Chemical Journal of Kazakhstan
Volume 1, Number 89(2025), 150-159 https://doi.org/10.51580/2025-1.2710-1185.15

УДК 665.662.36

CHEMICAL ASPECTS OF BIOMOLECULE EXTRACTION FROM *ZOPHOBAS MORIO* LARVAE: LIPID AND PROTEIN EXTRACTION MECHANISMS AND SOLVENT EFFICIENCY ANALYSIS

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Abstract. *Introduction.* This study deals with the chemical aspects of biomolecule extraction from *Zophobas morio*, including the influence of different solvents, lipid, and protein extraction mechanisms, and comparative analysis of solvents on their efficiency and safety. *The study aims* to optimize the extraction of lipids and proteins from *Zophobas morio* larvae, emphasizing the choice of solvents, protein precipitation methods, and amino acid composition analysis. *Results and discussion.* Optimization of lipid extraction conditions showed that the most effective extractants were petroleum ether, providing maximum lipid yield (65.55%), and chloroform with ejection capacity (44.85%). Among the studied methods of protein extraction and precipitation, precipitation at the isoelectric point after alkaline extraction was the most effective, which gave a protein yield of 66.09% of the initial dry matter, much higher than that of acetone precipitation after aqueous extraction (36.9%). Analysis of amino acid composition of protein concentrate revealed the presence of 15 amino acids, 10 of which are essential. The essential amino acid index was 1.81, which is significantly higher than traditional protein sources such as fishmeal and soya, and comparable to casein. These results confirm the high purity and efficiency of the isoelectric precipitation method applied for the first time to *Zophobas morio*, highlighting its industrial potential. *Conclusion.* The study confirmed that the choice of solvent and precipitation method significantly affects the efficiency of lipid extraction and protein precipitation from *Zophobas morio*, with petroleum ether proving to be the most efficient extractant for lipids and precipitation at the isoelectric point, the optimal method for protein extraction. The research results demonstrate the promising potential of the methods used to obtain protein concentrate from *Zophobas morio* for the food and feed industry as a sustainable and high-quality alternative to traditional protein sources.

Keywords: lipids, extraction, solvents, protein concentrate, insects.

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Citation: Zhatkanbayeva Zh., Kurtibay K., Zhatkanbayev Ye., Kappassuly A., Fedeli R. Chemical aspects of biomolecule extraction from *Zophobas morio* larvae: lipid and protein extraction mechanisms and solvent efficiency analysis. *Chem. J. Kaz.*, **2025**, 1*(89)*, 150-159. DOI: https://doi.org/10.51580/2025-1.2710-1185.15

Introduction

According to the projections of the Food and Agriculture Organization (FAO) of the United Nations, the global population will reach 9 billion by 2050 [1], and protein consumption in the diet is expected to increase by 22% by 2030 and by 25% by 2050 [2]. However, the growth in the production of traditional protein sources (meat, fish, and dairy products) is associated with significant environmental consequences, including greenhouse gas emissions, increased water consumption, and land degradation [3-5]. Therefore, there is a need to identify alternative, environmentally sustainable protein sources. One promising solution is the use of insects, which serve as a valuable source of proteins, fats, and minerals while having a minimal environmental footprint [6].

The larvae of *Zophobas Morio* (superworms) are already utilized in the feed industry due to their high nutritional value, as their protein composition and amino acid profile are often comparable to or even superior to conventional feed ingredients. Their biomass contains 43.13–51.62 g of protein and 32.8–43.54 g of lipids per 100 g of dry weight [7,8]. Studies indicate that *Zophobas morio* surpasses traditional feed ingredients in protein content, reaching 68.05 g/100 g [9], making them a promising resource for the food and feed industries. During the larval stage, the protein content varies between 39.4 and 49.96 g/100 g [8,10-15].

Despite the high potential of *Zophobas morio*, selecting optimal lipid and protein extraction methods remains a challenge. This is primarily due to variations in solvent efficiency, toxicity, and industrial applicability. This study aims to determine the optimal conditions for lipid and protein extraction from *Zophobas morio* larvae, analyze the amino acid composition of the protein concentrate, and assess its potential applications in the biotechnology and food industries.

2. Experimental Part

2.1 Research Object.

Larvae of the insect Zophobas morio were reared under laboratory insectarium conditions using wheat bran, selected fruits and vegetables as feed. Sample preparation stages: preparation of live larvae samples in Petri dishes without feeding for 24 hours, rinsing with distilled water to remove external contaminants (dirt, sand, and organic residues) and drying in a convection oven at 70°C for 24 hours, grinding of dry larvae with a laboratory blender and storage at -20°C until experiments) [16].

2.2 Lipid extraction from Zophobas morio larvae.

Representative samples weighing 100 g of dried larval powder were used for extraction. Extraction was carried out for 6 h using the following solvents: petroleum ether (70-100°C, reagent), n-hexane (reagent), chloroform (GOST 20015-88), and ethanol (96.3%) using a Soxhlet apparatus (DWK Life Sciences, Germany) [17]. The solvent was removed using a rotary evaporator (Labconco No 600310, USA) equipped with a water bath at the following temperature

conditions: 40°C for 30 min at 800 mbar and 60°C until complete evaporation of the solvent [16].

2.3 Protein extraction from Zophobas morio larvae

Distilled water and 1M NaOH were used for protein extraction after preliminary defatting of larval biomass. The extraction was carried out at 40°C for 60 min as follows: 10 g of dry sample was mixed with 200 ml of water and 0.2 g of ascorbic acid, then centrifuged at 10,000 rpm for 30 min at 4°C and filtered. The supernatant was used for protein extraction and protein concentrate preparation. Protein extraction was carried out by isoelectric and acetone precipitation methods [18].

For isoelectric precipitation, 100 ml of the supernatant of the extract was centrifuged (10,000 rpm, 4°C) for 10 minutes. The separated precipitate was dried at 60°C to constant weight. The procedure was repeated for different pH values of the extract solution at pH 6, 5, 4, 3, 1. For protein precipitation with acetone, 40 ml of ice-cold acetone was added to 10 ml of aqueous protein extract. The mixture was incubated at -20°C for 1 hour, then centrifuged (10,000 rpm, 4°C) and the precipitate was dried at 60°C for 24 hours until a constant weight was reached [18].

2.4 Hydrolysis method for protein concentrate

Hydrolysis of the protein concentrate, weighing 0.100±0.001 g, was performed in 10.0 mL of hydrochloric acid (1:1) in a hydrolysis vial with a screw cap under stirring and heating at 110°C for 14–16 hours. The hydrolysate samples were then cooled to room temperature and filtered using «blue ribbon» filter paper, collecting the filtrates in airtight containers [19, 20].

2.5 Protein quality assessment

Protein quality assessment was carried out using the Essential Amino Acid Index (EAAI), which reflects the content of all essential amino acids relative to a reference protein [21]. The Essential Amino Acid Index (EAAI) was calculated using the following formula [22]:

$$EAAI = \sqrt[n]{\frac{aa_1}{AA_1} \times \frac{aa_2}{AA_2} \times \ldots \times \frac{aa_n}{AA_n}}$$

Where *aa* - content of essential amino acid in the sample under study; *AA* - content of essential amino acid according to literature data; n - number of essential amino acids.

3. Results and Discussion

Studies on the nutritional composition of edible insect species, such as *Zophobas morio*, remain limited. This species belongs to the order Coleoptera, which includes darkling beetles [12]. Insects are rich in both proteins and lipids, which are released during protein extraction and contribute significantly to nutrition [22]. Although much research has focused on the fatty acid composition

of wild edible insects [23, 24], some studies have examined insects used for animal feed [25].

Zophobas morio larvae are recognized as a valuable source of high-quality protein and lipids. The nutritional composition of this species has been studied by various authors (Table 1). Lipid extraction from these larvae is essential, as residual lipids can reduce the efficiency of protein fraction isolation. This is due to the formation of lipid-protein complexes stabilized by hydrophobic and Van der Waals interactions, as well as ionic bonds between amino acids and phospholipids in lipid membranes [26].

Table 1 - Protein and lipid content in the dry matter (g/100g) of *Zophobas morio* (ZM) larvae

Lipids, g/100g	Proteins, g/100g	Country	Source
43.64 ± 0.47	46.80 ± 1.78	Brazil	[14]
40.8	43.13	USA	[24]
14.25	68.05	Netherlands	[8]
35 ± 0.1	46 ± 1.0	Indonesia	[11]
34 ± 1.8	48.1 ± 0.6	Czech Republic	[12]
39.1 ± 0.4	39.4 ± 0.1	Poland	[26]
28.98	49.96	Indonesia	[13]

The high hydrophobicity of lipids and their propensity to clot reduce protein solubility, and lipids can change their isoelectric points and promote protein denaturation [27, 28]. Therefore, effective removal of lipids is necessary for successful protein fraction isolation.

In this study, the following organic solvents were used for lipid extraction: petroleum ether, chloroform, n-hexane, and ethanol. The results allow for the selection of optimal extraction conditions that enable the efficient removal of lipids while minimizing their impact on protein structures and improving process efficiency. The analysis of different solvents' effectiveness demonstrated that the highest lipid yield (65.55 ± 2.43) was achieved using petroleum ether, attributed to its low solvating ability when applied to nonpolar compounds. Chloroform also exhibited relatively high extraction efficiency (44.58 ± 2.41), confirming its reactivity in lipid extraction. Meanwhile, n-hexane and ethanol showed comparatively lower lipid yields (35.8 ± 1.60 and 34.3 ± 2.28, respectively), which may be due to their lower solubility for lipid components in larvae

Optimization of the extraction conditions and protein concentration showed that the most effective method is deposition at an isoelectric point during alkaline extraction (1M NaOH), which provided a protein yield of 66.09%, significantly exceeding the result with acetone (36.9%). The efficiency of the isoelectric deposition is explained by the pH stabilization, which facilitates the aggregation of protein molecules and their precipitation. In contrast, acetone deposition is based on the dehydration of the macromolecules resulting in lower output.

Moreover, the deposition of acetone allows the purity of the product up to 99.19% by removing the water associated with the protein [22]. Studies have shown that the type of solvent plays a key role in protein composition and output, while temperature and duration of extraction do not have a significant influence. This is due to the natural solubility of proteins in aqueous solutions at elevated pH, which is caused by their polyampholite structure [29,30] and promotes deposition. The protein yield depends more on the ionic composition of the medium than on temperature conditions [31, 32]. The protein concentrate obtained by isoelectric deposition was a dark brown amorphous substance. Isoelectric precipitation achieved a protein yield of 66.09% from 100 g of the initial dry matter

Amino acids in protein concentrate obtained by isoelectric deposition were identified by capillary electrophoresis (Fig. 1, Table 2).

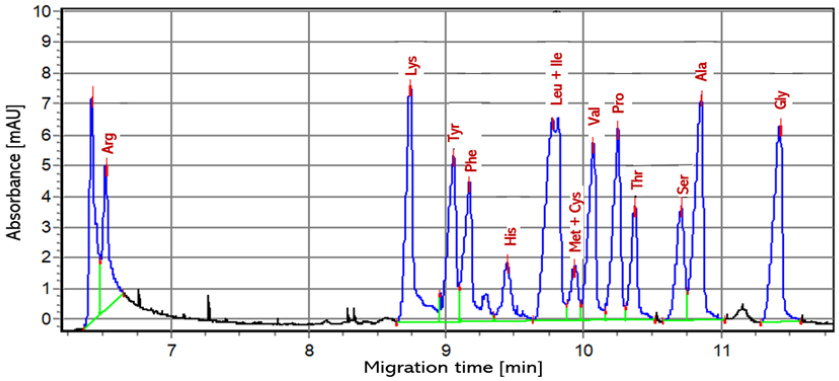

Figure 1 - Electropherogram of the amino acid composition of the protein concentrate

The obtained results, presented in Table 2, provide a detailed characterization of the amino acid composition of the protein concentrate and allow for an assessment of its nutritional value.

Table 2 - Amino acid composition of the protein concentrate

№	Amino acid	Content, %
1	Arginine (Arg)	7.696±3.078
2	Lysine (Lys)	6.790±2.309
3	Tyrosine (Tyr)	10.412±3.124
4	Phenylalanine (Phe)	9.507±2.852
5	Histidine (His)	3.893±1.947
6	Leucine + Isoleucine (Leu + Ile)	8.148±2.119
7	Methionine + Cysteine (Met + Cys)	2.626±0.893
8	Valine (Val)	6.790±2.716
9	Proline (Pro)	6.790±1.766
10	Threonine (Thr)	3.848±1.539
11	Serine (Ser)	3.803±0.989
12	Alanine (Ala)	6.338±1.648
13	Glycine (Gly)	4.391±1.493

The amino acid composition of the resulting protein concentrate was compared with other literature data and known sources of protein for assessment of its quality and biological value (Table. 3).

In the amino acid composition study of protein concentrate, 15 amino acids were identified, 10 of which belong to a group of essential amino acids. These amino acids play a fundamental role in the metabolism and biosynthesis processes of the body, serving as key components for maintaining normal physiological functions.

The obtained data confirm that the protein concentrate extracted from the larvae of *Zophobas morio* has high nutritional characteristics in terms of both essential and substitutable amino acids. The essential amino acid index (EAAI) was 1.81, which is significantly higher than values in traditional protein sources such as fish meal and soy, and comparable to casein.

Table 3 - Amino acid content in the protein concentrate compared to literature data

№	Amino Acids	*Zophobas morio* (studied)	*Zophobas morio* [22]	Fish Meal [32]	Soy [22]	Casein [22]	1985 FAO/WHO /UNU [21]
Essential Amino Acids							
1	Histidine	3.9	3.1	1.37	2.5	3.2	1.5
2	Isoleucine	8.1	4.6	2.35	4.7	5.4	3.0
3	Leucine		7.1	3.83	8.5	9.5	5.9
4	Lysine	6.8	5.4	4.10	6.3	8.5	4.5
5	Methionine	2.6	2.4	2.34	2.4	3.5	2.2
6	Cysteine						
7	Phenylalanine	9.5	11.1	2.15	9.7	11.1	3.8
8	Tyrosine	10.4		1.91			
9	Threonine	3.8	4.0	2.62	3.8	4.2	2.3
10	Tryptophan	–	1.4	–	1.1	1.4	0.6
11	Valine	6.8	6.3	2.62	4.9	6.3	3.9
Total		77.22	45.4	23.29	43.9	53.1	27.7
Non-Essential Amino Acids							
12	Alanine	6.3	6.8	3.76	–	–	–
13	Arginine	7.7	5.4	3.87	–	–	–
14	Aspartic Acid	–	8.2	5.32	–	–	–
15	Glycine	4.4	12.7	4.85	–	–	–
16	Serine	3.8	4.8	2.82	–	–	–
17	Proline	6.8	5.6	3.13	–	–	–
18	Glutamic Acid	–	4.2	7.64	–	–	–
Total		29	47.7	31.39	–	–	–
EAAI		1.81	1.66	0.88	1.56	1.93	–

This demonstrates the high quality of the protein, making it promising for use in food and feed additives, as well as opening up new applications in biotechnology and the food industry. The highest levels of amino acids are found

in tyrosine (10.41%), phenylalanine (9.51%), and leucine + isoleucine (8.15%), which highlights their importance in biosynthesis and metabolic processes. Also, high levels of arginine (7.70%), lysine (6.79%), and valine (6.79%) confirm the potential of protein concentrate as a rich source of essential amino acids. Proline (6.79%), alanine (6.34%), serine (3.80%), and threonine (3.85%) play key roles in collagenogenesis and carbohydrate exchange, and low levels of histidine (3.89%) and methionine + cysteine (2.63%) may indicate a deficiency of these amino acids, but their level is sufficient for normal metabolism. These results underline the high quality of the protein from *Zophobas morio* and its potential for use in dietary supplements, biotechnology, nutrition, and feed production.

The findings of this study may serve as a basis for further scientific research and the development of innovative products in the fields of biotechnology, nutraceuticals, and feed production.

4. Conclusion

The study successfully identified optimal conditions for lipid and protein extraction from *Zophobas morio* larvae using various solvents. Chloroform demonstrated a high extraction efficiency (44.85%), while petroleum ether showed the highest lipid yield (65.55%). In protein concentration, isoelectric precipitation proved to be the most effective method, yielding 66.09%, significantly outperforming acetone precipitation (36.9%). This emphasizes the importance of protein aggregation properties and pH stability for efficient precipitation with minimal loss. The study confirms that isoelectric precipitation offers an optimal combination of high yield and protein purity for separating proteins from Zophobas morio. Additionally, the lipid and protein fractions derived from these larvae have promising applications in the food and biotechnology industries, with lipid extracts useful for food ingredients, feed formulations, and pharmaceuticals, while protein concentrates provide a valuable alternative protein source.

Conflict of Interest: All authors declare that they have no conflict of interest.

ZOPHOBAS MORIO ДЕРНӘСІЛДЕРІНЕН БИОМОЛЕКУЛАЛАРДЫ АЛУДЫҢ ХИМИЯЛЫҚ АСПЕКТІЛЕРІ: ЛИПИДТЕР МЕН АҚУЫЗДАРДЫ АЛУ МЕХАНИЗМДЕРІ ЖӘНЕ ЕРІТКІШ ТИІМДІЛІГІН ТАЛДАУ

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Түйіндеме. *Кіріспе.* Бұл зерттеуде биомолекулаларды экстракциялау процесінің химиялық аспектілері, соның ішінде әртүрлі еріткіштердің әсері, липидтер мен ақуыздарды бөліп алу механизмдері, сондай-ақ еріткіштердің экстракциялық тиімділігі мен қауіпсіздігі тұрғысынан салыстырмалы талдауы қарастырылады. Жұмыстың *мақсаты Zophobas morio* дернәсілдерінен

липидтер мен ақуыздарды экстракциялау процесін оңтайландыру, оның ішінде еріткіштерді таңдау, ақуыздарды тұндыру әдістері және аминқышқылдық құрамды талдау. *Нәтижелер және талқылау.* Липидтерді экстракциялау шарттарын оңтайландыру нәтижесінде ең тиімді экстрагенттер петролей эфирі (65.55% максималды шығым) және хлороформ (44.85% экстракциялық қабілеті) екені анықталды. Ақуыздарды экстракциялау және тұндыру әдістері ішінде ең тиімдісі – сілтілік экстракциядан кейінгі изоэлектрлік нүктеде тұндыру болды, бұл ақуыз шығымын 66.09% дейін жеткізуге мүмкіндік берді. Бұл көрсеткіш ацетонмен тұндыру әдісінен кейінгі су экстракциясына қарағанда айтарлықтай жоғары (36,9%). Ақуыз концентратының аминқышқылдық құрамы 15 аминқышқылынан тұратынын көрсетті, оның 10-ы – алмастырылмайтын аминқышқылдары. Алмастырылмайтын аминқышқылдарының индексі 1.81-ге тең болды, бұл дәстүрлі ақуыз көздері – балық ұны мен соядан әлдеқайда жоғары және казеинге жақын. Бұл нәтижелер *Zophobas morio* үшін алғаш рет қолданылған изоэлектрлік тұндыру әдісінің жоғары тазалығы мен тиімділігін растайды, сондай-ақ оның өнеркәсіптік әлеуетін айқындайды. *Қорытынды.* Зерттеу көрсеткендей, липидтер мен ақуыздарды экстракциялау тиімділігі еріткішті таңдау және тұндыру әдістеріне айтарлықтай тәуелді. Петролей эфирі липидтерді экстракциялауда ең тиімді экстрагент болып табылса, ал ақуыздарды бөліп алудың оңтайлы әдісі ретінде изоэлектрлік нүктеде тұндыру анықталды. Зерттеу нәтижелері *Zophobas morio* негізіндегі ақуыз концентратын азық-түлік және мал азығы өнеркәсібінде дәстүрлі ақуыз көздеріне орнықты және жоғары сапалы балама ретінде пайдаланудың перспективалы екенін дәлелдейді.

Түйінді сөздер: липидтер, экстракция, еріткіштер, ақуыз концентраты, жәндіктер.

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ХИМИЧЕСКИЕ АСПЕКТЫ ЭКСТРАКЦИИ БИОМОЛЕКУЛ ИЗ ЛИЧИНОК *ZOPHOBAS MORIO*: МЕХАНИЗМЫ ИЗВЛЕЧЕНИЯ ЛИПИДОВ И БЕЛКОВ И АНАЛИЗ ЭФФЕКТИВНОСТИ РАСТВОРИТЕЛЕЙ

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Резюме. *Введение.* В данном исследовании рассматриваются химические аспекты экстракции биомолекул, включая влияние различных растворителей, механизмы выделения липидов и белков, а также сравнительный анализ растворителей на их экстракционную эффективность и безопасность. *Целью исследования является о*птимизация экстракции липидов и белков из личинок *Zophobas morio* с акцентом на выбор растворителей, методы осаждения белков и анализ аминокислотного состава. *Результаты и обсуждение.* Оптимизация условий экстракции липидов показала, что наиболее эффективными экстрагентами являются петролейный эфир, обеспечивающий максимальный выход липидов (65.55%) и хлороформ с эктракционной способностью (44.85%). Среди изученных методов экстракции и осаждения белков наиболее эффективным оказалось осаждение при изоэлектрической точке после щелочной экстракции, что обеспечило выход белка 66.09% от исходного сухого вещества, значительно превышая выход при осаждении ацетоном после водной экстракции (36.9%). Анализ аминокислотного состава белкового концентрата выявил наличие 15 аминокислот, из которых 10 являются незаменимыми. Индекс незаменимых аминокислот составил 1.81, что существенно превышает показатели

традиционных белковых источников, таких как рыбная мука и соя, и сопоставимо с казеином. Данные результаты подтверждают высокую чистоту и эффективность метода изоэлектрического осаждения, впервые примененного к *Zophobas morio*, что подчеркивает его промышленный потенциал. *Заключение.* Исследование подтвердило, что выбор растворителя и метода осаждения существенно влияет на эффективность экстракции липидов и осаждения белков из Zophobas morio, при этом петролейный эфир оказался наиболее эффективным экстрагентом для липидов, а осаждение при изоэлектрической точке – оптимальным методом выделения белков. Полученные результаты демонстрируют перспективность используемых методов для получения белкового концентрата из *Zophobas morio* в пищевой и кормовой промышленности как устойчивой и высококачественной альтернативы традиционным источникам белка.

Ключевые слова: липиды, экстракция, растворители, белковый концентрат, насекомые.

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