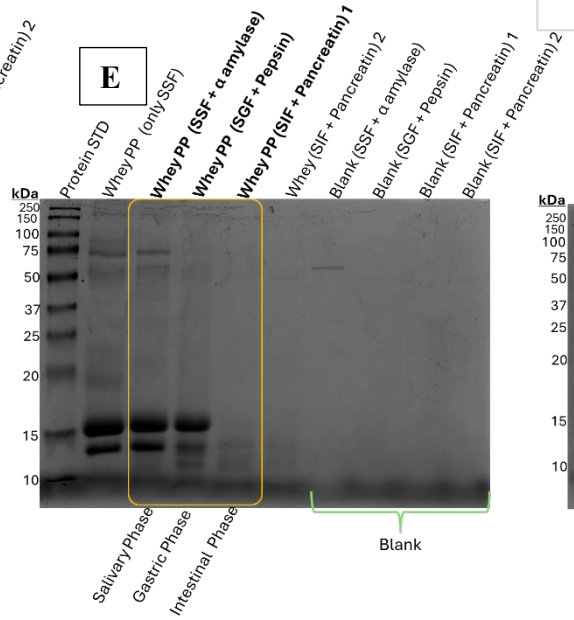
In Vitro Simulated Digestion Protocol – INFOGEST model.











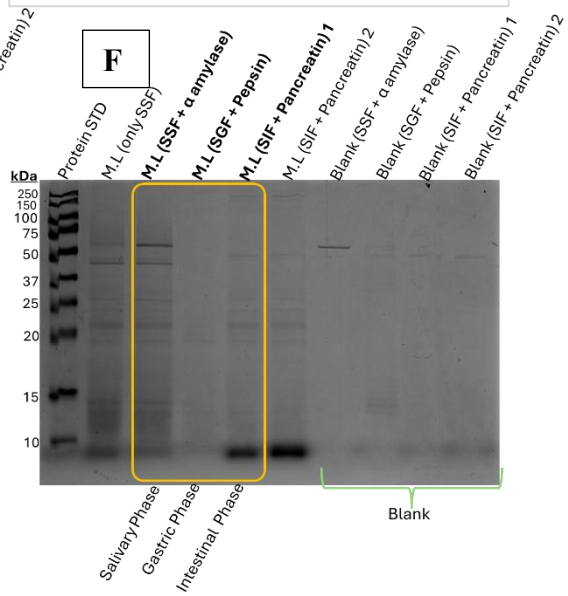


Figure 3. Gel electrophoresis analysis of the Mealworm sample (**A**) at different stages of simulated digestion (Oral, Gastric, and Intestinal phases) including corresponding blanks containing only the enzymes in the appropriate digestion fluids. The Gel image of mealworm sample represents steps taken to analyse protein band patterns and digestibility calculation depending on their intensity using Image Lab software. A graphical representation of protein digestibility for the Mealworm sample based on band intensity (**B**). Gel electrophoresis images showing simulated digestion patterns of Fish (**C**), Peas (**D**), Whey Protein Powder (**E**), and Migratory Locust (**F**).

Table 6. Amino acid content of House cricket, Migratory Locust, Black soldier fly and Mealworm samples measured after extraction using 6M HCl acid, stimulated gastrointestinal digestion, Enzymatic digestion i.e., Pronase, and the free Amino acids.

	Acid Digestion					Pronase-Digestion					Simulated-Digestion					Free Amino acids				
	House Cricket (mg/g)	Migratory Locust (mg/g)	Black Soldier Fly (mg/g)	Mealworm – BT (mg/g)	Mealworm – PT (mg/g)	House Cricket (mg/g)	Migratory Locust (mg/g)	Black Soldier Fly (mg/g)	Mealworm – BT (mg/g)	Mealworm – PT (mg/g)	House Cricket (mg/g)	Migratory Locust (mg/g)	Black Soldier Fly (mg/g)	Mealworm – BT (mg/g)	Mealworm – PT (mg/g)	House Cricket (mg/g)	Migratory Locust (mg/g)	Black Soldier Fly (mg/g)	Mealworm – BT (mg/g)	Mealworm – PT (mg/g)
Indispensable Amino Acids																				
Arg	54.29	35.67	15.40	32.45	29.15	32.23	27.45	5.25	21.69	16.3	31.62	25.67	13.59	22.78	20.05	4.52	6.72	1.97	4.5	3.41
His	15.83	12.61	7.08	15.79	15.43	8.94	14.35	2.47	10.68	6.19	3.13	3.13	2.72	4.85	4.36	0.76	0.97	0.71	1.74	1.35
Isoleucine	32.42	27.15	14.08	27.07	26.7	15.06	27.73	3.22	14.32	6.83	3.92	5.05	2.89	4.55	3.39	0.29	1.22	0.38	1.3	0.87
Leucin	66.38	50.78	20.98	45.6	44.04	30.48	46.94	6.84	23.61	19.36	16.71	17.36	7.54	12.5	12.58	0.29	3.54	0.35	0.64	0.65
Lysin	42.49	27.84	15.94	33.11	28.46	24.27	34.76	4.87	17.66	9.81	18.35	19.63	10.43	16.76	14	0.91	7.97	0.67	1.08	1.16
Met	12.26	7.1	3.92	5.45	5.16	6.88	10.04	1.97	5.98	0.34	3.34	3.4	1.28	1.93	1.47	0.16	1.35	0.06	0.17	0.13
Phe	25.4	19.54	11.24	22.11	21.57	12.95	19.83	5.12	13.47	12.54	11.1	10.73	6.07	10.63	10.19	0.18	5.03	0.31	1.54	1.3
Thr	21.38	16.96	9.48	18.95	18.76	10.04	15.45	3.96	10.55	9.79	8.6	8.4	4.56	7.96	7.94	0.17	5.03	0.30	1.45	1.25
Trp	0.21	0.16	0.20	0.21	0.18	5.52	7.7	2.41	6.04	4.96	3.86	3.77	3.21	4.32	4.44	0.22	1.06	0.55	2.44	3.5
Valin	39.14	34.75	17.35	32.34	33.32	20.53	39.76	6.21	20.8	11.2	4.07	5.53	3.04	4.93	4.7	0.47	1.14	0.74	1.91	1.92
Dispensable amino acids																				
Ala	85.08	88.69	29.37	48.24	43.21	29.45	64.82	16.95	28.84	17.57	4.01	5.92	5.02	4.1	2.6	1.55	2.89	5.20	3.8	2.57
Asp	85.75	34.25	18.79	47.14	37.25	3.82	7.41	0.87	3.34	1.39	0.75	1.11	0.89	0.59	2.2	0.4	0.53	1.34	0.11	0.09
Cysteine						21.36	54.29	6.03	12.66	5.53	1.87	0.67	4.21	0.53	1.89	0.83	1.58	7.52	1.44	1.72
Cystin	4.9	2.83	1.70	3.35	2.09	1.78	2.86	0.38	1.04	0.61	0.3	0.25	0.43	0.44	0.46	0.05	0.05	0.00	0.02	0
Glu	41.67	23.81	13.99	30.76	24.63	8.01	13.35	2.09	7.71	3.73	3.69	2.83	1.59	2.61	2.19	1.35	0.65	0.51	1.43	1.01
Gly	43.78	38.7	17.83	31.14	29.6	5.85	14.11	2.48	5.39	1.8	2.98	2.83	0.82	1.21	1.1	2.02	2.76	0.32	0.31	0.39
Pro	41.73	37.28	19.44	34.55	30.24	4.95	9.44	3.35	14.08	11.25	4.85	4.65	3.94	10.79	7.45	2.86	3.28	3.07	6.2	5.04
Ser	33.66	18.45	10.67	22.04	18.46	8.9	11.85	1.43	7.99	3.64	1.76	2.44	1.19	1.25	1.03	0.27	0.42	0.17	0.21	0.24
Tyr	33.5	28.04	15.03	36.84	36.16	14.97	27.49	7.95	23.53	17.5	13.05	10.29	10.46	12.61	15.42	0.72	2.43	0.89	4.4	2.39
Asn	0.38	0.32	0.26	0.31	0.47	6.34	6.87	0.95	3.47	1.02	0.75	0.73	0.41	0.7	0.25	0.06	0.04	0.03	0	0.01
Gln	0	0	0.00	0	0	7.31	11.34	0.80	4.92	3.05	3.43	4.75	2.88	4.18	4.12	2.23	1.93	1.88	2.31	2.37
Total Amino Acid (mg/g)	**680.25**	**504.93**	**242.75**	**487.45**	**444.88**	**279.64**	**467.84**	**85.60**	**257.77**	**164.41**	**142.14**	**139.14**	**87.18**	**130.22**	**121.83**	**20.31**	**50.59**	**26.97**	**37.00**	**31.37**

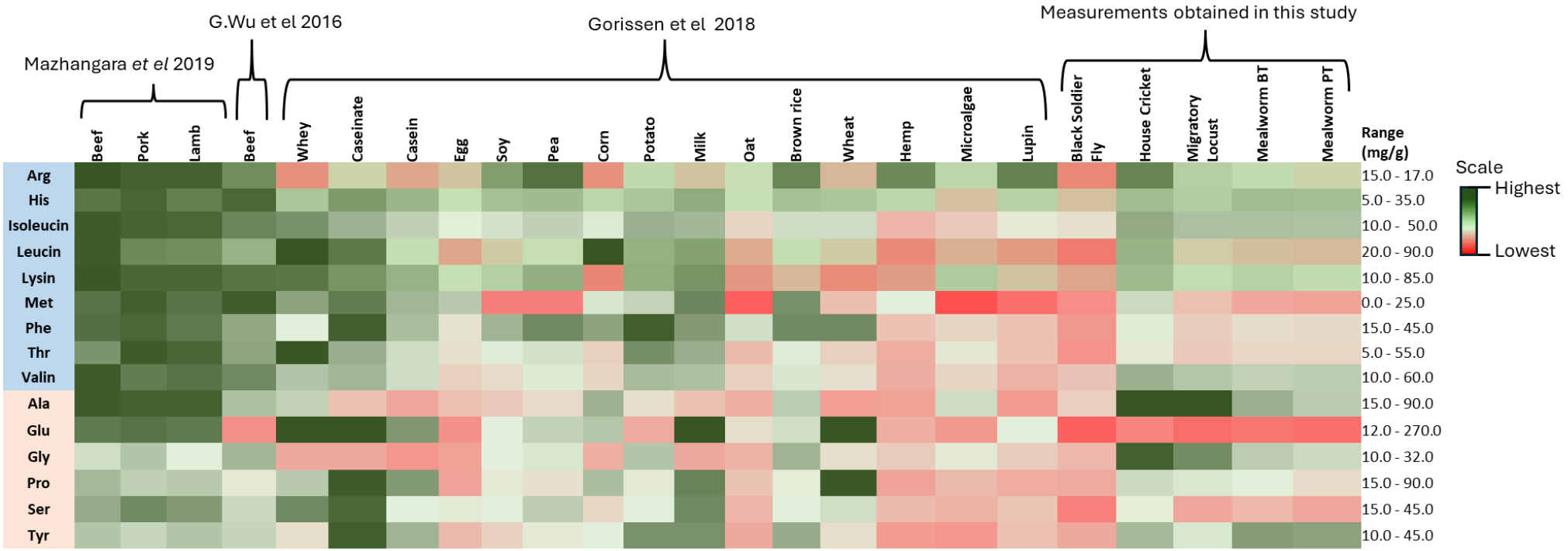


Figure 4. Gel electrophoresis analysis of the Mealworm sample (A) at different stages of simulated digestion (Oral, Gastric, and Intestinal phases) including corresponding blanks containing only the enzymes in the appropriate digestion fluids. The Gel image of mealworm sample represents steps taken to analyse protein band patterns and digestibility calculation depending on their intensity using Image Lab software. A graphical representation of protein digestibility for the Mealworm sample based on band intensity (B). Gel electrophoresis images showing simulated digestion patterns of Fish (C), Peas (D), Whey Protein Powder (E), and Migratory Locust (F).

References:

A. Van Huis; F. V. Dunkel (2017): Edible Insects: A Neglected and Promising Food Source. In *Sustainable Protein Sources*, pp. 341–355. DOI: 10.1016/B978-0-12-802778-3.00021-4.

André Brodkorb; Lotti Egger; Marie Alminger; Paula Alvito; Ricardo Assunção; Simon Ballance et al. (2019): INFOGEST static in vitro simulation of gastrointestinal food digestion. In *Nature Protocols* 14 (4), pp. 991–1014. DOI: 10.1038/S41596-018-0119-1.

Anna Bordiean; Michał Krzyżaniak; Mariusz Jerzy Stolarski (2022): Bioconversion Potential of Agro-Industrial Byproducts by Tenebrio molitor—Long-Term Results. In *Insects* 13 (9). DOI: 10.3390/INSECTS13090810.

Bahar, Nur H.A.; Lo, Michaela; Sanjaya, Made; van Vianen, Josh; Alexander, Peter; Ickowitz, Amy; Sunderland, Terry (2020): Meeting the food security challenge for nine billion people in 2050: What impact on forests? In *Global Environmental Change* 62, p. 102056. DOI: 10.1016/j.gloenvcha.2020.102056.

Barre, Annick; Pichereaux, Carole; Simplicien, Mathias; Burlet-Schiltz, Odile; Benoist, Hervé; Rougé, Pierre (2021): A Proteomic- and Bioinformatic-Based Identification of Specific Allergens from Edible Insects: Probes for Future Detection as Food Ingredients. In *Foods (Basel, Switzerland)* 10 (2), p. 280. DOI: 10.3390/foods10020280.

EU-Novel Food (2015). Available online at https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2015:327:FULL, checked on 11/30/2025.

Hsiaoling Wang; Nagarani Pampati; William M. McCormick; Lokesh Bhattacharyya (2016): Protein Nitrogen Determination by Kjeldahl Digestion and Ion Chromatography. In *Journal of Pharmaceutical Sciences* 105 (6), pp. 1851–1857. DOI: 10.1016/j.xphs.2016.03.039.

Janssen, Renske H.; Vincken, Jean-Paul; van den Broek, Lambertus A. M.; Fogliano, Vincenzo; Lakemond, Catriona M. M. (2017): Nitrogen-to-Protein Conversion Factors for Three Edible Insects: Tenebrio molitor, Alphitobius diaperinus, and Hermetia illucens. In *Journal of Agricultural and Food Chemistry* 65 (11), pp. 2275–2278. DOI: 10.1021/acs.jafc.7b00471.

Michiel van Dijk; Tom Morley; Marie Luise Rau; Yashar Saghai (2021): A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. In *Nature Food 2021 2:7* 2 (7), pp. 494–501. DOI: 10.1038/s43016-021-00322-9.

Rumpold, Birgit A.; Schlüter, Oliver K. (2013): Nutritional composition and safety aspects of edible insects. In *Molecular nutrition & food research* 57 (5), pp. 802–823. DOI: 10.1002/mnfr.201200735.

Samy Boulos; Anina Tännler; Laura Nyström (2020): Nitrogen-to-Protein Conversion Factors for Edible Insects on the Swiss Market: T. molitor, A. domesticus, and L. migratoria. In *Frontiers in Nutrition* 7. DOI: 10.3389/FNUT.2020.00089.

WHO (2007): Protein and amino acid requirements in human nutrition. In *World Health Organization technical report series* (935), 1-265, back cover.

Xu, Miaomiao; Hu, Danting; Liu, Xiaoguang; Li, Zhaowei; Lu, Liming (2025): Branched-Chain Amino Acids and Inflammation Management in Endurance Sports: Molecular Mechanisms and Practical Implications. In *Nutrients* 17 (8), p. 1335. DOI: 10.3390/nu17081335.