NTNU | Faculty of Natural Sciences
Department of Biotechnology and Food Science

BACHELOROPPGAVE 2023

15 studiepoeng

Comparison of chemical and enzymatic chitin extraction methods on mealworm *Tenebrio molitor* and its exoskeleton

Sammenligning av kjemisk og enzymatisk ekstraksjonsmetoder for melorm *Tenebrio molitor* og melormskall

Utført av
Hedda Fosseng Traa
Sofie Solås Winsnes
Ida Viktoria Pilskog Øvrelid

Dette arbeidet er gjennomført som ledd i bachelorutdanningen i matvitenskap, teknologi og bærekraft ved Institutt for bioteknologi og matvitenskap, NTNU. Bruk av oppgavens innhold skjer på eget ansvar.

Abstract

Background - Chitin is a polysaccharide that can be utilised in the food industry that can be extracted from mealworm.

Aim - The aim of the project was to chemically and enzymatically extract chitin from mealworm and its exoskeleton.

Materials and method - The raw materials were analysed with Kjeldahl method and CHNS. A chemical and enzymatic protocol was used to extract chitin. CHNS analysis was used to characterise the extracts and the supernatants. Amine detection analysis was also used to characterise the supernatants and the raw materials.

Results - The protein content of the mealworm was calculated to 59.50% with Kjeldahl and 68.20% with CHNS. For the exoskeleton the protein content was calculated to 49.8% with Kjeldahl and 59.70% with CHNS. The protein content from the chemical extraction was calculated to 65.70% for the exoskeleton and 68.80% for the mealworm. In the chemical extraction, the protein content from the supernatants were 56.20% for the exoskeleton and 59.90% for the mealworm. The protein content from the enzymatic method was 59.60% for the exoskeleton and 55.20% for the mealworm. For the supernatants from the enzymatic extraction the protein content was calculated to 59.30% for the exoskeleton and 59.40% for the mealworm. From the enzymatic extraction, the carbon/nitrogen ratio was 6.24±0.36 for the exoskeleton, 4.46±0.03 for the exoskeleton supernatant, 5.12±0.24 for the mealworm and 4.62±0.16 for the mealworm supernatant. For the chemical extracton, the carbon/nitrogen ratio was 5.25±0.04 for the exoskeleton, 4.91±0.14 for the supernatant of exoskeleton, 5.50±0.04 for the mealworm and 4.75±0.01 for the mealworm supernatant.

Conclusion - There were no notable differences in the composition of the extracts. The presence of protein in the supernatants show that the protein content in the extracts could be lower than the raw materials.

Sammendrag

Bakgrunn - Kitin er et polysakkarid som det finnes rikelig av i naturen. Det kan ekstraheres fra melorm og brukes i næringsmiddelindustrien.

Mål - Målet for prosjektet var å ekstrahere kitin fra melorm og melormskall ved hjelp av en kjemisk og en enzymatisk protokoll.

Materialer og metoder - Råstoffet ble analysert med Kjeldahls metode og CHNS. En kjemisk og en enzymatisk prosedyre ble gjennomført for å ekstrahere kitin. CHNS ble brukt for å karakterisere ekstraktene og supernatantene. Det ble og brukt en analyse for amindeteksjon med OPA-reagens for begge prøvene.

Resultat - Proteininnholdet i melormen ble 59.50% med Kjeldahl og 68.20% med CHNS. I skallet ble det 49.8% med Kjeldahl og 59.70% med CHNS. Kjemisk ekstrahert skallprøve hadde 65.70% protein og melormprøven hadde 68.80%. Supernatanten fra kjemisk behandlet skallprøve inneholdt 56.20% protein og supernatanten fra kjemisk behandlet melormprøve inneholdt 59.90% protein. Proteininnholdet for melormprøven fra enzymatisk ekstraksjon ble 55.20%, og 59.60% for kjemisk ekstrahert skallprøve. Supernatanten fra enzymatisk behandlet skallprøve inneholdt 59.30% protein, og tilsvarende behandlet melormprøve inneholdt 59.40%. Forholdet mellom karbon/nitrogen var 6.24±0.36 i skallprøve fra enzymatisk ekstraksjon, 4.46±0.03 i supernatanten for skallprøve fra enzymatisk ekstraksjon, 5.12±0.24 i melormprøve fra enzymatisk ekstraksjon og 4.62±0.16 i supernatanten fra melormprøve fra enzymatisk ekstraksjon. Forholdet mellom karbon/nitrogen var 5.25±0.04 i skallprøven fra kjemsik ektraskjon, 4.91±0.14 for supernatanten fra skallprøven fra kjemisk ektraskjon, 5.50±0.04 i melormprøven fra kjemisk ekstraksjon og 4.75±0.01 i supernatanten fra kjemisk ekstrahert melormprøve.

Konklusjon - Det var ingen bemerkelsesverdige forandringer i sammensetningen av ekstraktene. Tilstedeværelse av protein i supernatantene kan vise at proteininnholdet i ekstraktene er lavere enn de er i råmaterialet.

Acknowledgements

The research presented in this thesis was conducted in the Department of Biotechnology and Food Science at the Norwegian University of Science and Technology (NTNU) in cooperation with the company Invertapro. The practical work of the thesis is done at the laboratories at Akrinn and Gløshaugen, Trondheim, Norway. The work and research that shaped this thesis was conducted from January 2023 until May 2023.

We would like to thank our supervisors Ashkan Madadlou, Finn Lillelund Aachmann and Gaston Courtade for guiding us through this thesis and for providing helpful feedback and support. We want to give our gratitude to Gerd Inger Sætrom, Anne Kathrine Streitlien and Siri Stavrum for supporting us with our practical work in the laboratories, encouragement and advice. Thank you to Jakob Irgens Blakstad from Invertapro for providing us with the raw material for the project, and a thanks to NTNU for providing materials for laboratory experimenting and analytical work. Lastly, we would like to thank our friends and family for supporting us from the very start of our project. A special thanks to Kirsten Solås for proof reading our work and motivating us until the end.

29.05.2023, TRONDHEIM

Hedda Fosseng Traa Sofie Solås Winsnes Ida Viktoria Pilskog Øvrelid

List of Abbreviations

Abbrevation	Definition
EE	Exoskeleton from enzymatic method
EESN	Exoskeleton from enzymatic method, supernatant
EC	Exoskeleton from chemical method
ECSN	Exoskeleton from chemical method, supernatant
ME	Mealworm from enzymatic method
MESN	Mealworm from enzymatic method, supernatant
MC	Mealworm from chemical method
MCSN1	Mealworm from chemical method, supernatant after first step
MCSN2	Mealworm from chemical method, supernatant after second step
rpm	Rounds per minute

Table of Contents

List of Figures

[1] Chawla et al. 2015.

List of Tables

Appendix

1 Introduction

The main goal for this project were to extract chitin from whole mealworm and its exoskeleton. The methods to be used was a chemical extraction method, which involves a demineralisation and deproteinisation step, and an enzymatic extraction with a deproteinisation step with Alcalase®. The thesis was defined in cooperation with Invertapro and NTNU.

During the project following interim goals were defined:

- Producing a suitable and effective protocol based in previous literature that is reproducable.
- Successfully extract chitin from the raw materials by demineralising in acidic conditions and deproteinising in alkaline conditions.
- Successfully extract chitin by deproteinising the raw materials with the enzyme Alcalase®.
- Characterise extracts and supernatants to find chitin yield.

The topic researched in this project was interesting because it has the possibility to introduce more raw materials to chitin production. Mealworm has the potential of entering the market to a increasingly degree of exploitation. Several forecasts has implied that insects and production of insects, such as mealworm, can dominate a larger part of the food industry[2]. It was also interesting because the exoskeleton is a waste product for the company as of today. If it is possible to obtain chitin from the exoskeleton, this could be further developed as a solution to what to do with the accumulated waste products. In previous research it is shown that chitin extraction is commonly practised with waste products from the seafood industry such as crab and shrimp. Optimising a process for extracting chitin to make it more sustainable would have been beneficial.

The thesis quesiton was “How can chitin be extracted from mealworm and its exoskeleton by a chemical method and an enzymatic method?”. It was interesting to research this since there was little to none information about this being done before. A successful procedure could make it possible to research the topic further and optimise the procedures to make it more sustainable.

[2] Siemianowska et al. 2013.

2 Theoretical background

Chitin and chitosan are versatile substances that can be extracted through differenet methods from sources such as insects cuticle, fungi cell walls and exoskeleton of crustaceans. Chitin is applied in different ways in the food industry, capsules in medicine and agriculture.[3]

2.1 Chitin in mealworm

Mealworm consists of carbohydrates, protein, fat and other nutrients. Invertapro analysed the composition of the mealworms they produce, see appendix 1. The fiber content in fresh mealworm is 1.9g per 100g mealworm, while for dried mealworm it is 4.9g per 100g. It is assumed that most of the fiber is chitin. Similar amounts of chitin in mealworm was reported by another study where they obtained a yield of $4.72 \pm 0.21g$ chitin per $100g$ dried mealworm[4].

With regards to biopolymers, chitin is the second most abundant one on earth. The most abundant biopolymer is cellulose, which chitin shares structural similarities with (see figure 1). Chitin is a linear polymer of the amino sugar N-acetyl-D-glucoseamine ($poly\ \beta-(1\rightarrow 4)-N-acetyl-D-glucosamine$)[5]. Chitin occurs in three polymorphic crystalline structures where α-chitin is the most abundant and is the one usually found in for instance crustaceans and insect cuticles. The less abundant forms are β-chitin and γ-chitin[6]. Because of its crystalline structure, chitin has poor solubility in water which limits possibilities for application[7]. Chitin is organic and contains nitrogen, hydrogen and carbon which is illustrated in figure 1. Some commercial chitin was analysed and the results showed that it consisted of 46.12% carbon and 6.65% nitrogen[8]. A different study reported their findings for hydrogen content, which showed 0.39%[9].

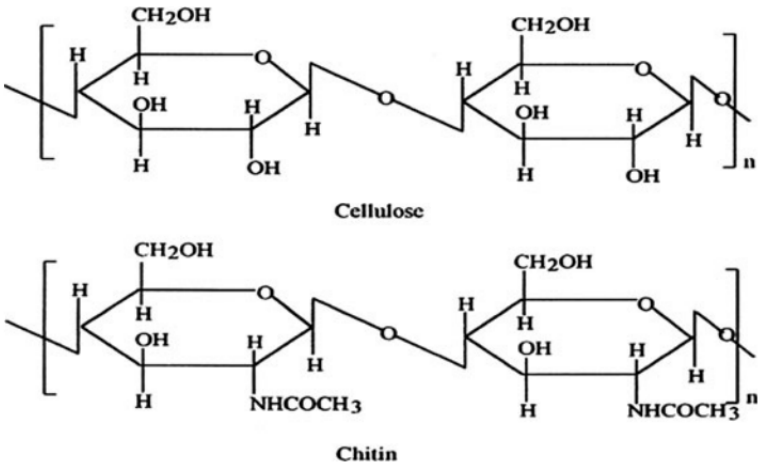

Figure 1: The chemical structures of cellulose and chitin (Chawla et al. 2015)

Deacetylation can remove acetyl groups to convert chitin into chitosan[10]. Compared to chitin, chitosan has higher potential to be applied in food industry because it has higher solubility in water due to its free amino groups (see figure 2)[11]. Chitin and chitosan has gained attention because of potential applications in the food industry. The substances and their derivatives has shown antimicrobial activity. Some studies have reported that bacteria such as *Eschericia coli, Staphylococcus aureus* and *Bacillus*

[3] Shahidi et al. 1999.
[4] Son et al. 2021.
[5] Moussian 2019.
[6] Pakizeh et al. 2021.
[7] Jang et al. 2004.
[8] Tan et al. 2020.
[9] Varma and Vasudevan 2020.
[10] Shin et al. 2019.
[11] Fabris et al. 2010.

cereus can be inhibited by relatively low concentrations of chitosan, rainging from 0.005% to 1.5%. The concentration of chitosan for antimicrobial application is dependent on factors such as degree of acetylation, type of bacteria and pH. It can also be used as an alternative for clarification and deacidification in fruit juices. Chitosan can form films that protect fresh, frozen and fabricated food products which can help prolong the product's shelf life.[12] Chitosan has also been found to be effective in the process of removing particles during drinking water treatment[13].

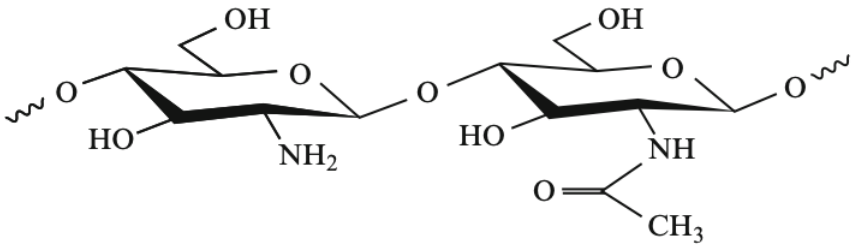

Figure 2: The chemical structure of chitosan[14].

2.2 Extraction methods

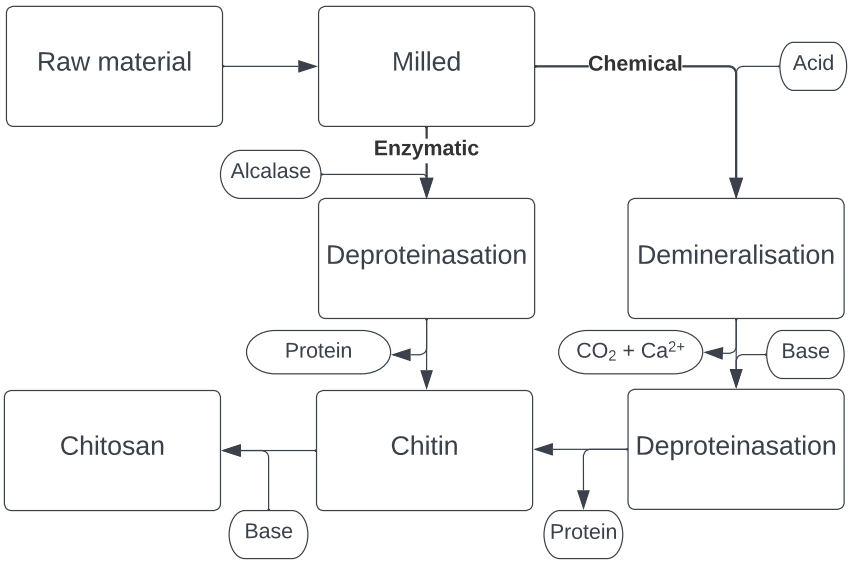

Figure 3: Overall process of both enzymatic and chemical extraction.

Chitin can be extracted chemically. The steps to extract chitin and chitosan is by demineralisation and deproteinisation, which can be followed by deacetylation if necessary.[15] The raw material is usually in powder form to maximise surface area to volume ratio, and then treated with the chemicals. Demineralisation is a process that is used to remove minerals from the sample such as $CaCO_3$ by treating the raw material with an acid. Afterwards, an alkaline solution of NaOH or an enzyme such as alcalase can be used to deproteinise the raw material. Alcalase contains a protease extract that can deproteinise materials by breaking peptide bonds, which releases bioactive petides[16]. Optimal coniditions for alcalase is at pH 10.00 with a maximum activity at 70°C, though it can maintain activity in room temperature at

[12] Shahidi et al. 1999.
[13] Fabris et al. 2010.
[15] Zaku et al. 2011.
[16] Tacias-Pascacio et al. 2020.

pH between 5.00 and 11.00[17]. The enzyme is effective in most of animal protein application and also plant protein, it has a variety of applications and can safely be used for food production[18]. One study achieved an efficiency of 85% with enzymatic extraction of chitin[19].

The treatment conditions for each step of the extraction methods varies and are dependant on desired outcome. However, one study found that higher temperatures increased reaction time without affecting the product significantly.[20]

Another possible method for chitin extraction is a microbiological process using bacteria. Fermentation with enzyme producing bacteria is researched as an alternative to deproteinisation with alkaline chemicals[21]. The proteolytic bacterium *Pseudomonas maltophilia* synthesises a protease that is able to remove protein by breaking peptide bonds during chitin production with crustacean shell waste as the raw material[22].

2.3 Analytical methods used for characterisation

Protein and nitrogen content in the samples can be determined with KjelMaster, which builds on the original Kjeldahl method. The factor used to calculate protein content in samples with high nitrogen contents such as meat is 6.25[23]. CHNS elemental analysis, which builds on the Dumas method, can be used to measure the carbon, hydrogen, nitrogen and sulphur content in an organic sample[24]. O-Phthaldialdehyde (OPA) is an amine detecting reagent which reacts with primary amines, amino acids peptides and protein to form fluorescent product. This product can be used to quantify the beforementioned components in a sample. Chitin does not have primary amine groups and should not react with OPA, but chitosan does have a primary amine group and can react with the reagent.[25]

2.4 Sustainability and life cycle assessment

As of today, general consumption is high and a large amount of the food being produced goes to waste. United Nation's sustainability goal 12 aims to ensure sustainable consumption and production, for example by reducing food waste within a set time period. Norway has one of the highest consumptions per capita. This indicates that resources and products from for instance the food industry needs to be repaired, reused or recycled more effectively. Sustainablility goal 14 is also related, it aims to preserve and use marine resources in a sustainable matter. This includes reducing pollution of the ocean, especially from industries on-shore. Plastic based products, sewage and fertilisers are frequently discharged of in the ocean, which warrants the need to replace plastic in these products with a biodegradable source such as chitin and chitosan.[26]

Life cycle assessment is closely linked to sustainability as it analyses the whole process. It is applied as a tool to assess the impacts a process, product or a service has on the environment. This can include how the raw material is obtained, production stages, transportation emissions and waste management.[27]

The manufaturing and use of NaOH and HCl requires a lot of energy. Both electric and fossil energy which can lead to negative environmental impacts[28]. These chemicals can create hazardous, toxic waste[29].

A study that aimed to compare chemical and enzymatic extractions methods found that the extract

[17] Yang et al. 2017.
[18] Novozymes 2023.
[19] Jantzen da Silva Lucas et al. 2021.
[20] Percot et al. 2003.
[21] Jung et al. 2006.
[22] Hayes et al. 2008.
[23] Mihaljev et al. 2015.
[24] GEOMAR 2023.
[25] Interchim 2023.
[26] FN 2023.
[27] Hauschild et al. 2018.
[28] Ponnusamy and Mani 2022.
[29] Płotka-Wasylka et al. 2017.

yields were higher for the chemical method. Extraction time for the enzymatic approach was longer to achieve similar yields to the chemical method, which meant that the enzymatic method had a worse impact on sustainability. The conclusion states that an enzymatic approach cannot definitively be considered as a greener alternative.[30]

[30] Ponnusamy and Mani 2022.

3 Materials and methods

The method that was used in this project was based on already existing protocols for chemical and enzymatic extraction of chitin. Our method was mainly based on protocols from Zaku et al. (2011) and Einbu (2007). Finally, the methods were applied to our raw materials which were whole mealworm and mealworm exoskeleton. The results from the two different raw materials were compared. The contents of the raw material was mapped by analysing Kjeldahl, CHNS and OPA reagent. The chitin extract was analysed with CHNS, and the supernatants were also analysed with CHNS and OPA reagent.

3.1 Raw material

The raw material consisted of two different materials; the whole mealworm and the exoskeleton of the mealworm. The mealworm arrived as a milled powder from the producer. The exoskeleton was received whole and not in a homogenous state. The exoskeletons were mixed with wood shavings, plastic pieces and other wastes from the production. This is because these materials are used in the boxes where the mealworm is grown. Moreover, at the production site the exoskeleton is treated as waste material which can also explain why it contained other residues. The exoskeletons were sifted manually and any visible pieces were removed before the exoskeleton was milled with IKA M20 universal mill, this is the first step for the exoskeleton in figure 4. It was milled in small portions to avoid aggregation due to moisture. The milled exoskeletons were stored in a refrigerator at 4°C for the entire duration of the project.

BUCHI KjelMaster K-375 was used to determine the nitrogen content and protein content in the samples by using the Kjeldahl method. Three parallels of mealworm and three of exoskeleton were prepared. Each sample was measured to 1g and added to tubes with 15mL of 95-97% sulphuric acid and two BUCHI Kjeldahl-tablets, 4g each. MERCK glycine GR for analysis was used for internal control, and tubes with acid and tablets without any sample was used as blank. The final nitrogen content in the samples were recorded and the protein content was calculated by the KjelMaster.

CHNS/O Elemental Analyzer was used to characterise the dried mealworm and exoskeleton. The samples were weighed to 3-4 mg, then packed inside weighing dishes and placed in the instrument for analysis. Each sample had three parallels.

The raw materials and the supernatants from the chemical and enzymatic extraction methods were analysed with Fluoraldehyde™ o-Phthaldialdehyde reagent solution (OPA)[31]. Three parallels of 100μL from each sample was analysed for this method. Firstly, a carbonate buffer with pH 10.5 at 0.05M was prepared to be used as a diluent. For 1L of buffer 760.459 mg sodium bicarbonate and 4.34 g anhydrous sodium carbonate was added to 800mL of deionised water, then the remaining deionised water was added to reach 1L[32]. The final pH of the buffer was measured to 10.47. Then 10mL of standard solution was made by adding 500μg of MERCK glycine GR for analysis to 10mL of carbonate buffer, with a final concentration of 500$\mu g/mL$. From this, five standards were made at the concentrations 500, 250, 125, 62.5 and 31.3 $\mu g/mL$. The diluent was used as the blank sample. 100μL of each standard was added to 1mL of OPA reagent and mixed carefully. The samples were left in room temperature for 2 minutes and then measured with a VWR V-3000PC spectrophotometer at 340nm. The dried mealworm and exoskeleton was boiled in HCl 1M for one hour to release proteins, and cooled down in room temperature before samples were taken from the supernatant. The supernatant samples from the chemical and enzymatic extractions were prepared by adding 0.01g of freeze dried supernatant to 10mL of diluent. Then the samples were analysed with the same protocol used for the standards.

[31] Interchim 2023.
[32] AAT Bioquest 2023.

3.2 Chemical extraction

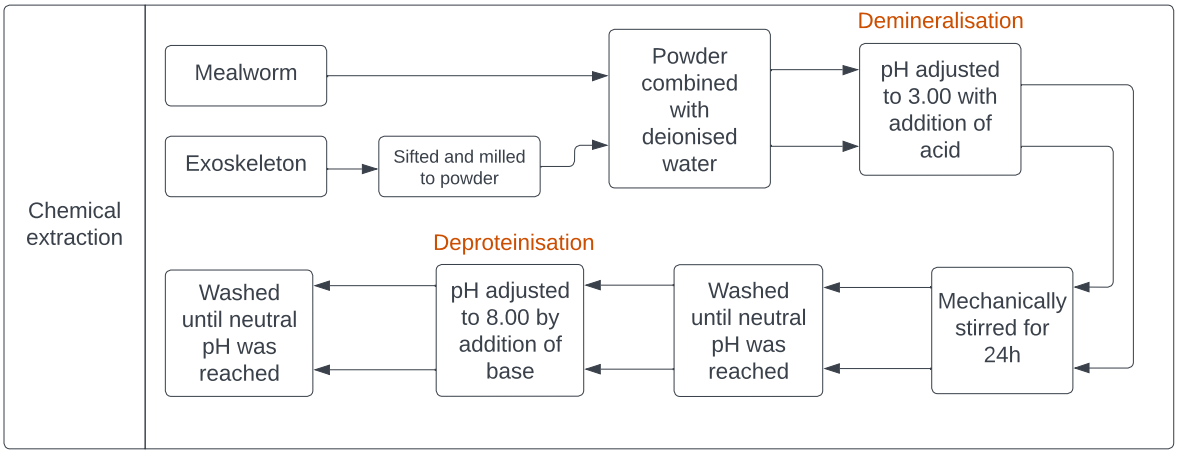

Figure 4: Overall process of chemical extraction.

Each step for the chemical extraction is outlined in figure 4. 50g of whole mealworm powder and exoskeleton powder was added to 0.95L deionised water to hydrate the samples. The pH was adjusted to 3.00 for the demineralisation step by adding 1M HCl, the pH was monitored continously with pH electrode during acidification until pH 3.00 was reached. They were left in room temperature and mechanically stirred for 24h. The samples were washed until pH 7.00 was reached. This was done by centrifuging them in a Rotina 380 Centrifuge at 9000 rpm for 20 minutes at 20°C. A sample of 100mL was collected from the supernatant for both the whole mealworm and the exoskeleton. The supernatant and sediment was separated, then deionised water was added to the sediment and centrifuged. This was repeated for an additional four rounds of five minutes each at the same temperature and rpm to reach pH 7.00.

The next step was deproteinisation. The sediments were added to 0.95L of deionised water to rehydrate the samples. The pH was adjusted to 8.00 by adding 1M NaOH, it was continuously monitored with a pH electrode until the desired pH was reached. The samples were then left in the same conditions as they were in the demineralisation step, and they were also washed using the same protocol until pH 7.00 was reached. Another sample of 100mL was collected from the supernatants after the first centrifugation. The sediments and supernatant samples were freeze dried with a FreeZone freeze dryer for further analysis. The samples were put in 50mL centrifuge tubes, they were filled until the 15mL mark. The tubes were covered with parafilm that was punctured. All of the samples were analysed with CHNS/O Elemental Analyzer.

3.3 Enzymatic extraction

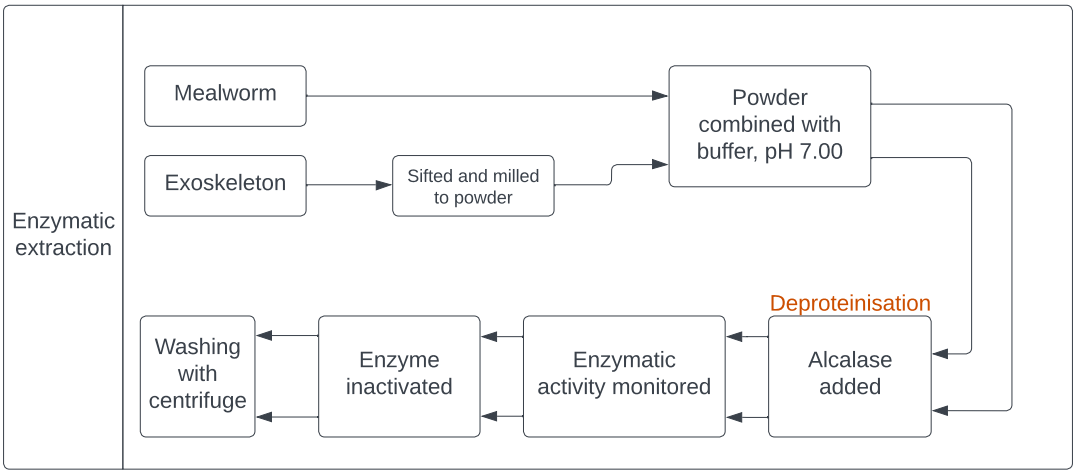

Figure 5: Overall process for enzymatic extraction.

Each step for the enzymatic method is presented in figure 5. 50g of mealworm and exoskeleton in a powdered state were diluted with Certipur buffer solution pH 7.00 (di-sodium hydrogen phosphate/ potassium dihydrogen phosphate) until it covered the powder sufficiently. Whole mealworm powder required 250mL of buffer and the exoskeleton powder required 450mL. Novozymes Alcalase enzyme from *Bacillus licheniformis* was added with a an enzyme substrate ratio of 1:200. The enzymatic activity was not monitored after the alcalase was added as illustrated in the figure, because it was not possible to take samples from the solutions. After 90 minutes the samples were kept at 70°C for 10 minutes to inactivate any remaining enzyme. Then they were centrifuged in a Rotina 380 Centrifuge at 9000 rpm for 20 minutes at 20°C. A sample of 100mL was collected from the supernatant for both the whole mealworm and the exoskeleton. The sediments and supernatant samples were freeze dried for further analysis, the protocol was the same as for the chemical method. All of the samples were analysed with CHNS/O Elemental Analyzer.

Table 1: Overview of the chemicals and solutions used for each analysis in the project.

Analysis	**Chemical**	**Formula**	**Distributor**	**CAS-number**
Kjeldahl	Kjeldahl tablets	K_2SO_4 $CuSO_4 \cdot 5H_2O$	BUCHI	7778-80-5 (K_2SO_4) 7758-99-8 ($CuSO_4 \cdot 5H_2O$)
	Sulfuric acid 95-97%	H_2SO_4	MERCK	7664-93-9
	Glycine	$C_2H_5NO_2$	MERCK	56-40-6
Extraction	Hydrochloric acid 1M (of 36.5%)	HCl*	-	-
	Sodium hydroxide 1M (of 4%)	NaOH*	-	-
	Alcalase	$C_3H_8O_3$	Sigma-Aldrich	56-81-5
	Bradford reagent	H_3PO_4 CH_3OH	Bio-Rad Laboratories	7664-38-2 (Phosphoric acid) 67-56-1 (Methanol)
	Certipur Buffer pH 7.00	-	Supelco	-
Amine detection	O-phthaldialdehyde	$C_8H_6O_2$	Sigma-Aldrich	643-79-8
	Buffer pH 10.50*	$NaHCO_3$ Na_2CO_3	-	-

**Hydrochloric acid (HCl), sodium hydroxide (NaOH) and buffer pH 10.50 were all pre-made in the laboratory.*

4 Results

The following section will show results from characterisation of the whole mealworm powder and the exoskeleton powder, extracts from the chemical and enzymatic method and their respective supernatants.

The calculated means and standard deviations that were used to generate the figures for Kjeldahl analysis can be found in appendix 2, the raw data is presented here as well. The raw data from CHNS elemental analysis is presented in appendix 3, the calculated means and standard deviations can be found in appendix 4. The raw data, calculated means and standard deviations for the samples from amine detection analysis can be found in appendix 5. The raw data from the standards and the standard curve can be found in appendix 6.

4.1 Characterisation of the raw material

In preparation for the extraction methods and analytical methods some observations were made. The starting pH for the mealworm solution and exoskeleton solution were not the same and required different amount of chemicals in the extraction methods. The pH for the exoskeleton solution was between 5-6, while the mealworm solution was between pH 6.5-7. Therefore the mealworm solution required a higher volume of HCl to reach pH 3.00. This was notable when NaOH was added too. The exoskeleton solution required higher volumes of NaOH to reach pH 8.00. The exoskeleton samples were noticeably more static than the mealworm samples.

The raw material was analysed with the KjelMaster. In the mealworm the nitrogen content was 9.52% ±0.03, for the exoskeleton it was 7.97% ±0.05, see fig 6. The protein content was determined by the KjelMaster with nitrogen-protein factor of 6.25, the mealworm had an average of 59.50% protein and the exoskeleton had an average of 49.8% protein. The nitrogen content for the mealworm was slightly higher than the content for the exoskeleton, which is reflected in the calculated protein content. The standard deviation for nitrogen content in the mealworm and the exoskeleton indicates low variability in the datasets.

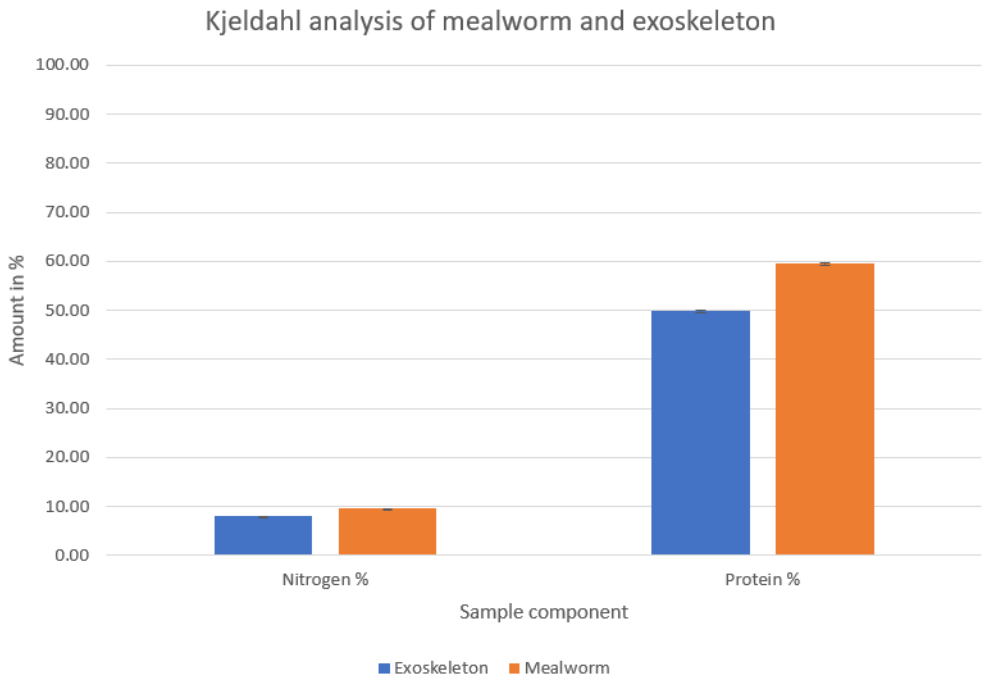

Figure 6: The results for nitrogen % and protein % in whole mealworm and exoskeleton from Kjeldahl analysis.

The raw material was analysed by CHNS analysis to determine the composition of nitrogen, carbon, hydrogen and sulfur in the samples. The factor used to calculate the protein content was 6.25, the same that was used for Kjeldahl analysis. The results are presented in figure 7. The results show that the mealworm has 10.90% ±1.32 nitrogen, 59.60% ±6.54 carbon and 9.43% ±1.02 hydrogen. The protein content was found to be 68.20%. The exoskeleton has 9.54% ±1.08 nitrogen, 45.5% ±4.80 carbon and 6.99% ±0.78 hydrogen. The calculated protein content was 59.70%. The values for sulfur content is included in the raw data, but not figure 7. The CHNS elemental analyzer could not produce reliable results for sulfur content, indicated by the "Su" in the info-column in appendix 3, page 2.

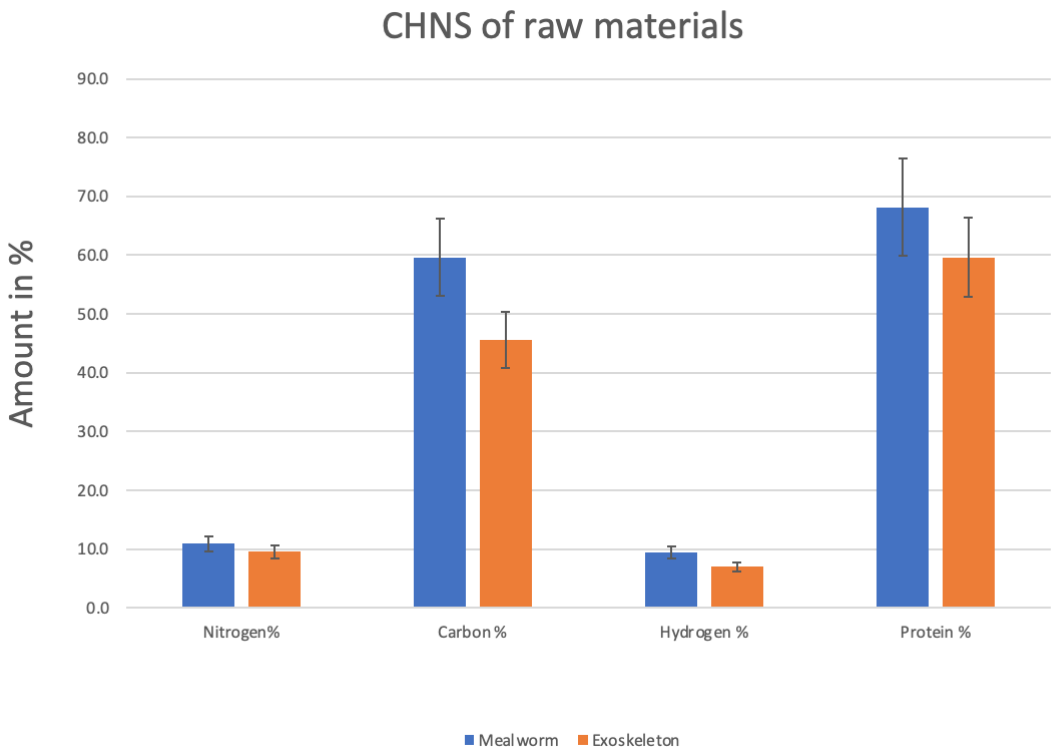

Figure 7: Nitrogen, carbon, hydrogen and protein content of the raw materials

Amine detection analysis was used to find primary amino acids in the raw materials. A standard curve was generated with the spectrophotometric results from serial dilutions of glycine. The raw data and the standard curve are presented in appendix 6. From the standard curve the linear equation was found to be:

$$y = 0.0013x + 0.537 \tag{1}$$

The equation was used to calculate the concentration of primary amines in the mealworm and the exoskeleton prior to extraction. According to figure 8, a higher concentration of primary amines were detected in the exoskeleton compared to the mealworm. In the exoskeleton a concentration of $235.40 \mu g/mL \pm$ 121.80 primary amines was calculated, the standard deviation indicates substantial variability in the dataset. In the mealworm a concentration of 22.70 $\mu g/mL \pm 1.63$ primary amines was calculated.

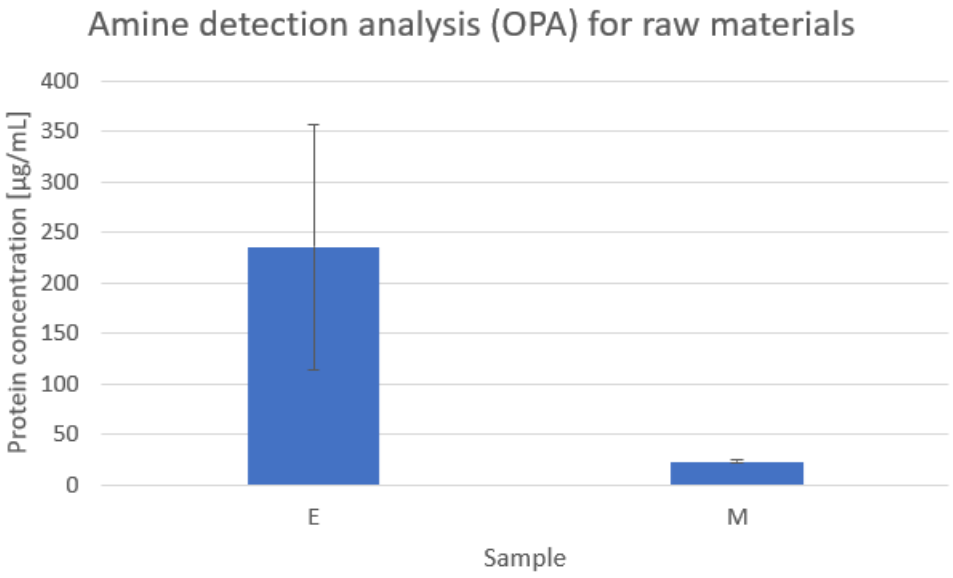

Figure 8: Amine detection analysis of whole mealworm and exoskeleton.

4.2 Characterisation of the processed materials

After the materials had been processed the exoskeleton samples were still static. The wood shavings from the mealworm production remained in the exoskeleton samples after extraction. The processed materials were analysed with CHNS to see if the composition of the samples changed. The supernatants were also analysed with CHNS and amine detection analysis.

After chemical extraction the EC sample's nitrogen content was calculated to be 10.50% ±0.05, carbon content was 55.20% ±0.16, hydrogen content was 8.31% ±0.06 and the protein content was 65.70%. From the MC, the contents were 11.00% ±0.17 nitrogen, 60.50% ±1.16 carbon, 9.41% ±0.22 hydrogen and 68.80% protein. The results are presented in figure 9. There was low variability within the datasets for each component in both extracts, which is indicated by their standard deviation.

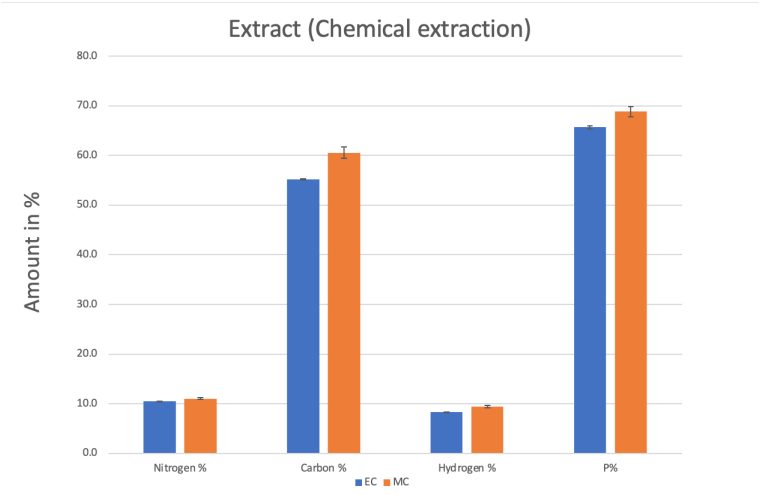

Figure 9: Extract from chemical extraction.

The mean composition of the ECSN was calculated and the results showed that it contained 9.00% ±1.19 nitrogen, 44.10% ±4.58 carbon, 7.64% ±0.96 hydrogen and the protein content was 56.20%. For the supernatant from the MCSN2, the contents were calculated and found to be 9.59% ±0.01 nitrogen, 45.50% ±0.10 carbon, 8.32% ±0.07 hydrogen and the protein content was 59.90%. The results are presented in figure 10.

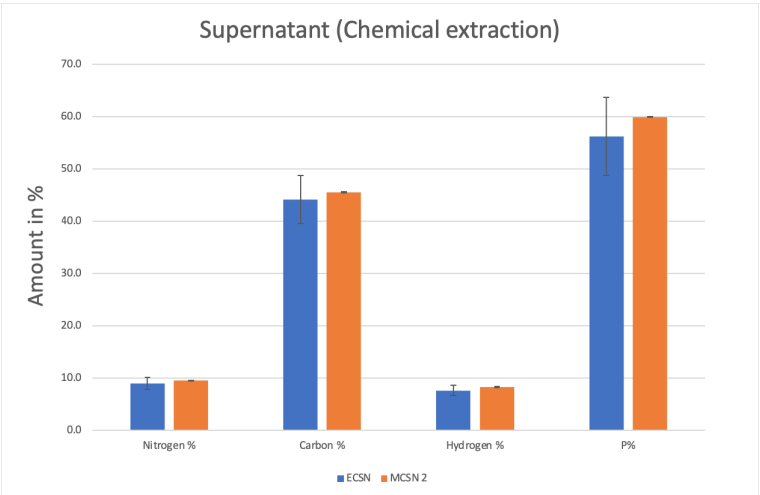

Figure 10: Supernatant from chemical extraction.

The mean composition of ME was calculated and the results showed that it contained 8.83% ±0.24 nitrogen, 45.20% ±0.91 carbon, 7.95% ±0.09 hydrogen and the protein content was 55.20%. The EE from the enzymatic extraction was calculated and found to be 9.49% ±1.69 nitrogen, 42.38% ±7.28 carbon, 7.67% ±1.14 hydrogen and had a protein content of 59.60%. The results are presented in figure 11.

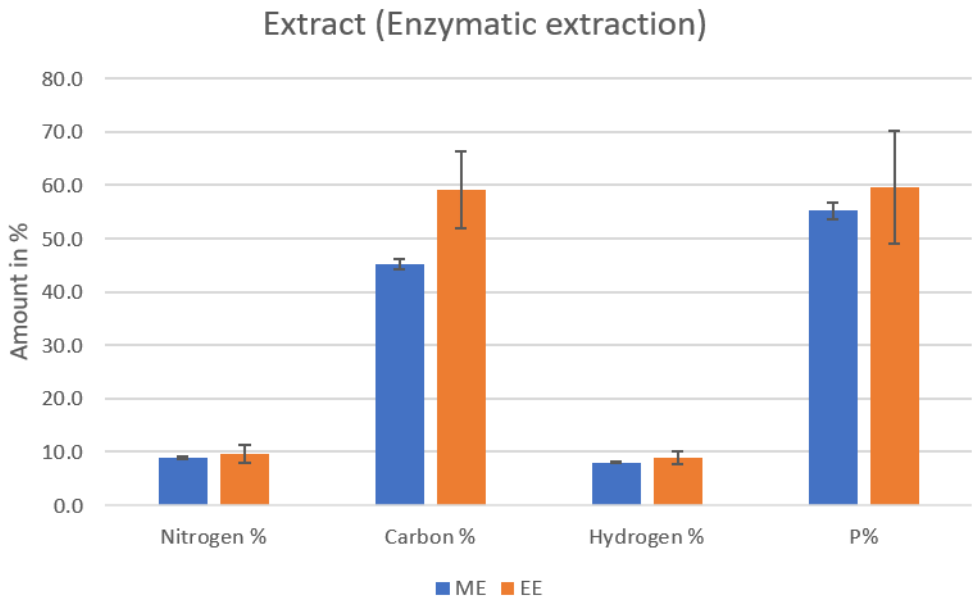

Figure 11: Extract from enzymatic extraction.

From the enzymatic extraction the EESN was calculated to 9.49% ±1.69 nitrogen, 42.40% ±7.67 carbon, 7.67% ±1.36 hydrogen and the protein content was 59.30%. For the MESN the results were calculated to 9.51% ±0.16 nitrogen, 44.00% ±0.76 carbon, 7.67% ±0.10 hydrogen and the protein content was 59.40%. The results are presented in figure 12.

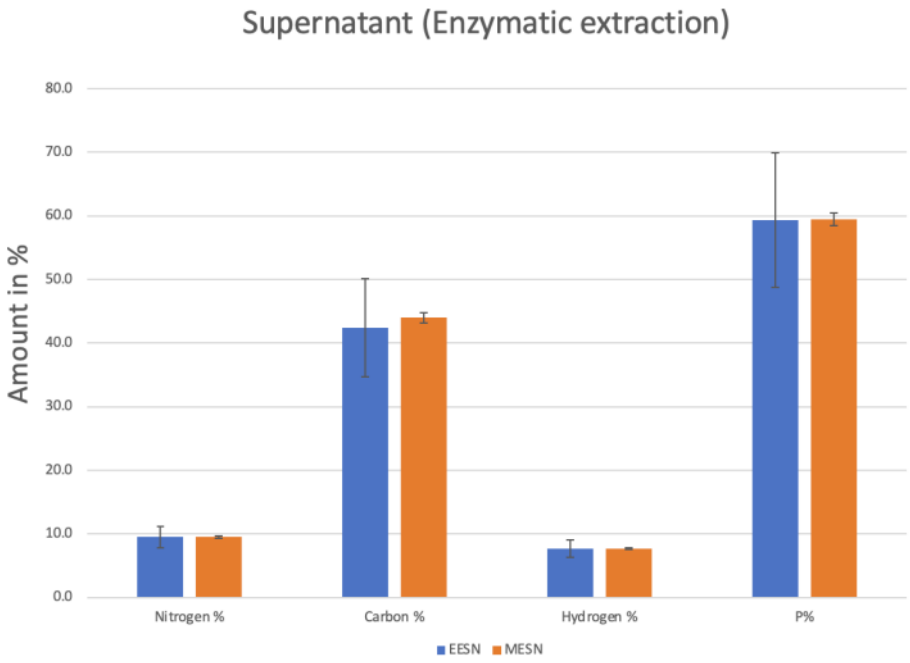

Figure 12: Supernatant from enzymatic extraction.

The carbon/nitrogen ratio was calculated to be 6.24±0.36 for EE, 4.46±0.03 for EESN, 4.91±0.14 for ECSN, 5.25±0.04 for EC, 5.50±0.04 for MC, 4.75±0.01 for MCSN2, 4.62±0.16 for MESN and 5.12±0.24 for ME. The results are presented in figure 13.

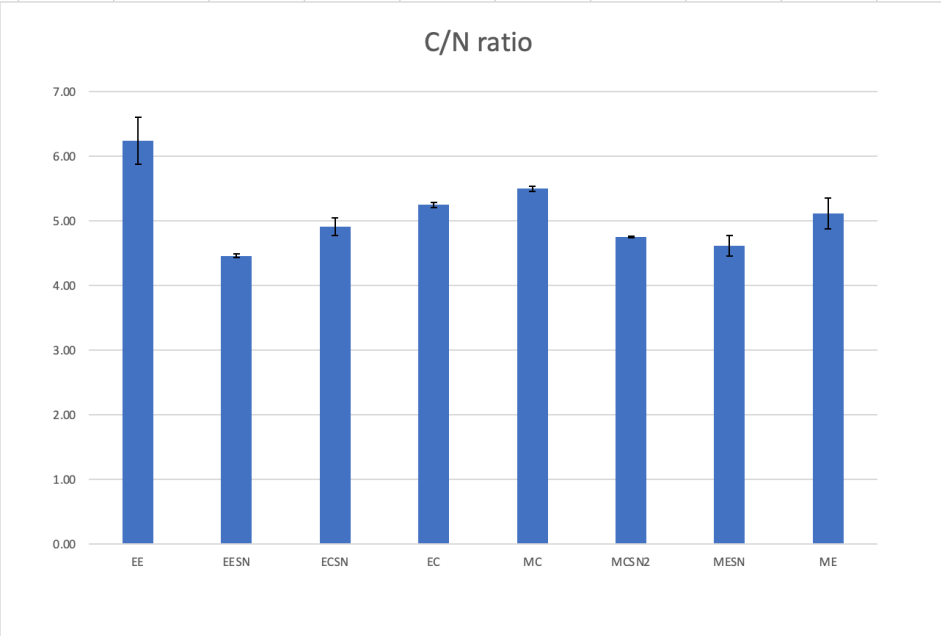

Figure 13: Carbon and nitrogen ratio of all samples

The supernatants from both extraction methods were analysed with amine detection analysis. Results were calculated with the linear equation from the standard curve in appendix 6. The mean of the results are calculated and presented in figure 14. Sample EESN R has the lowest concentration of 78.80 $\mu g/mL\pm$ 24.48. Sample MESN R has the highest concentration of 152.10 $\mu g/mL\pm$96.56. The supernatant samples from the chemical extraction have similar consentrations. ECSN1 is 117.70$\mu g/mL\pm$74.93 and MCSN1 is 121.30 $\mu g/mL\pm$27.10. The observed data from the two supernatant samples with the highest calculated concentrations has large standard deviations, which indicates high variability in the datasets.

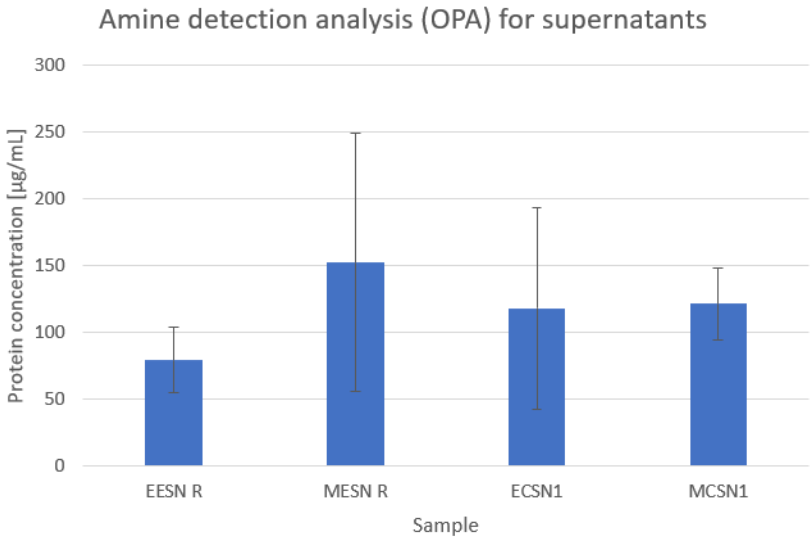

Figure 14: Amine detection analysis, supernatants

5 Discussion

The results for all of the analyses and observations that were made while handling the mealworm and exoskeleton as raw materials will be discussed in this section. This includes results from different analyses of the raw materials, analysis of the extracts from the chemical and enzymatic methods, and results from different analyses of the supernatants. The sulfur content in all samples analysed with CHNS was neglible, this was indicated in the raw data produced by the CHNS Elemental Analyzer and can be found in the column "info", see appendix 3 and 4.

5.1 Raw material

Different observations were made for the whole mealworm and the exoskeleton as raw materials. The exoskeletons were mixed with different wastes from production and had to be manually removed, which was time consuming. The samples were not free from waste when they were analysed, wood shavings still remained because it was impossible to remove it all. Another environment for growth could be considered if there is a wish to utilise the exoskeleton for chitin extraction.

Dried mealworm powder was less static than the whole exoskeleton and the exoskeleton powder, which made it easier to weigh and prepare for analysis. Neither of the raw materials are soluble in water, but the mealworm powder was easier to disperse in water than the exoskeleton powder. This could be due to the difference in density, as the volume of 50g of exoskeleton powder was visibly larger than the same amount of mealworm powder. It can also be related to the outer layer of the exoskeleton which for most insects is composed of wax[33]. The exoskeleton powder would have a much higher concentration of wax than the mealworm powder. This affects its ability to be evenly dispersed in water. A lower mass of exoskeleton powder in the same amount of liquid could improve its ability to be dispersed evenly, but if the main challenge is due to the wax then the sample should be washed in solvent to remove the wax and other fats in the sample. The mealworm powder could also benefit from fat removal, as the fat content in the mealworm is high, around 32% (appendix 1).

Another observed difference was the pH of the solutions once the raw materials were dispersed in water, as the pH of the exoskeleton solution was lower than the mealworm powder. One study reported that the decrease in pH for stored mealworm can be due to acid and gas production by microbes[34]. The mealworm arrived as a powder which indicates some form of pretreatment, this could involve a step to inhibit microbial growth. The exoskeletons did not appear to have gone through any pretreatment to inhibit growth, which could explain that the starting pH for the exoskeleton solution was lower than the mealworm solution.

Both Kjeldahl analysis and CHNS analysis of the raw material showed that the mealworm has approximately 10% more protein than the exoskeleton. Protein content for dried mealworm was calculated from the nitrogen content to 59.50% with Kjeldahl and 68.20% with CHNS. The discrepancy between the results from these two analyses can be due to heterogenous samples. The sample size for CHNS was between 3-4mg, which is relatively low compared to the sample size in Kjeldahl which was 1g. According to Invertapro's data, the whole mealworm consists of 50.40% protein. This is lower than the number calculated by the Kjeldahl method and CHNS analysis. The cause of the difference between the protein contents from this report and Invertapro is unknown. The method for protein determination used by Invertparo is not known. The protein content was calculated by multiplying the nitrogen content with 6.25, which is commonly used for Kjeldahl analysis of meat. This factor was used for all of the samples where protein content is calculated. This was used because there was not a factor available for mealworm and exoskeleton. However, the protein levels in mealworm reported by Invertapro is relatively high which made the factor for meat most applicable.

In comparison, the protein content in the exoskeleton was calculated to 49.78% with Kjeldahl and 59.65% with CHNS. Again, this discrepancy can attributed to heteregenous samples and difference in sample size. Some of the nitrogen in the sample can be attributed to other sources than protein, but the exact composition is unknown.

[33] *NC State University* 2023.
[34] Borremans et al. 2020.

An amine detection analysis with OPA reagent was carried out to determine how much protein was extracted from the raw materials. The samples were broken down with high temperature and acid to get a supernatant of proteins and amino acids. The concentrations for the mealworm and the exoskeleton were quite different. The exoskeleton had a concentration of 141.50 $\mu g/mL$ compared to the mealworm which had a much lower concentration at 9.20 $\mu g/mL$. However, the mean value for the exoskeleton has a large standard deviation which indicates that the results are highly unreliable because of the variability in the samples.

According to Sivashankari and Prabaharan (2017), the C/N ratio for chitin is 6.861 and it varies down to 5.145 for completely deacetylated chitosan. In this study the C/N ratio for dried mealworm is 4.78 ±0.07 and exoskeleton is 5.47 ±0.08. The samples are not similar to the C/N ratio of chitin, but the exoskeleton ratio is somewhere between chitin and chitosan. However, this is an analysis of the raw material which means that these samples are not expected to be pure chitin or chitosan.

5.2 Chemical method

The chemical extraction was done twice because different protocols were used. In the first extraction protocol NaOH was added to adjust the pH to 8.00 as the first step, followed by acidification to pH 3.00 by addition of HCl. The second chemical extraction was done according to the protocol in section 3.2, which is the protocol that is referenced in section 3. The results from both protocols are presented in the raw data, but only the results from the second protocol are processed and presented in the results section. This is due to the reason that the first protocol was done in an incorrect order according to our final protocol.

The solids were mixed with deionised water, 50g of solids dispersed in 950mL deionised water. As mentioned previously this was sufficient for the mealworm powder, but not for the exoskeleton powder. An additional step to remove fat and wax prior to extraction and adjust protocols to the powders difference in density could be beneficial. The samples had to be continuously mechanically stirred to ensure that the powders were evenly dispersed in the solutions.

The nitrogen content in the extracts of mealworm and exoskeleton had similar mean values to the raw material, approximately 11%. The amine detection results of the supernatants from the chemical extractions contains over 100.00$\mu g/mL$, which could indicate that protein has been removed from the raw materials and that a higher part of the nitrogen content in the extracts is from chitin. However, these amine detection results have large standard deviations because of high variability in concentrations in the samples which indicates that they are not reliable. According to the CHNS results for the supernatants there was approximately 10% nitrogen in the mealworm supernatant and a calculated protein content of approximately 60%. This can also indicate that protein has been removed from the raw material, and that a larger part of the nitrogen content in the extract is chitin compared to the raw material. The exoskeleton supernatant had similar results with a nitrogen content of 9% and a calculated protein content of approximately 56%. If the total volume of the supernatants had been measured it could have been used to estimate how much of the protein in total that was extracted from the raw materials.

The C/N ratio for EC is 5.25 ±0.04 and MC is 5.5 ±0.04. These values are not close to what the reference value of C/N ratio for chitin is. This indicates that the samples do not contain pure chitin. There are multiple steps in both methods that could have caused this. The raw material of both samples were not a homogeneous powder, which could affect the ability of the raw materials to completely react with the chemicals. The deproteinisation and demineralisation reactions were not monitored continuously, so the efficacy of the reactions are unknown.

5.3 Enzymatic method

The enzymatic extraction was done twice. The first enzymatic extraction had a lower amount of enzyme than the second, this was due to a faulty measurement in the execution of the protocol the first time. The raw data from the incorrect execution of the protocol is included in the appendix, but only the correct execution is processed and included in the results section. It should be noted that the enzymes activity was not measured beforehand, it had been stored for an unknown amount of time and the initial activity

could have decreased.

The raw materials were mixed with buffer solution and enzyme. This gave a dense and thick mixture that was difficult to mechanically stir, the samples were not evenly dispersed. Because of limited access to buffer solution the amount of solution could not be majorly increased, which would likely have helped the efficacy of the method. It was difficult to pipette samples from both solutions to measure the activity during extraction, therefore the results are not presented as the pipetted samples were not presentable of the solutions.

It was difficult to obtain clear results from the spectrophotometric measurements, as the samples where very dark and viscous. There were big particles of mealworm and exoskeleton in the samples. This also showed in the measurements when there was more particles in one of the samples compared to the rest, it was difficult to ensure that the extracted samples were the same. This method can be improved to make it more reliable when reading results from spectrophotometric measurements during the enzymatic extraction. Adding more buffer or less sample to be able to pipette when the enzyme is added will improve the method. The samples were only recorded for one hour, so to map the activity more accurately it can be measured for more than 60 minutes before termination. Alternatively the activity could be measured with another method.

The nitrogen content in the mealworm extract is lower than the mean value for the raw material, approximately 2% below. This can indicate that some protein has been removed from the extract. The nitrogen content in the exoskeleton extract was approximately 9.5%, which is similar to the mean value from the raw exoskeleton's nitrogen content. The amine detection results of the supernatant from the enzymatic extraction of mealworm contains over $100.00 \mu g/mL$, which could indicate that protein has been removed from the raw materials and that a higher part of the nitrogen content in the extracts is from chitin compared to the raw material. The results from the supernatant from the exoskeleton is approximately $75 \mu g/mL$ which indicates the same. However, these amine detection results have large standard deviations because of high variability in concentrations in the samples which indicates that they are not reliable.

According to the CHNS results for the supernatants from the enzymatic extraction there was approximately 9.5% nitrogen in the mealworm supernatant. The calculated protein content is approximately 60%. This can also indicate that protein has been removed from the raw material, and that a larger part of the nitrogen content in the extract is chitin than in the untreated, whole mealworm. The exoskeleton supernatant had similar results with a nitrogen content of approximately 9% and a calculated protein content of approximately 60%. If the total volume of the supernatants had been measured it could have been used to estimate how much of the protein in total that was extracted from the raw materials.

The C/N ration for EE is 6.24 $\pm$0.36 and for ME is 5.12 $\pm$0.24. The mean C/N ratio calculated from EE is the sample that is closest to the reference value of chitin. ME is not close to the C/N ratio of chitin. This could be due to incomplete deproteinisation, as the nitrogen levels in the extracts did not change significantly. However, the presence of nitrogen in the supernatants and the calculated protein content suggests that it was at least partially complete. It is possible that the alcalase was not properly distributed in the sample because the amount of buffer solution was too low to cover the whole sample, which made it difficult to agitate.

5.4 Sustainability and life cycle assessment

With regards to sustainability the chemical method utilises amounts of chemicals which are not sustainable. However, using the waste materials from mealworm production can be beneficial. As of today, the exoskeleton is a waste product which is composted. Since the mealworm and its exoskeleton is organic and can be composted successfully is composting a sustainable method to discard the waste from production[35]. Comparing these alternatives, the composting has a lower impact on the surrounding environment.

If it is possible to use the exoskeleton as a raw material for chitin extraction, this will match with the United Nations sustainability goal 12. It highlights the importance of responsible consumption and

[35] Cooperband n.d.

production patterns. This can contribute to a circular economy by recycling of materials and utilising them for their unused components. With a successful chitin extraction it can be possible to use the chitin as a component in other materials. The fact that chitin is able to make films together with being biodegradable is perceived as beneficial because it can factor in on the use of materials, specifically it could reduce the use of petroleum-based plastics. If petroleum based materials are reduced, it will contribute more to sustainability goal 12 as well as sustainability goal 14 which aims to reduce pollution of the ocean.

However, the process of extracting chitin from the exoskeleton of mealworm can be expensive. The high costs will not be sustainable over time. It is a process that requires time, resources, space and workforce. Since the chitin content in mealworm is relatively low, this can seem disadvantegous and composting can be considered a more sustainable option. Despite the low possible yield, chitin from mealworm can possess different properties compared to the chitin that can be extracted from crustaceans which could expand the areas of use. However, one study that compared the methods found that the enzymatic approach could not be guaranteed as a greener alternative because the extract yields were higher for the chemical method[36]. Chitin extraction from mealworm requires similar amounts of resources as the exoskeleton. A third, possibly more sustainable option, is to utilise the microbial extraction method.

5.5 Further research

There is further research that can be conducted for chitin extraction from mealworm and its exoskeleton based on the results that have been presented. Chitin has versatile properties which can be utilised in different industries, so it could be very beneficial to use what is normally waste as a raw material for chitin extraction. A third, combined method of enzymatic and chemical extraction which was not investigated in this project but could possibly give a pure product of chitin from mealworm.

Kjeldahl was not used to analyse the processed materials because the data from CHNS showed similar results, but it had lower standard deviations and can be considered a more precise way to measure nitrogen and protein content. Kjeldahl could have been used to analyse the supernatans, but the time was allocated towards analysing the extracts. Extraction of other components of the mealworm is possible, such as proteins from the supernatants and lipids.

The supernatant could be monitored during demineralisation by extraction several samples over time and checking their ash content. The ash content in the supernatant should increase over time if it is successfully extracted from the raw material. During deproteinisation the supernatant could be monitored with OPA og Bradford analysis for example, the protein concentration of the supernatant should increase over time if the deproteinisation is successful. The extracts were not pure or homogeneous enough to be prepared as samples for NMR analysis. If the demineralisation and deproteinisation steps were monitored to ensure that they were successful, perhaps a more pure form of chitin could be extracted. It should be possible to analyse this extract with NMR.

Another way to extract chitin is with the use of bacteria. However, a microbiological approach for extracting chitin can be complicated due to the required conditions for microbial growth. The samples have to be held at specific and different optimum temperatures in the correct conditions for an efficient extraction. This method is more time consuming due to the growing of bacteria and fermentation of samples. That is why it was not chosen to be done in this project. For the characterisation of the processed materials, pure chitin could be analysed with the same methods to have a reference. This would make it easier to see if the extracts compare to the characteristics of pure chitin. Measuring a total yield of chitin in the different extractions would be useful. It obtains quantitative data that gives a concrete measurement of the desired product. This makes it easier to analyse the effectiveness of the extraction. Comparing yields of multiple extractions makes it possible to determine what method has the highest yield of chitin. The samples were quite viscous, possibly because of the presence of fat and wax in the samples, which would have made it difficult to get accurate measurements of the yield from the extracts that were analysed in this research.

[36] Ponnusamy and Mani 2022.

6 Conclusion

To conclude, the protocol that was used for chemical extraction required more time than the protocol that was used for the enzymatic extraction. The fat and wax in the raw materials were not removed, this could be a beneficial step to include before the chitin extraction happens. There were no notable, large differences in the results from the extraction methods. There were slight variations between the mealworm powder and the exoskeleton powder for each method, with the largest difference being between the raw materials in the chemical extraction. This indicates show that the exoskeleton is as good a raw material as the whole mealworm for chitin. The CHNS analysis of the supernatants indicated that the samples had been deproteinised to some degree, the exact measurement is unknown. This could imply that the chitin concentration in the extracts were higher than the concentrations in the raw materials. However, the extracts were not sufficiently characterised to conclude with this and further research is needed.

The interim goals that were defined in the beginning of the thesis were partly reached. The efficacy of the chemical and enzymatic protocols for chitin extraction could not be totally proven, so steps for improvement were discussed. This affected the following goals which was to extract chitin from the raw materials by demineralisation and deproteionisation in the chemical protocol, and deproteinisation in the enzymatic protocol. Deproteinisation could be seen as partly successful because of the protein content in the supernantants, but the characterisation of the extracts was lacking and could not support this. The last goal was to characterise the extracts and supernatants to find chitin yield, despite not finding a chitin yield the extracts and supernatants were partially characterised. So the answer the thesis question, theoretically the chemical and enzymatic extraction protocols should work but they will need some practical adjustments and more research. The monitoring of reactions and the characterisation of samples have to be more thorough to reach a clearer answer to the thesis question. We recommend that the protocols for chitin extraction specifically from the mealworm exoskeleton are further explored, as this could be a good source for waste recycling. This could have positive effects on the environment and establish a circular economy for this product.

Bibliography

AAT Bioquest, Inc. (2023). *Carbonate-Bicarbonate Buffer (pH 9.2 to 10.6) Preparation and Recipe*. AAT Bioquest. URL: https://www.aatbio.com/resources/buffer-preparations-and-recipes/carbonate-bicarbonate-buffer-ph-9-2-to-10-6 (visited on 26/05/2023).

Borremans, An, Ruben Smets and Leen Van Campenhout (15th July 2020). 'Fermentation Versus Meat Preservatives to Extend the Shelf Life of Mealworm (Tenebrio molitor) Paste for Feed and Food Applications'. In: *Frontiers in Microbiology* 11, p. 1510. ISSN: 1664-302X. DOI: 10.3389/fmicb.2020.01510.

Chawla, Surinder, Sweetie Kanatt and A.K. Sharma (5th June 2015). 'Chitosan'. In: pp. 219–246. ISSN: 978-3-319-16297-3. DOI: 10.1007/978-3-319-16298-0_13.

Cooperband, Leslie (n.d.). 'The Art and Science of Composting'. In: ().

Einbu, Aslak (2007). 'Characterisation of Chitin and a Study of its Acid-Catalysed Hydrolysis'. Accepted: 2014-12-19T13:14:05Z ISBN: 9788247116432. Doctoral thesis. Fakultet for naturvitenskap og teknologi.

Fabris, R., C. W. K. Chow and M. Drikas (1st Apr. 2010). 'Evaluation of chitosan as a natural coagulant for drinking water treatment'. In: *Water Science and Technology* 61.8, pp. 2119–2128. ISSN: 0273-1223. DOI: 10.2166/wst.2010.833.

FN (15th May 2023). *Ansvarlig forbruk og produksjon*. URL: https://www.fn.no/om-fn/fns-baerekraftsmaal/ansvarlig-forbruk-og-produksjon (visited on 16/05/2023).

GEOMAR (2023). *Determination of CNS in sediment samples with the CARLO ERBA Elemental Analyzer*. GEOMAR. URL: https://www.geomar.de/en/research/fb2/fb2-mg/benthic-biogeochemistry/mg-analytik/determination-of-cns (visited on 14/05/2023).

Hauschild, Michael, Ralph Rosenbaum and Stig Olsen (2018). *Life Cycle Assessment, Theory and Practice*. Springer International Publishing. ISBN: 978-3-319-56474-6.

Hayes, Maria et al. (2008). 'Mining marine shellfish wastes for bioactive molecules: Chitin and chitosan ndash; Part A: extraction methods'. In: *Biotechnology Journal* 3.7, pp. 871–877. ISSN: 1860-7314. DOI: 10.1002/biot.200700197.

Interchim (2023). *OPA, amine detection reagent*. URL: https://www.interchim.fr/ft/0/02727A.pdf (visited on 15/05/2023).

Jang, Mi-Kyeong et al. (2004). 'Physicochemical characterization of -chitin, -chitin, and -chitin separated from natural resources'. In: *Journal of Polymer Science Part A: Polymer Chemistry* 42.14. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/pola.20176, pp. 3423–3432. ISSN: 1099-0518. DOI: 10.1002/pola.20176.

Jantzen da Silva Lucas, Andressa et al. (1st May 2021). 'Extraction, physicochemical characterization, and morphological properties of chitin and chitosan from cuticles of edible insects'. In: *Food Chemistry* 343, p. 128550. ISSN: 0308-8146. DOI: 10.1016/j.foodchem.2020.128550.

Jung, W. J. et al. (1st June 2006). 'Extraction of chitin from red crab shell waste by cofermentation with Lactobacillus paracasei subsp. tolerans KCTC-3074 and Serratia marcescens FS-3'. In: *Applied Microbiology and Biotechnology* 71.2, pp. 234–237. ISSN: 1432-0614. DOI: 10.1007/s00253-005-0126-3.

Mihaljev, Željko A et al. (2015). 'Comparison of the Kjeldahl method, Dumas method and NIR method for total nitrogen determination in meat and meat products'. In: *Journal of Agroalimentary Processes and Technologies*.

Moussian, Bernard (2019). 'Chitin: Structure, Chemistry and Biology'. In: *Targeting Chitin-containing Organisms*. Ed. by Qing Yang and Tamo Fukamizo. Advances in Experimental Medicine and Biology. Singapore: Springer, pp. 5–18. ISBN: 9789811373183. DOI: 10.1007/978-981-13-7318-3_2.

NC State University (2023). The Exoskeleton. URL: https://genent.cals.ncsu.edu/bug-bytes/exoskeleton/ (visited on 28/05/2023).

Novozymes (2023). *Alcalase®*. URL: https://www.novozymes.com/en/products/plant-based-foods/alcalase (visited on 16/05/2023).

Pakizeh, Majid, Ali Moradi and Toktam Ghassemi (5th Oct. 2021). 'Chemical extraction and modification of chitin and chitosan from shrimp shells'. In: *European Polymer Journal* 159, p. 110709. ISSN: 0014-3057. DOI: 10.1016/j.eurpolymj.2021.110709.

Percot, Aline, Christophe Viton and Alain Domard (1st Jan. 2003). 'Optimization of Chitin Extraction from Shrimp Shells'. In: *Biomacromolecules* 4.1. Publisher: American Chemical Society, pp. 12–18. ISSN: 1525-7797. DOI: 10.1021/bm025602k.

Płotka-Wasylka, Justyna et al. (1st June 2017). 'Extraction with environmentally friendly solvents'. In: *TrAC Trends in Analytical Chemistry* 91, pp. 12–25. ISSN: 0165-9936. DOI: 10.1016/j.trac.2017.03.006.

Ponnusamy, Prabaharan Graceraj and Sudhagar Mani (1st Mar. 2022). 'Life cycle assessment of manufacturing cellulose nanofibril-reinforced chitosan composite films for packaging applications'. In: *The International Journal of Life Cycle Assessment* 27.3, pp. 380–394. ISSN: 1614-7502. DOI: 10.1007/s11367-022-02035-y.

Shahidi, Fereidoon, Janak Kamil Vidana Arachchi and You-Jin Jeon (1st Feb. 1999). 'Food applications of chitin and chitosans'. In: *Trends in Food Science & Technology* 10.2, pp. 37–51. ISSN: 0924-2244. DOI: 10.1016/S0924-2244(99)00017-5.

Shin, Chae-Shim, Do-Yeong Kim and Weon-Sun Shin (15th Mar. 2019). 'Characterization of chitosan extracted from Mealworm Beetle (Tenebrio molitor, Zophobas morio) and Rhinoceros Beetle (Allomyrina dichotoma) and their antibacterial activities'. In: *International Journal of Biological Macromolecules* 125, pp. 72–77. ISSN: 0141-8130. DOI: 10.1016/j.ijbiomac.2018.11.242.

Siemianowska, Ewa et al. (27th June 2013). 'Larvae of mealworm (*Tenebrio molitor* L.) as European novel food'. In: 2013. Publisher: Scientific Research Publishing. DOI: 10.4236/as.2013.46041.

Sivashankari, P. R. and M. Prabaharan (1st Jan. 2017). '5 - Deacetylation modification techniques of chitin and chitosan'. In: *Chitosan Based Biomaterials Volume 1*. Ed. by J. Amber Jennings and Joel D. Bumgardner. Woodhead Publishing, pp. 117–133. ISBN: 978-0-08-100230-8. DOI: 10.1016/B978-0-08-100230-8.00005-4.

Son, Yang-Ju et al. (Mar. 2021). 'Determination of Carbohydrate Composition in Mealworm (Tenebrio molitor L.) Larvae and Characterization of Mealworm Chitin and Chitosan'. In: *Foods* 10.3. Number: 3 Publisher: Multidisciplinary Digital Publishing Institute, p. 640. ISSN: 2304-8158. DOI: 10.3390/foods10030640.

Tacias-Pascacio, Veymar G. et al. (15th Dec. 2020). 'Use of Alcalase in the production of bioactive peptides: A review'. In: *International Journal of Biological Macromolecules* 165, pp. 2143–2196. ISSN: 0141-8130. DOI: 10.1016/j.ijbiomac.2020.10.060.

Tan, Yun Nian, Pei Pei Lee and Wei Ning Chen (28th Jan. 2020). 'Microbial extraction of chitin from seafood waste using sugars derived from fruit waste-stream'. In: *AMB Express* 10.1, p. 17. ISSN: 2191-0855. DOI: 10.1186/s13568-020-0954-7.

Varma, Rahul and Sugumar Vasudevan (6th Aug. 2020). 'Extraction, Characterization, and Antimicrobial Activity of Chitosan from Horse Mussel Modiolus modiolus'. In: *ACS Omega* 5.32, pp. 20224–20230. ISSN: 2470-1343. DOI: 10.1021/acsomega.0c01903.

Yang, Anshu et al. (2017). 'Enzymatic characterisation of the immobilised Alcalase to hydrolyse egg white protein for potential allergenicity reduction'. In: *Journal of the Science of Food and Agriculture* 97.1. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/jsfa.7712, pp. 199–206. ISSN: 1097-0010. DOI: 10.1002/jsfa.7712.

Zaku, S G et al. (13th July 2011). 'Extraction and characterization of chitin; a functional biopolymer obtained from scales of common carp fish (Cyprinus carpio l.): A lesser known source'. In: *Afr. J. Food Sci.*

Appendix 1: Nutritional contents for fresh and dried mealworm

Næringsinnhold tørket melorm

Generelt

Analyse av næringsinnhold gjennomført av Eurofins i 2021. Fiber regnes å være hovedsakelig kitin**.

Næringsinnhold	per 100g	% av referanse*
Energi kJ	2181 kJ	26 %
Energi kcal	521 kcal	26 %
Fett	31,9 g	46 %
- hvorav mettet fett	8,3 g	42 %
Karbohydrater	6,1 g	2 %
- hvorav sukkerarter	0 g	0 %
Fiber	4,9 g	16 %
Protein	50,4 g	101 %
Salt	0,45 g	8 %
Aske	3,6 g	-
Vanninnhold	3,1 g	-

Mineraler

Analyse av mineraler gjennomført av Eurofins i 2021.

Mineral	Mengde (mg/100 g)	% av referanse*
Kalium (K)	1100	55 %
Fosfor (P)	900	129 %
Magnesium (Mg)	250	67 %
Kalsium (Ca)	75	9 %
Sink (Zn)	16	160 %
Jern (Fe)	4,8	34 %
Molybden (Mo)	0,11	220 %
Mangan (Mn)	0,97	49 %
Kobber (Cu)	2,2	220 %
Jod (I)	0,009	6 %
Fluor (F)	< 0,1	-
Bly (Pb)	< 0,002	-
Selen (Se)	< 0,005	-

*Referanseverdier: https://lovdata.no/dokument/SF/forskrift/2014-11-28-1497/KAPITTEL_4-1-1-20-1#KAPITTEL_4-1-1-20-1

** Yang-Ju et al. (2021). **Determination of Carbohydrate Composition in Mealworm (*Tenebrio molitor* L.) Larvae and Characterization of Mealworm Chitin and Chitosan**. https://doi.org/10.3390/foods10030640

Næringsinnhold ferske larver

Basert på verdier fra tørkede larver, omregnet til ferske (frosne larver).
Vanninnhold kan variere fra batch til batch. Ligger mellom 60% og 70%.

Næringsinnhold		**Mineraler**	**mg/100 g**
Vann	63%	Potassium (K)	420,0
Tørrstoff	37%	Phosphorus (P)	343,7
Energi kJ	833	Magnesium (Mg)	95,5
Energi kcal	199	Sodium (Na)	68,7
		Calcium (Ca)	28,6
	g/100g	Zinc (Zn)	6,11
Protein	19,2	Iron (Fe)	1,83
Fett	12,2	Molybdenum (Mo)	0,04
- hvorav mettet fett	3,2	Manganese (Mn)	0,37
Karbohydrater	2,3	Copper (Cu)	0,84
- hvorav sukkerarter	0,0	Iodine (I)	0,003
Fiber	1,9	Fluoride (F)	less than 0,1
Salt	0,2	Lead (Pb)	less than 0,002
		Selenium (Se)	less than 0,005

Fettsyreprofil

Enkelte fettsyrer er ekskludert fra denne tabellen da de kun fins i veldig små mengder.
Analysert av Eurofins i 2021

Fettsyre	**Struktur**	**% av total FA**
Mettet fett	**Totalt**	**27,2 %**
Myristidinsyre	C14:0	2,1 %
Palmitinsyre	C15:0	21,1 %
Stearinsyre	C18:0	3,2 %
Enumettet fett	**Totalt**	**43,6 %**
Palmitoleinsyre	C16:1 n-7	1,3 %
Oljesyre	C18:1 n-9	42,1 %
Flerumettet fett	**Totalt**	**27,4 %**
Linolsyre	C18:2 n-6	26,0 %
Alfa-linolensyre	C18:3 n-3	1,3 %
Sum omega-6		26,1 %
Sum omega-3		1,3 %
Omega-6/Omega-3 rate		20,04

Aminosyreprofil

Analysert av Eurofins i 2021.

Aminosyre	g/100g tørket larve	mg/g protein	% av total protein
Alanin	5,41	80	8 %
Arginin	3,48	60	6 %
Asparginsyre	5,24	77	7 %
Glutaminsyre	8,06	133	13 %
Glysin	3,3	60	6 %
Histidin	1,83	38	4 %
Hydroksyprolin	0	0	0 %
Isoleusin	2,77	49	5 %
Leusin	4,7	82	8 %
Lysin	3,57	65	6 %
Ornitin	0	0	0 %
Phenylalanin	2,16	44	4 %
Prolin	4,34	74	7 %
Serin	2,87	55	5 %
Treonin	2,51	41	4 %
Tyrosin	3,76	80	8 %
Valin	3,92	69	7 %
Tryptofan	0,685	10	1 %
Cystein + cystine	0,514	11	1 %
Metionin	0,765	20	2 %

Appendix 2: Raw data and mean values with standard deviation from Kjeldahl analysis

Raw data

Tube	Sample	Weight (g)	Titr. volum cm^3	Nitrogen %	Protein %
1	Blind	-	0.318	-	-
2	Blind	-	0.247	-	-
3	IK - Glycin	0.2054	14.277	19.04	-
4	Parallell 1 - exoskeleton	1.0757	30.78	7.93	49.58
5	Parallell 2 - exoskeleton	1.0154	29.09	7.94	49.61
6	Parallell 3 - exoskeleton	1.036	29.997	8.03	50.16
7	Parallell 1 - mealworm	1.0283	35.347	9.54	59.64
8	Parallell 2 - mealworm	1.2015	34.214	9.48	59.26
9	Parallell 3 - mealworm	1.0966	37.592	9.52	59.51
10	IK - Glycin	0.2119	13.856	17.9	-

Mean values with standard deviation

	Nitrogen %	Protein %
Mealworm	9.52 ±0.03	59.5 ±0.20
Exoskeleton	7.97 ±0.05	49.8 ±0.32

Appendix 3: Raw data from CHNS analysis of mealworm and exoskeleton

No.	Weight [mg]	Name	Method	Moisture [%]	N Area	C Area	H Area	S Area	N [%]	C [%]	H [%]	S [%]	C/N ratio
1	1	RunIn	Blank with O	0	946	9268	14364	0	2.59	39.59	18.618	0	15.2573
2	1	RunIn	Blank with O	0	46	211	354	7	0.12	0.85	0.657	0.064	7.2375
3	1	Blnk	Blank with O	0	9	116	231	0	0	0	0	0	0
4	1	Blnk	Blank with O	0	13	90	144	27	0	0	0	0	0
5	4.237	RunIn	5mg90s	0	2806	28027	11289	314	1.99	28.11	3.49	0.706	14.1041
6	3.9805	RunIn	5mg90s	0	2566	26230	11057	271	1.93	27.96	3.639	0.643	14.4653
7	4.1015	sulfanilic acid	5mg90s	0	22703	41433	13217	7301	16.26	41.81	4.65	18.62	2.5713
8	3.8705	sulfanilic acid	5mg90s	0	21260	38882	12253	7048	16.26	41.81	4.65	18.62	2.5713
9	3.9005	sulfanilic acid	5mg90s	0	21708	39531	12467	7246	16.26	41.81	4.65	18.62	2.5713
22	3.237	Exoskeleton	5mg90s	0	14108	53312	24600	116	12.71	68.43	10.8	0.35	5.3836
23	4.053	Exoskeleton	5mg90s	0	13284	51513	23781	109	9.58	52.8	8.348	0.261	5.5104
24	4.1045	Exoskeleton	5mg90s	0	14649	56865	26477	106	10.42	57.6	9.144	0.252	5.5281
25	4.192	Mealworm	5mg90s	0	11516	39593	17305	29	08.07	39.12	5.938	0.068	4.8461
26	4.025	Mealworm	5mg90s	0	14643	49093	22048	37	10.62	50.64	7.814	0.09	4.7681
27	4.4025	Mealworm	5mg90s	0	14985	49696	22242	52	9.94	46.87	7.204	0.114	4.7143
28	0			0	0	0	0	0	0	0	0	0	0

C/H ratio	N Factor	C Factor	H Factor	S Factor	N Blank	C Blank	H Blank	S Blank	Prot. Factor	Prot. [%]	Memo	Info	Date	Time
2.1264	1	1	1	1	0	0	0	0	3.8	9.86		Snp	12.04.2023	13:13
1.2953	1	1	1	1	0	0	0	0	3.8	0.447		Nu,Cu,Su	12.04.2023	13:22
0	1	1	1	1	9	116	231	0	3.8		0		12.04.2023	13:31
0	1	1	1	1	13	90	144	27	3.8		0		12.04.2023	13:40
8.0532	1	1	1	1	0	0	0	0	3.8	7.572			12.04.2023	13:50
7.6837	1	1	1	1	0	0	0	0	3.8	7.345			12.04.2023	14:00
8.9914	0.9722	0.9681	1.1098	1.073	0	0	0	0	3.8	61.788			12.04.2023	14:09
8.9914	0.9804	0.9743	1.1282	1.0493	0	0	0	0	3.8	61.788			12.04.2023	14:19
8.9914	0.9673	0.9655	1.1162	1.0283	0	0	0	0	3.8	61.788			12.04.2023	14:29
6.3365	0.9733	0.9693	1.1174	1.0502	0	0	0	0	3.8	48.304		Su	12.04.2023	16:36
6.3243	0.9733	0.9693	1.1174	1.0502	0	0	0	0	3.8	36.408		Su	12.04.2023	16:45
6.299	0.9733	0.9693	1.1174	1.0502	0	0	0	0	3.8	39.594		Su	12.04.2023	16:55
6.5876	0.9733	0.9693	1.1174	1.0502	0	0	0	0	3.8	30.673		Su	12.04.2023	17:05
6.4811	0.9733	0.9693	1.1174	1.0502	0	0	0	0	3.8	40.359		Su	12.04.2023	17:15
6.5062	0.9733	0.9693	1.1174	1.0502	0	0	0	0	3.8	37.783		Su	12.04.2023	17:25
0	0.9733	0.9693	1.1174	1.0502	0	0	0	0	3.8		0			

Appendix 4: Raw data from CHNS analysis of processed materials and supernatants

The samples marked with red and marked with F at the end of the name are samples from methods done incorrectly.

No.	Weight [mg]	Name	Method	Moisture [%]	N Area	C Area	H Area	S Area	N [%]	C [%]	H [%]	S [%]	C/N ratio	C/H ratio
1	1	RunIn	Blank with O	0	15	235	794	31	0,04	0,95	1,297	291	249,942	7,309
2	1	RunIn	Blank with O	0	19	63	370	28	0,05	0,25	0,68	256	53,016	3,719
3	1	Blnk	Blank with O	0	18	59	267	32	0	0	0	0	0	0
4	1	Blnk	Blank with O	0	4	54	266	29	0	0	0	0	0	0
5	35,745	RunIn	5mg90s	0	14319	43828	18024	155	12,01	52,46	6,482	401	4,368	80,927
6	3,161	RunIn	5mg90s	0	12597	38744	17279	152	11,99	52,36	7,037	445	43,655	74,398
7	46,225	sulfanilic acid	5mg90s	0	25696	46659	15166	8511	16,26	41,81	4,65	18,62	25,713	89,914
8	4,292	sulfanilic acid	5mg90s	0	19669	35936	11115	6765	16,26	41,81	4,65	18,62	25,713	89,914
9	37,595	sulfanilic acid	5mg90s	0	20808	37992	11807	7182	16,26	41,81	4,65	18,62	25,713	89,914
10	3.447	EE	5mg90s	0	10374	46995	20406	70	9.47	60.45	9.093	0.202	6.3847	6.6476
11	4.1735	EE	5mg90s	0	10441	48215	20969	39	7.87	51.23	7.71	0.094	6.5112	6.645
12	3.1225	EE	5mg90s	0	11192	46208	20269	32	11.25	65.6	9.973	0.102	5.8305	6.5777
13	3.295	EESN	5mg90s	0	11735	37130	19348	139	11.16	49.82	9.036	0.422	4.4626	5.5131
14	4.1475	EESN	5mg90s	0	10258	32437	16943	124	7.78	34.5	6.316	0.301	4.4331	5.4629
15	3.3685	EESN	5mg90s	0	10191	32702	16681	115	9.52	42.83	7.66	0.341	4.4983	5.5915
16	4.198	EESN F	5mg90s	0	10293	31535	17556	110	7.71	33.12	6.457	0.263	4.2934	5.1297
17	3.1835	EESN F	5mg90s	0	9476	29463	16269	103	9.38	40.76	7.913	0.324	4.3433	5.1512
18	4.0095	EESN F	5mg90s	0	9911	30421	16899	99	7.79	33.43	6.517	0.248	4.2945	5.1306
19	3.1905	ECSN	5mg90s	0	10418	35250	17937	176	10.27	48.81	8.674	0.554	4.7517	5.6263
20	3.3365	ECSN	5mg90s	0	9337	33159	16073	142	8.83	43.86	7.462	0.426	4.9696	5.8776
21	3.877	ECSN	5mg90s	0	9723	34825	16980	146	7.9	39.67	6.771	0.377	5.0205	5.8593
22	3.516	EC F	5mg90s	0	10412	39288	16288	24	9.31	49.44	7.172	0.067	5.3073	6.8926
23	3.6765	EC F	5mg90s	0	10857	41081	17209	22	9.28	49.46	7.233	0.06	5.331	6.8389
24	3.604	EC F	5mg90s	0	10476	39642	16561	19	9.14	48.67	7.11	0.052	5.3238	6.8451
25	4.687	EC	5mg90s	0	15715	58193	25453	24	10.46	55.16	8.279	0.052	5.2707	6.6625
26	4.694	EC	5mg90s	0	15778	58495	25826	32	10.49	55.36	8.384	0.069	5.2768	6.6039
27	4.0305	EC	5mg90s	0	13664	50011	21742	24	10.56	55.05	8.268	0.059	5.2107	6.6583
28	3.97	EC F	5mg90s	0	10189	50988	22434	10	8.08	56.99	8.652	0.026	7.0552	6.5871
29	3.5885	EC F	5mg90s	0	9614	46259	20002	14	8.44	57.15	8.567	0.04	6.7675	6.6701
30	3.7925	EC F	5mg90s	0	11468	49983	22086	14	9.49	58.47	8.921	0.038	6.1645	6.5543
31	3.845	MCSN 1	5mg90s	0	6764	45420	23791	55	5.57	52.36	9.455	0.142	9.3968	5.5375
32	3.1245	MCSN 1	5mg90s	0	5407	37504	18983	39	5.48	53.07	9.356	0.127	9.6816	5.6726
33	3.74	MCSN 1	5mg90s	0	6954	42747	22663	59	5.89	50.62	9.275	0.159	8.598	5.4581
34	3.645	MC	5mg90s	0	12788	49894	22437	114	10.96	60.73	9.424	0.312	5.5395	6.4436
35	3.6885	MC	5mg90s	0	12841	49285	22113	101	10.88	59.27	9.184	0.274	5.4497	6.4541
36	3.729	MC	5mg90s	0	13388	51735	23480	114	11.2	61.57	9.626	0.306	5.4984	6.3967
37	3.7095	MC F	5mg90s	0	15097	47780	21300	131	12.69	57.12	8.807	0.355	4.5007	6.4854
38	3.8005	MC F	5mg90s	0	15485	48772	21750	138	12.71	56.92	8.771	0.365	4.4772	6.4893
39	4.0275	MC F	5mg90s	0	16457	51868	23386	153	12.77	57.16	8.878	0.38	4.4771	6.4381
40	3.5005	MCSN 2	5mg90s	0	10682	36115	19062	23	9.59	45.59	8.384	0.066	4.7531	5.4378
41	3.4485	MCSN 2	5mg90s	0	10519	35582	18589	30	9.59	45.58	8.307	0.086	4.7523	5.4876
42	3.686	MCSN 2	5mg90s	0	11250	37849	19787	38	9.58	45.41	8.254	0.103	4.7403	5.5009
43	3.6755	MESN	5mg90s	0	10934	37082	18479	121	9.34	44.6	7.749	0.331	4.773	5.7556
44	3.5855	MESN	5mg90s	0	11034	35001	17567	117	9.66	43.12	7.565	0.328	4.4613	5.6995
45	3.4105	MESN	5mg90s	0	10325	34123	16996	101	9.52	44.17	7.704	0.296	4.6378	5.7341
46	3.9575	ME F	5mg90s	0	14604	53821	24235	126	11.5	60.38	9.351	0.32	5.2512	6.4567
47	3.5945	MC F	5mg90s	0	13173	48909	21806	115	11.44	60.35	9.297	0.322	5.2766	6.4916
48	3.695	MC F	5mg90s	0	13339	50046	22408	123	11.26	60.09	9.285	0.335	5.3361	6.4714
49	3.428	ME	5mg90s	0	9654	34715	17447	91	8.87	44.72	7.86	0.266	5.0396	5.6898
50	3.755	ME	5mg90s	0	10819	37891	19441	103	9.05	44.62	7.966	0.276	4.9286	5.6017
51	3.448	ME	5mg90s	0	9375	36080	17953	87	8.57	46.24	8.033	0.255	5.393	5.756
52	3.431	MC F	5mg90s	0	13911	44677	19408	126	12.62	57.7	8.704	0.367	4.5731	6.6294
53	3.3395	MC F	5mg90s	0	13380	43901	19111	114	12.5	58.24	8.81	0.342	4.6604	6.6107
54	3.6075	MC F	5mg90s	0	14548	47264	20763	123	12.56	58.09	8.835	0.343	4.6237	6.575

N Factor	C Factor	H Factor	S Factor	N Blank	C Blank	H Blank	S Blank	Prot. Factor	Prot. [%]	SD	Info	Date Time
1	1	1	1	0	0	0	0	6,25	237		Nu,Cu,Su	23.05.2023 12:46
1	1	1	1	0	0	0	0	6,25	298		Nu,Cu,Su	23.05.2023 12:55
1	1	1	1	18	59	267	32	6,25	0			23.05.2023 13:04
1	1	1	1	4	54	266	29	6,25	0			23.05.2023 13:13
1	1	1	1	0	0	0	0	6,25	75,058		Su	23.05.2023 13:23
1	1	1	1	0	0	0	0	6,25	74,958		Su	23.05.2023 13:33
9,672	9,675	10,957	10,359	0	0	0	0	6,25	101,625			23.05.2023 13:43
11,762	11,703	13,711	12,127	0	0	0	0	6,25	101,625			23.05.2023 13:52
9,732	9,688	11,335	1	0	0	0	0	6,25	101,625			23.05.2023 14:02
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	59.173		Su	5/23/2023 14:12
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	49.179		Su	5/23/2023 14:22
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	70.32		Su	5/23/2023 14:32
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	69.769		Su	5/23/2023 14:42
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	48.643		Su	5/23/2023 14:52
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	59.513		Su	5/23/2023 15:02
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	48.218		Su	5/23/2023 15:12
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	58.653		Su	5/23/2023 15:22
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	48.657		Su	5/23/2023 15:31
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	64.194		Su	5/23/2023 15:41
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	55.159		Su	5/23/2023 15:51
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	49.387		Su	5/23/2023 16:01
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	58.218		Su	5/23/2023 16:11
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	57.991		Su	5/23/2023 16:21
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	57.136		Su	5/23/2023 16:31
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	65.406		Su	5/23/2023 16:41
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	65.575		Su	5/23/2023 16:51
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	66.03		Su	5/23/2023 17:01
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	50.487		Su	5/23/2023 17:11
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	52.776		Su	5/23/2023 17:21
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	59.282		Su	5/23/2023 17:31
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	34.822		Su	5/23/2023 17:41
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	34.26		Su	5/23/2023 17:51
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	36.797		Su	5/23/2023 18:01
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	68.517		Su	5/23/2023 18:11
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	67.976		Su	5/23/2023 18:21
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	69.988		Su	5/23/2023 18:31
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	79.318		Su	5/23/2023 18:41
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	79.458		Su	5/23/2023 18:51
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	79.789		Su	5/23/2023 19:01
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	59.95		Su	5/23/2023 19:11
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	59.951		Su	5/23/2023 19:21
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	59.867		Su	5/23/2023 19:31
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	58.402		Su	5/23/2023 19:41
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	60.403		Su	5/23/2023 19:51
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	59.53		Su	5/23/2023 20:01
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	71.862		Su	5/23/2023 20:11
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	71.487		Su	5/23/2023 20:21
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	70.381		Su	5/23/2023 20:31
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	55.466		Su	5/23/2023 20:41
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	56.585		Su	5/23/2023 20:51
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	53.587		Su	5/23/2023 21:01
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	78.859		Su	5/23/2023 21:11
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	78.107		Su	5/23/2023 21:21
1.0388	1.0356	1.2001	1.0829	0	0	0	0	6.25	78.525		Su	5/23/2023 21:31

Appendix 5: Calculated means and standard deviations from CHNS analysis

	Nitrogen %	Carbon %	Hydrogen %	Sulfur %	Protein %	C/N ratio
Dried mealworm	10.90 ±1.32	59.61 ±6.54	9.43 ±1.02	0.09 ±0.02	68.15 ±8.27	4.78 ± 0.07
Exoskeleton	9.54 ±1.08	45.54 ±4.80	6.99 ±0.78	0.27 ±0.07	59.65 ±6.74	5,47 ±0.08
EE	9.49 ±1.69	42.38 ±7.28	7.67 ±1.14	0.13 ± 0.06	59.56 ±10.58	6.24 ± 0.36
EESN	8.57 ±1.69	46.24 ±7.67	8.033 ±1.36	0.35 ± 0.06	59.31 ±10.56	4.46 ± 0.03
ME	8.83 ±0.24	45.19 ±0.91	7.95 ±0.09	0.27 ± 0.01	55.21 ±1.51	5.12 ± 0.24
MESN	9.51 ±0.16	43.96 ±0.76	7.67 ±0.10	0.32 ± 0.02	59.45 ±1.00	4.62 ± 0.16
EC	10.50 ±0.05	55.19 ±0.16	8.31 ±0.06	0.06 ± 0.01	65.67 ±0.32	5.25 ± 0.04
ECSN	9.00 ±1.19	44.11 ±4.58	7.64 ±0.96	0.45 ± 0.09	56.25 ±7.46	4.91 ± 0.14
MC	11.00 ±0.17	60.5 ±1.16	9.4 ±0.22	0.3 ±0.02	68.8 ±1.04	5.5 ±0.04
MCSN1	5.65 ±0.22	52.02 ±1.26	9.36 ±0.09	0.14 ± 0.01	35.29 ±1.33	9.23 ± 0.56
MCSN2	9.59 ±0.01	45.53 ±0.10	8.32 ±0.07	0.08 ± 0.02	59.92 ±0.05	4.75 ± 0.01

Appendix 6: Raw data and mean values with standard deviation from amine detection analysis (OPA) of the raw materials and supernatants from the enzymatic and chemical extraction methods

The samples marked with R are from the extraction methods that were done correctly, the samples marked with an F and outlined in red is from methods done incorrectly and are not presented in the results. Values marked dark red are outside of the area of the standard curve.

		Sample conc. (µg/mL)	Absorbance (340nm)	Calc. conc.	Mean	Standard deviation
1	EESN R	100	1.227			
2	EESN R	100	0.662	96.15		
3	EESN R	100	0.617	61.54	78.85	24.48
4	MESN R	100	0.674	105.38		
5	MESN R	100	0.651	87.69		
6	MESN R	100	0.879	263.08	152.05	96.56
7	ECSN1	100	0.643	81.54		
8	ECSN1	100	0.625	67.69		
9	ECSN1	100	0.802	203.85	117.69	74.93
10	MCSN1	100	0.662	96.15		
11	MCSN1	100	0.732	150.00		
12	MCSN1	100	0.690	117.69	121.28	27.10
13	EESN F	100	0.625	67.69		
14	EESN F	100	0.642	80.77		
15	EESN F	100	0.594	43.85	64.10	18.72
16	MESN F	100	0.563	20.00		
17	MESN F	100	0.596	45.38		
18	MESN F	100	0.579	32.31	32.56	12.69
19	E	100	0.955	321.54		
20	E	100	0.477			
21	E	100	0.731	149.23	235.38	121.84
22	M	100	0.515			
23	M	100	0.568	23.85		
24	M	100	0.565	21.54	22.69	1.63

Appendix 7: Raw data for standards and the standard curve from amine detection analysis (OPA)

Raw data

Protein standard ($\mu g/mL$)	Absorbance (340nm)
31.3	0.635
52.5	0.636
125	0.665
250	0.753
500	1.220

Standard curve