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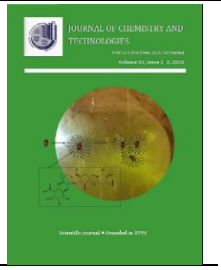

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# ASPECTS OF RESEARCH ON PROTEIN COMPOSITION OF CRICKET FLOUR

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## Abstract

**This study presents data on the biological value of wheat and cricket flour, focusing on their amino acid composition and protein digestibility. With rising demand for high-protein foods, alternative sources like insect flour particularly from *Acheta domesticus* – are gaining attention. Crickets contain 40–75 % protein, 15–40 % fats (including essential fatty acids), and 3–10 % minerals per 100 g of dry matter. Despite consumer resistance to entomophagy, processing insects into flour improves acceptability. The main goal of this study was to evaluate the protein content, amino acid profile, and digestibility of cricket flour compared to wheat flour. Protein content was measured using standard chemical methods, including the Kjeldahl method. Amino acid composition was analysed using high-performance liquid chromatography (HPLC), and digestibility was assessed through amino acid indices, which reflect the body's ability to utilise proteins. Results show that while wheat flour contains key amino acids, its profile is not fully balanced to meet human nutritional needs. In contrast, cricket flour demonstrates a richer and more balanced amino acid composition, particularly in essential amino acids. Chromatographic analysis confirmed higher amino acid content in cricket flour, supporting its superior biological value. Moreover, cricket flour exhibited higher digestibility than wheat flour, highlighting its potential in dietary applications. In conclusion, cricket flour is a promising alternative protein source due to its balanced amino acid profile, higher digestibility, and greater nutritional value compared to wheat flour.**

*Keywords:* cricket flour; wheat flour; proteins; amino acid composition; functional product chromatogram; digestibility.

# АСПЕКТИ ДОСЛІДЖЕННЯ БІЛКОВОГО СКЛАДУ БОРОШНА ІЗ ЦВІРКУНІВ

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## Анотація

**У цьому дослідженні представлені дані про біологічну цінність пшеничного та цвіркунового борошна, з акцентом на їх амінокислотний склад та засвоюваність білка. Зі зростанням попиту на продукти з високим вмістом білка все більшої уваги привертають альтернативні джерела, такі як борошно з комах, зокрема, з Acheta domesticus. Цвіркуни містять 40–75% білка, 15–40 % жирів (включаючи незамінні жирні кислоти) і 3–10 % мінералів на 100 г сухої речовини. Незважаючи на стійкість споживачів до ентомофагії, переробка комах на борошно покращує їхню прийнятність. Основною метою цього дослідження було оцінити вміст білка, амінокислотний профіль та засвоюваність борошна з цвіркунів у порівнянні з пшеничним борошном. Вміст білка вимірювали за допомогою стандартних хімічних методів, включаючи метод К'єльдаля. Амінокислотний склад аналізували за допомогою високоефективної рідинної хроматографії (HPLC), а засвоюваність оцінювали за допомогою амінокислотних індексів, які відображають здатність організму утилізувати білки. Результати показують, що хоча пшеничне борошно містить ключові амінокислоти, його профіль не є повністю збалансованим для задоволення харчових потреб людини. На противагу цьому, крикетне борошно демонструє багатший і збалансованіший амінокислотний склад, особливо в незамінних амінокислотах. Хроматографічний аналіз підтвердив вищий вміст амінокислот у крикетному борошні, що**

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**підтверджує його вищу біологічну цінність. Крім того, крикетне борошно має вищу засвоюваність, ніж пшеничне, що підкреслює його потенціал у дієтичному харчуванні. Отже, крикетне борошно є перспективним альтернативним джерелом білка завдяки своєму збалансованому амінокислотному профілю, кращій засвоюваності та більшій поживній цінності порівняно з пшеничним борошном.**

*Ключові слова:* борошно із цвіркунів; пшеничне борошно; білки; амінокислотний склад; функціональний продукт; хроматограма; засвоюваність.

## Introduction

Protein deficiency in the daily human diet is a major public health problem that arises due to insufficient intake of protein substances contained in food products. Proteins are considered to be one of the main components of the human diet, which perform important biological functions and are the main structural components of muscles and other tissues in the human body. In turn, amino acids are considered to be the building blocks of protein in cellular mechanisms that act as intermediate links on the path to metabolic health. Proteins and protein compounds are essential for life [1], as they are broken down into amino acids that are absorbed by the human body. In addition, proteins play an important role in the regulation of gene expression, but unlike fats (in the form of lipids stored in adipose tissue) and carbohydrates (in the form of glycogen in muscles and liver), the body does not have a special tissue for storing proteins [2]. Some studies indicate that protein restriction has a positive effect on human metabolic health, and the quality and properties of protein are determined by the ratio of essential to non-essential amino acids. Branched-chain amino acids are considered the main source of protein for metabolic health, and there is a relationship between the amount of protein consumed and the level of branched-chain amino acids in the blood of a person [3]. High-quality proteins contain all essential amino acids in an easily digestible form that meets the needs of the human body, but insufficient protein intake can lead to malnutrition and, with the rest, to hidden hunger [4]. Therefore, the search for alternative sources of protein is a priority for nutritionists and researchers.

Recently, insect proteins have been highlighted as an alternative source of protein. Statistically, several hundred million people worldwide consume insects as food [5]. The most common edible insects are those of the following orders: Coleoptera (31 % of all insects consumed), Lepidoptera (18 %), Hymenoptera (14 %), Orthoptera (13 %), Hemiptera (10%), Odonata (3 %) and Diptera (2 %) [6].

The nutritional composition of edible insects, including protein content, can be comparable to or even exceed traditional sources of protein used in human nutrition, such as salmon, chicken, beef, and pork. According to a study by Liceaga (2022), the protein content (as a percentage of dry matter) in eight orders of edible insects ranges from 35 % in Blattodea, 40 % in Coleoptera, 45 % in Lepidoptera, 46 % in Hymenoptera, 48 % in Hemiptera, 49 % in Diptera, 55 % in Odonata and up to 61 % in Orthoptera. These values are significantly higher than the protein content in fresh products (~100 g), such as pork (21.0 %), beef (22.5 %), and chicken (22.2 %) [10]. It is worth noting that traditional protein sources are usually consumed fresh, while insects are mainly processed into flour. One of these is cricket flour, which is made from the domestic cricket of the genus Acheta domesticus, widely used in the food industry [7, 15], which is due to its high nutritional value, namely protein content of 40.0–75.0 % per 100 g of dry matter [8], mineral content within 3.0–10.0 %, among which calcium, iron, magnesium predominate, as well as fat and essential fatty acid content of 15.0–40.0 % per 100 g of dry matter [9].

Due to its concentrated content, the flour has an increased nutritional value compared to fresh products, which is one of the key factors in increasing the nutritional value of the products in which it is included. After all, cricket flour contains high-quality proteins [11], which are easily digestible and more bioavailable than vegetable and animal proteins [12]. In powdered form, cricket flour has gained popularity in countries such as the USA, Canada, and the European Union. According to its organoleptic indicators, it has a greyish-brown colour with a delicate nutty flavour [13]. In addition to high-quality proteins, the flour contains unsaturated fatty acids, vitamins, and minerals and has a low carbohydrate content [14].

It should be noted that, according to the literature, studies on the digestibility of cricket flour from the genus Acheta domesticus were conducted by researchers at the National Agricultural University, Olancho, Honduras [16]. It was found that the in vitro digestibility of cricket flour is within 87.7 %.

Given that cricket flour from the genus Acheta domesticus contains a significant amount of protein, it is considered one of the most famous

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types of flour obtained from insects intended for human consumption, which is widely used in products such as bread, pasta, extruded snacks, biscuit semi-finished products, however, studies on its biological value and amino acid composition are quite limited. Therefore, the purpose of this work is to study the amino acid composition and in vitro digestibility of cricket flour of the genus Acheta domesticus provided by TM SENS (London, Great Britain) for recommendations on its further use in food production.

## Materials and methods

*Materials*

The used cricket flour (Acheta domesticus) (CF) is sold in Ukraine under the trademark "SENS" by the manufacturer, Great Britain. According to the documentation provided by the manufacturer, to produce the flour, crickets underwent a process of degreasing, microwave drying, and grinding (120–250 microns), after which the flour was sieved on sieves with a sieve diameter of 1.25 mm. It is also stated that the flour does not contain artificial dyes, flavours, preservatives, monosodium glutamate, gluten, or dairy products.

According to previously conducted studies, cricket flour contains protein—40.0–75.0 % per 100 g of dry matter, fats – 13.7 g, carbohydrates – 0.5 g, and chitin—5.9 g per 100 g of dry matter [29].

The study used premium wheat flour of the “Khutorok” brand, produced in Ukraine. It was determined that the wheat flour of this brand contains 25.0 % satisfactorily weak gluten, protein content – 11.0 %, fat – 1.5 g, carbohydrates – 70 g per 100 g of dry matter.

*Methods*

*Determination of protein content in cricket flour*

The study involved the use of a classical titrimetric method for determining the total nitrogen content by the Kjeldahl method in accordance with the requirements of DSTU 7169 [17]. A feature of this study is that the described method was adapted, taking into account the biochemical properties of cricket flour, which differ from traditional wheat flour. In particular, to ensure complete decomposition of nitrogen-containing compounds of animal origin (chitin, proteins), the mineralization of cricket flour samples was accompanied by prolonged heating after decolorization of the reaction mixture, which is not provided for by the standard method. Additional control of the amount of ammonia after distillation was also carried out to avoid underestimation of the results.

However, due to the specific chemical composition of cricket flour, which is significantly different from traditional wheat flour, additional measures were introduced in the research process aimed at increasing the accuracy and reproducibility of the results.

To increase the accuracy of measurements, high-precision analytical equipment was used, such as: analytical balances for sample dosing (accuracy 0.1 mg), a burette with an accuracy of 0.01 ml for titration, a pH meter for controlling the end point of titration, and a fume hood for safe operation with volatile and corrosive substances.

The high content of chitin structures in cricket flour, as well as the presence of animal proteins with a special structure, required an extended mineralization time after decolorization of the solution, which guaranteed the complete decomposition of nitrogen-containing organic compounds. To prevent nitrogen losses and ensure process stability, ammonia release was monitored at each stage of distillation.

In addition, when studying the ash content of cricket flour samples, an extended calcination cycle was applied, since their mineral composition is characterized by an increased content of components that are more slowly oxidized at standard temperature regimes of the muffle furnace. This made it possible to avoid residual charred particles and obtain correct results.

The expansion of methodological approaches to determining the mass fraction of proteins and ash content allowed for adequate adaptation of classical standardized methods for the analysis of flour from entomological raw materials and ensured the correctness of comparing its protein composition with traditional wheat flour.

*Determination of the amino acid composition of cricket flour*

Amino acid composition (AC) of cricket flour was determined by the content of free (synthetic and natural) amino acids and the total content (free and bound forms in the sum) of certain amino acids using an amino acid analyzer according to DSTU ISO 13903 [18].

*Determination of free forms of amino acids*

Free forms of amino acids were extracted with diluted hydrochloric acid. Laboratory glassware (volumetric flasks, test tubes, pipettes) and a

heated magnetic stirrer were used to prepare solutions and mix samples.

The nitrogenous macromolecules extracted together with the amino acids were precipitated by adding sulfosalicylic acid, after which the samples were centrifuged in a laboratory centrifuge at 4000 rpm for 10 min. The resulting precipitate was separated by filtration using a vacuum filter. The filtrate was adjusted to pH – 2.20 using a Hanna Instruments HI 2211 pH meter.

Ion exchange chromatography was used to separate the amino acids on a Biochrom 30+, Sykam S433 amino acid analyzer equipped with an ion exchange resin column.

After chromatographic separation, the amino acids were reacted with ninhydrin, for which a thermostat was used for incubation at a temperature of about 100 °C. Quantitative determination of amino acids was carried out using a Specord 200 spectrophotometer at a wavelength of 570 nm.

*Determination of the total content (free and bound forms in the sum) of individual AAs*

The determination of the total free and bound forms in the sum of individual amino acids consisted of the fact that before hydrolysis, cystine (cysteine) and methionine were oxidized to cysteic acid and methionine sulfone, respectively. Tyrosine was determined in hydrolysates of unoxidized samples. All other amino acids were determined in both oxidized and unoxidized samples. Oxidation was carried out at a temperature of 0 °C with a mixture of performic acid with phenol. Excess oxidant was decomposed with sodium disulfide. Oxidized or unoxidized samples were subjected to hydrolysis with hydrochloric acid of a molar concentration of 6 mol/dm$^3$ for 23 hours. The hydrolysate was adjusted to pH 2.20 units. Amino acids were separated by ion exchange chromatography, derivatized with ninhydrin, and detected at a wavelength of 570 nm (440 nm for proline). The sample was thoroughly mixed and ground to pass through a 0.5 mm sieve. Before grinding, samples with high humidity were dried at a temperature not exceeding 50 °C.

*Methodology for calculating the amino acid score*

The calculation of the amino acid score for cricket flour and wheat flour was carried out using formula 1:

$$ASS = ASS\frac{amino\ acid\ content\ of\ the\ test\ protein\ (mg/g\ protein)}{content\ of\ the\ same\ amino\ acid\ in\ the\ reference\ protein\ (mg/g\ protein)} \times 100\% \qquad (1)$$

As a reference protein, a protein whose composition meets the recommended human needs according to FAO/WHO for adults and children was chosen. The amino acid with the lowest SCOR was considered limiting.

*Conducting the study*

*Determination of the content of free forms of amino acids*

To determine the content of free forms of amino acids, a sample from (1.0000± 0.0002) g to (5.0000± 0.0002) g was weighed on a scale, the prepared sample was quantitatively transferred to a conical flask, 100.0 cm$^3$ of extraction solution was added, which was made from 8.2 cm$^3$ of hydrochloric acid (ρ = 1.19 g/cm$^3$) with the addition of 900 cm$^3$ of distilled water, then 20 cm$^3$ of thiodiglycol was added and the volume of the solution was brought to the mark with water in a volumetric flask with a capacity of 1000.0 cm$^3$. The mixture was shaken for 60 min using a mechanical shaker, and settled, after which 10.0 cm$^3$ of the supernatant (liquid phase) of the liquid was transferred with a pipette into a 100 cm$^3$ beaker. While stirring, 5.0 cm$^3$ of sulfosalicylic acid was added and stirring was continued using a magnetic stirrer for 5 min. The solution was filtered to remove the precipitate. Then 10.0 cm$^3$ of the resulting solution was placed in a 100 cm$^3$ beaker and adjusted to a pH of 2.20 units using sodium hydroxide solution. The solution was quantitatively transferred into a volumetric flask of the appropriate volume (required for chromatography) and the volume of the solution was adjusted to the mark with citrate buffer.

*Determination of the total content (free and bound forms in the sum) of individual amino acids*

The determination of the total content (free and bound forms in the sum) of individual amino acids began with oxidation. For open hydrolysis, a sample of (0.1000± 0.0002) g to (1.0000± 0.0002) g was weighed into a 100 cm$^3$ round-bottom flask, which is used for all amino acids except cysteine, tyrosine and tryptophan, and for closed hydrolysis in a closed vessel, for such AAs as cysteine, tyrosine and tryptophan.

The sample taken for analysis contained about 10 mg of nitrogen with a humidity of 95%. The flask or container with the sample was placed in an ice bath, after cooling to 0 °C, 5 cm$^3$ of the oxidation mixture was added and stirred using a glass rod with a bent tip, and then the flask with the rod was hermetically sealed with a lid. The ice

bath with the closed vessel was placed in a refrigerator at 0 °C. After 16 hours, the vessel was removed from the fridge, and the excess of the oxidation mixture was removed by adding 0.84 g of sodium disulfide.

Next, hydrolysis of non-oxidized samples for such AAS as cysteine and methionine and oxidized samples for all other AAS was carried out.

To carry out hydrolysis, 25 $cm^3$ of the hydrolysis mixture was added to the prepared oxidized sample, washing away the sample residues from the walls of the vessel and the rod. Three glass beads were added to the mixture in the flask and boiled with a reflux condenser continuously for 23 hours. After the hydrolysis was completed, the refrigerator was washed with 5 $cm^3$ of citrate buffer, the flask was disconnected and cooled in an ice bath.

For the hydrolysis of unoxidized samples, a sample of (0.1000± 0.0002) g to (1.0000± 0.0002) g was weighed into a 250 $cm^3$ round-bottom flask and a 100 $cm^3$ container with a screw cap. The sample contained about 10 mg of nitrogen. 25 $cm^3$ of the hydrolysis mixture was carefully added and mixed with the sample. The flask or container containing the mixture prepared accordingly was placed in a drying oven at a temperature of 110 °C. During the first hour, to prevent pressure build-up (due to the release of gaseous substances) and to avoid explosion, the container was not covered with a lid placed on top of the vessel. After an hour, the container was closed with a lid and left in a drying cabinet for 23 hours. Upon completion of the hydrolysis, the flask or container was removed from the drying cabinet, the lid was carefully opened and cooled in an ice bath. Using citrate buffer, the contents of the container were quantitatively transferred to a glass or round-bottomed flask with a capacity of 250 $cm^3$, and the ph value was adjusted depending on the method.

To determine the ph value, 2.0 $cm^3$ of the internal standard solution was added to the hydrolysate before evaporation (0.6560 g of norleucine was dissolved in citrate buffer in a 250 $cm^3$ volumetric flask, and the volume of the solution was brought to the mark with buffer). Two drops of 1-octanol were added to the hydrolysate described above, and the volume was reduced to 5–10 $cm^3$ using a rotary evaporator in vacuum at a temperature of 40 °C. The acidity of the solution was then adjusted to a pH equal to 2.20 units using a sodium hydroxide solution with a molar concentration of 1 $mol/dm^3$.

The hydrolysate with the established pH value was quantitatively transferred using citrate buffer into a 200 $cm^3$ volumetric flask and the volume of the solution in the flask was brought to the mark with buffer. The solution was thoroughly mixed and chromatography was performed.

Before chromatography, the temperature of the hydrolysate was brought to room temperature. The mixture was stirred and the required amount was filtered through a membrane filter. The resulting clear solution was used for ion-exchange chromatography. Sample introduction was performed automatically, the same amount (± 0.5 %) of the standard and sample solutions were introduced into the column. The frequency of calibration depended on the stability of the ninhydrin solutions and the analytical system.

*Results processing*

The peak areas of the standard sample and the sample were measured for each amino acid separately. The amino acid content in the test sample is w, g/kg was calculated by formula 2:

$$w = \frac{A_o \cdot c \cdot M \cdot V_o}{A_c \cdot m \cdot 1000} \cdot \frac{A_{ic}}{A_{io}} \qquad (2)$$

where $A_0$ – an area of the AK peak in the hydrolysate;

c – molar concentration of the amino acid in the standard solution, $mol/dm^3$;

M – molecular mass of the amino acid;

$V_0$ – total volume of the hydrolysate or calculated total volume of extract dilutions, $cm^3$;

$A_c$ – area of the amino acid peak in the standard solution;

m – mass of the analyzed sample (corrected for the initial mass for dry and/or fat-free samples), g.

1000 – conversion factor for volume units:

$A_{ic}$ – an area of the internal standard peak in the standard solution;

$A_{io}$ – an area of the internal standard peak in the extract or hydrolysate.

In the hydrolysates of the oxidized sample, cystine and cysteine were determined as cysteic acid but were calculated as the sum of cystine and cysteine, using the molecular weight of cystine M = 240.30 and calculating the molecular weight of cysteine: M = 0.5·242.30 =120.15.

In the hydrolysates of the oxidized sample, methionine was determined as methionine sulfone but was calculated as methionine with M = 149.21. The free form of methionine was determined after extraction as methionine. Therefore, the value of M = 149.21 was used for calculations.

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The total volume of dilution of extracts ($V_e$), cm³, for the determination of free forms of amino acids was calculated by formula 3:

$$V_e = 100 \cdot \frac{10+5}{10} \cdot \frac{V_{et}}{10}, \quad (3)$$

where $V_{et}$ – final extract volume, cm³.

The correctness of the method was checked by repeated measurements of standard samples. The assessment of the content of essential amino acids was carried out following the recommendations of FAO/WHO (1991) [19].

*The digestibility of flour protein by digestive enzymes in vitro* was carried out using the basic method of O.O. Pokrovsky – I.D. Yertanov [26]. The essence of this method is the sequential action on the protein of the studied object of the proteinase system and the removal by dialysis of some hydrolysis products to prevent inhibition of the reaction by low-molecular peptides and free amino acids. The efficiency of protein digestion by the used proteinase system was judged by the amount of tyrosine that accumulated in the dialysate, taking into account its mass fraction in the product itself. The mass fraction of tyrosine in the studied product and dialysate was determined by the Lowry method [27] on a spectrophotometer at a wavelength of λ = 750 nm.

The digestibility of the product protein, (%), was calculated by formula 13 [28]:

$$P = \frac{10 \cdot P_1}{T}, \quad (13)$$

where P – protein digestibility of the studied product, % to the initial mass fraction of tyrosine in it;

T – mass fraction of tyrosine in the protein of the tested product, g/100 g of protein;

10 – proportionality coefficient, (g protein g %)/(mg 100 g of protein);

$P_1$ – protein digestibility of the studied product, mg tyrosine/1g protein:

$$P_1 = A - B - C, \quad (14)$$

where A – concentration of hydrolysis products in hydrolysis with enzyme;

B – concentration of hydrolysis products in hydrolysis without enzyme;

C – concentration of hydrolysis products in the enzyme solution.

*Statistical analysis*

Statistical analysis of experimental data was performed using the Statistica 6 application package. Data are the mean of three replicates ± standard deviation. To create the chromatogram, specialized software Chomatogram Plot was used. Graphical representation of experimental data was performed using the Google Colab statistical processing package.

## Results and discussion

The nutritional value of food depends on its chemical composition, which determines its benefits to humans. The amino acid composition of products is an important indicator of their nutritional value, especially when it comes to the protein component. This study compared the proteins and amino acid profiles of wheat flour (WF) and cricket flour (CF). The analysis is based on the division of amino acids into groups: essential (those that the human body cannot synthesize on its own) and non-essential (those that can be formed in the body, but their sufficient amount in the diet is important for maintaining the physiological functions of the human body). The content of the amount of proteins, as well as non-essential and non-essential amino acids in wheat flour and cricket flour, is given in Table 1.

*Table 1*

**Protein and amino acid content in raw materials**

| Component, % | Type of flour | | | | |
|---|---|---|---|---|---|
| | WF | | | CR | |
| Protein content, g | 12,45±0,15 | | | 70,53±0,02 | |
| Essential amino acids, mg/100g | FAO scale | WF | amino acid score, % | CR | amino acid score, % |
| Val | 50 | 470±0.01 | 0.94 | 614±0.03 | 1.23 |
| Leu | 70 | 806±0.07 | 1.15 | 751.4±0.05 | 1.07 |
| Ile | 40 | 430±0.09 | 1.08 | 441.9±0.03 | 1.11 |
| Lys | 55 | 250±0.03 | 0.45 | 781.5±0.03 | 1.42 |
| Met | 35 | 153±0.01 | 0.44 | 197.0±0.01 | 0.56 |
| Thr | 40 | 311±0.01 | 0.78 | 400.4±0.02 | 1.00 |
| Phe | 60 | 500±0.02 | 0.83 | 388.0±0.02 | 0.65 |
| Try | 10 | 100±0.02 | 1.0 | - | - |
| *Replaceable amino acids, mg/100g* | | Type of flour | | | |
| | | WF | | CR | |
| Asp | | 340.0±0.02 | | 537.0±0.47 | |
| Ser | | 500.0±0.02 | | 251.0±0.60 | |
| Glu | | 308.00±0.04 | | 629.0±0.67 | |

*Continuation of Table 1*

| | | |
|---|---|---|
| Pro | 970.0±0.01 | 448.0±0.38 |
| Gly | 350.0±0.04 | 382.0±0.32 |
| Ala | 330.0±0.05 | 697.0±0.49 |
| His | 200.0±0.40 | 267.0±0.24 |
| Arg | 400.0±0.20 | 450.0±0.39 |
| Cys | 200.0±0.01 | 560.0±0.02 |
| Tyr | 250.0±0.30 | 521.0±0.32 |

Note: WF – wheat flour, CF – cricket flour.

Val - Valine; Leu – Leucine; Ile – Isoleucine; Lys – Lysine; Met – Methionine; Thr – Threonine; Phe – Phenylalanine; Try - Tryptophan; ASP – Aspartic acid; Ser - Serine; Glu – Glutamic acid; Pro – Proline; Gly – Glycine; Ala – Alanine; His – Histidine; Arg –Arginine; Cys -Cystine; Tyr - Tyrosine.

Gluten proteins of wheat flour contribute to the creation of a gluten framework, which provides the volume and shape of bakery and flour confectionery products. They also determine the biological value of products and affect their digestibility by the body. The use of raw materials with different protein compositions and the content of essential amino acids in the recipe significantly changes the properties of finished products. According to the results of Table 1, cricket flour contains 5.76 times more proteins than wheat flour. Therefore, it is likely that adding CF to food products can increase the nutritional and biological value of products.

Also, Table 1 shows that CF is better than WF in almost all essential amino acid compositions. The valine content in CF exceeds the amino acid content in wheat flour by 1.36 %. Also, the content of isoleucine is higher by 1.03 %, lysine – by 3.13 %, methionine – by 1.29 %, and threonine – by 1.29 %, respectively.

It is known that the consumption of products containing a significant amount of lysine is extremely important. After all, lysine is necessary for the normal functioning of the body, namely for the synthesis of proteins in the human body. Lysine is a component of proteins contained in muscles, bones, collagen and other structural components. It helps the body fight harmful infections and microorganisms, namely stimulates the production of antibodies and immune cells. Lysine helps regulate the level of arginine, which affects blood circulation and nitrogen synthesis in the body [20].

CF also contains more methionine, which is considered the most limited amino acid among the acids presented. In living organisms, methionine is found mainly in the L-form and is part of most proteins. In microorganisms, it is formed from L-aspartic acid. In mammalian cells, L-methionine, which comes with food, is itself a building block for another conditionally essential amino acid, L-cysteine. According to the recommendations of the World Health Organization, the consumption of L-methionine for healthy adults should be 13 mg per 1 kg of body weight per day. L-methionine is found in products of animal and plant origin. Except for sesame (methionine content 1656 mg/100 g) and Brazil nuts (1008 mg/100 g), the content of L-methionine in animal proteins is significantly higher than in plant proteins [21; 22].

It is known that valine is one of the 20 amino acids that are part of the L-isomers of many known proteins and is an important component of food for humans and animals. When there is a lack of carbohydrates in the body, they are converted into glucose (glycogen) or glycogen through styrene acetate and phosphoenolpyruvate. The daily intake of valine is 110 mg/1 kg for children, 0.8 g for men and 0.65 g for women [23]. The main sources of L-valine are products of animal origin. It is present in free form in all organisms and is also present in the composition of proteins (especially abundant in albumin). The content of valine in proteins usually ranges from 4.1 % (horse myoglobin) to 7–8 % (human serum albumin, bovine casein), and in some cases – 13–14 % (elastin of connective tissue). The absence of valine in food depletes it of protein and leads to a negative nitrogen balance of casein in connective tissue proteins. Valine is recommended for use in programs for athletes engaged in building muscle mass, as well as in conventional multicomponent complexes as an aid for protein metabolism [24; 25].

From the analysis of the obtained data, it is clear that the amino acid SCORE of cricket flour proteins is in the range of 0.56–1.42 % (Table 1). The highest SCORE in CF was recorded for valine, the limiting amino acid was found to be methionine, the SCORE of which is 0.56 %, although its content in CF is higher compared to wheat flour.

Regarding essential amino acids, the results of the study showed that cricket flour significantly

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exceeds wheat flour in amino acid composition in terms of such amino acids as aspartic acid – 15.79 times, serine – 5.02; alanine – 21.12, cystine – 2.8, tyrosine – 20.84, arginine – 11.25, glutamic acid – 2.04, proline – 4.61 (Table 1).

Thus, according to the results of the study, cricket flour has a better amino acid profile compared to wheat flour, especially in essential amino acids. The most significant advantages of CF are the high amount of lysine, alanine, aspartic and glutamic acids, and cysteine, which improves its biological value. In turn, wheat flour is inferior to CF in most indicators, especially due to the low content of lysine, which makes its protein less complete. WF has a higher content of proline and serine, which can be useful for the formation of collagen, but in general, does not compensate for the lack of other amino acids. Therefore, cricket flour is a rich source of high-quality protein, which makes it a valuable supplement for people who need a high-protein diet.

Visual confirmation of the chemical composition of wheat flour and cricket flour protein is presented in chromatograms, the analysis of which allows us to compare the amino acid balance of different types of flour. According to Table 1, a chromatogram of the cricket flour standard (Fig. 1) and wheat flour (Fig. 2) was created.

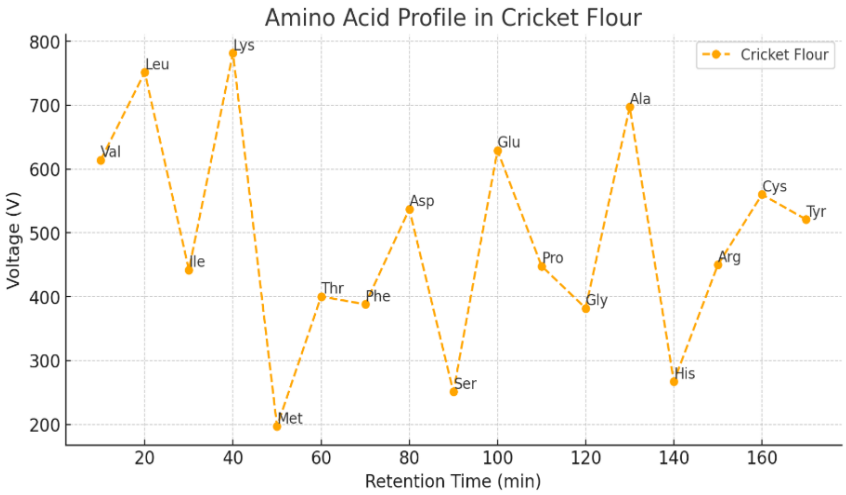


**Fig. 1. Chromatogram of amino acid standard for cricket flour (550 nm (440 nm for proline))**

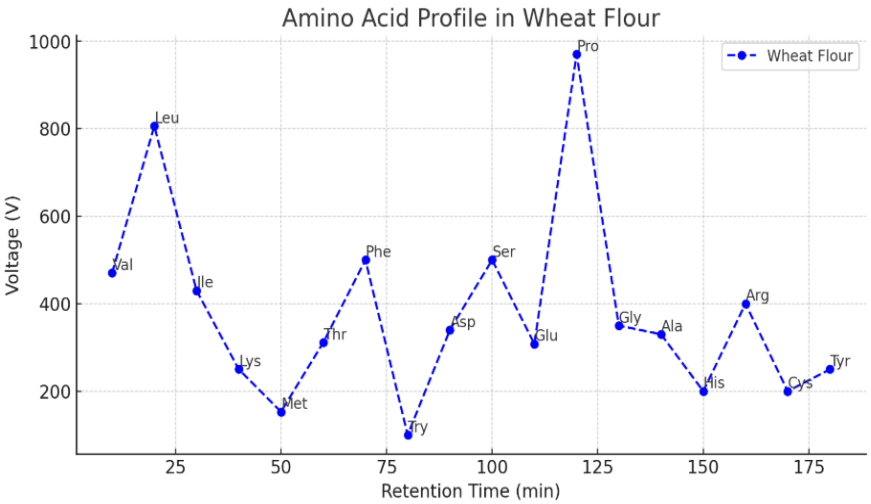


**Fig. 2. Chromatogram of amino acid standard for wheat flour (550 nm (440 nm for proline))**

Both graphs present the results of the amino acid analysis of flour. The chromatographic method with ninhydrin derivatization was applied according to DSTU ISO 13903. The analysis was carried out based on ion-exchange chromatography, which allows for the separation of amino acids and their detection at a wavelength of 570 nm (440 nm for proline).

Figure 1 shows the dependence of the electric voltage (V) on the retention time (min) for cricket flour. The data are visualized in the form of a dot plot connected by a dotted line, demonstrating

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the change in the parameter over time. Considering the characteristic of the dependence, a pronounced nonlinear change in voltage with numerous local maxima and minima is observed. The initial jump (about 700 V) in the first 10 minutes with a subsequent sharp decrease may indicate the first stages of the reaction in the system. Further voltage changes indicate the presence of possible transient processes, in particular oxidation reactions or structural changes of protein molecules.

The peak voltage values are probably associated with specific amino acid residues and chemical processes in the sample. Minima, such as around 25–30 min (where the voltage approaches zero), indicate certain stages of stabilization or decay of intermediate structures. In turn, the maximum voltage values (around 800 V at 10 min and 700 V at 60 min) indicate a peak of electrochemical activity.

The probable causes of the dynamics in cricket flour indicate the interaction of proteins with solvents and their denaturation, a change in the structure of amino acids during the ageing process, as well as possible participation in catalytic processes that change the electrical conductivity of the system.

Thus, the results obtained show a significant change in the electrical properties of cricket flour during the curing time. The presence of pronounced peaks and voltage drops is due to specific reactions of amino acids, which confirms the feasibility of further studies of the electrochemical characteristics of this product.

In turn, in Figure 2, significant voltage variability is observed throughout the entire experiment. Maximum values (approximately 900–1000 V) are observed at points corresponding to such AAs as leucine (10–15 min) and proline (50 min). A noticeable sharp decrease in voltage after these peak values indicates dynamic changes in the chemical composition of the system.

High voltage values for leucine, phenylalanine and proline indicate their higher electrical conductivity under the corresponding conditions. Low values (about 200 V) for lysine, methionine and cysteine are associated with weaker interaction of these amino acids in the system. However, strong voltage fluctuations after 50 min indicate possible reactions of amino acids with the environment.

Taking into account these indicators, the probable reasons for the dynamics of change are possible structural changes in protein molecules and amino acids under the influence of an electric field, the character of voltage distribution indicates different levels of polarity of amino acids in the flour composition, as well as the interaction of wheat proteins with solvents or their potential denaturation. Therefore, the obtained data demonstrate that wheat flour is characterized by pronounced changes in electrical activity, which is probably associated with the amino acid composition of proteins. The peak voltage values correspond to different electrochemical properties of amino acids, which makes further study of their role in structural transformations a promising direction of analysis.

Comparing the analysis of chromatograms of amino acids from cricket flour and wheat flour, we note that in terms of amplitude of voltage fluctuations, wheat flour demonstrates higher peak voltage values, with a maximum of ≈1000 V for proline with a duration of 50 min. In turn, cricket flour has smaller peak values, with a maximum value of ≈800 V for leucine lasting 10 min. This indicates a potentially higher electrochemical activity of amino acids in wheat flour, which may be associated with the specific composition of proteins.

In wheat flour, sharp voltage fluctuations alternate with stable areas, which indicates a complex protein structure with different levels of electrical conductivity. Also, wheat flour demonstrates more contrasting peak values and sharp declines, which indicates a more active distribution of amino acids in the protein matrix.

Cricket flour has fewer sharp drops, as it has a different protein structure and amino acid profile compared to wheat flour. Cricket flour has smoother changes in the stability of electrochemical processes. This indicates different types of protein interactions that are significantly different from wheat flour and the presence of chitin, which affects the electrochemical properties.

Some amino acids, in particular leucine, phenylalanine, proline and arginine, show high voltage values in both cases, which indicates their role in shaping the electrochemical activity of proteins. At the same time, in cricket flour, a lower level of electrochemical activity is observed for proline, which is key in structural proteins.

Regarding the chemical composition and the influence of the detection method, it should be noted that the analysis method indicates that both free and bound amino acids were determined, which affects the intensity of the peaks in the chromatogram. The content of

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individual amino acids, such as cysteine (Cys) and methionine (Met), was corrected according to their oxidized form. A wavelength of 440 nm was used to detect proline, which affects its high intensity in the results.

Therefore, both chromatograms (Fig. 1 and 2) demonstrate the presence of essential amino acids, but their quantitative characteristics differ. Wheat flour has a higher content of some amino acids, such as leucine and proline. Cricket flour has a higher content of essential amino acids, especially arginine (Arg) and tryptophan (Trp), which is reflected in the change of peak values. The difference in extraction and hydrolysis methods affects the results, especially for sulfur-containing amino acids (cysteine, methionine).

Therefore, the chromatography method is a scientific basis for determining the amino acid composition of various food raw materials and food systems, which is important for understanding the processes occurring during the interaction of different types of raw materials in the human body. The data obtained allowed a deeper analysis of the assessment of the amino acid composition of cricket flour compared to wheat flour, which have different physiological roles in the human body, since leucine in wheat flour takes an active part in protein synthesis, stimulates muscle growth, promotes recovery after physical exertion or injuries, and also regulates blood sugar levels, ensuring energy stability, while proline, in turn, performs a structural function, being the main component of collagen – a protein that forms connective tissues such as skin, ligaments, bones and cartilage. Due to its properties, proline promotes wound healing, tissue regeneration, strengthens blood vessels, and maintains skin health. In addition, it has antioxidant properties that help reduce the negative impact of stress on cells. Thus, leucine and proline perform complementary functions: the first is responsible for growth and energy processes, the second for tissue repair, structural stability, and protection, which in combination contribute to maintaining overall human health. Cricket flour proteins contain an increased amount of arginine and tryptophan, which play an important role in maintaining the physiological functions of the body, each of which has unique properties and effects on human health. Arginine is a conditionally essential amino acid, especially important during periods of stress, intensive growth, illness, or injury, when its endogenous production may be insufficient. Arginine is involved in the synthesis of nitric oxide, a molecule that dilates blood vessels, improves blood circulation, and helps normalize blood pressure. Arginine also stimulates the release of growth hormone, supports the immune system, promotes wound healing and tissue repair, and participates in the processes of detoxification and cleansing of the body. Due to these properties, arginine is often used in sports nutrition, cardio-metabolic disorders, and clinical nutrition. Tryptophan enters the body only with food. It is a precursor for the synthesis of serotonin, a neurotransmitter responsible for mood, emotional state, appetite, and sleep quality. Due to this, tryptophan plays a key role in maintaining psycho-emotional health. It also participates in the production of melatonin, a hormone that regulates biorhythms and ensures healthy sleep. Tryptophan is necessary for growth and development of the body, the synthesis of nicotinic acid (vitamin B3). Therefore, the amino acid composition of wheat and cricket flour has quantitative and qualitative differences, understanding of which will allow us to give recommendations on the use of the studied raw materials in the production of new products.

The next step was to study the protein digestibility coefficient in vitro. The essence of the method for determining protein digestibility in cricket flour by digestive enzymes is the sequential action of the proteinase system on the protein of the studied object and the removal of hydrolysis products by dialysis to avoid inhibition of the reaction by low-molecular peptides and free amino acids. Table 2 and Figure 3 show data characterizing the dynamics of cricket flour digestibility by low-molecular peptides and free amino acids.

*Table 2*

**Digestibility of cricket flour and wheat flour (in vitro)**

| Sample number | Mass fraction of tyrosine, g/100 g protein | Protein digestibility | | | |
|---|---|---|---|---|---|
| | | mg tyrosine/g protein | | | % |
| | | pepsin | trypsin | total | |
| Cricket flour | 3.55±0.17 | 5.59±0.05 | 21.88±0.18 | 27.47±0.27 | 77.37 |
| Wheat Flour | 0.77±0.02 | 1.17±0.01 | 2.50±0.02 | 3.67±0.02 | 47.69 |

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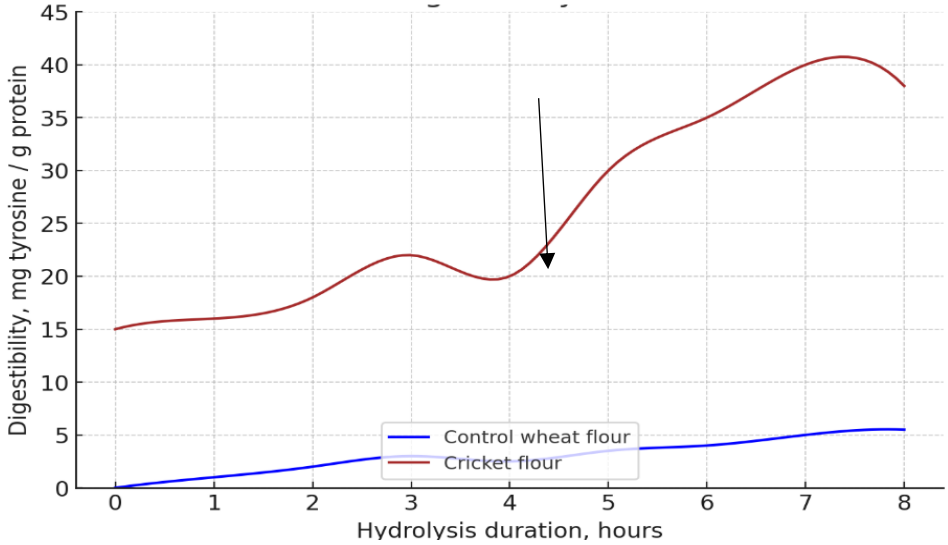


**Fig. 3. Dynamics of proteolysis process of protein systems in flour**

Note: The arrow indicates the moment of addition of trypsin to the pepsin digest.

Table 2 shows that the protein digestibility coefficient of cricket flour is 77.37 % compared to wheat flour, the digestibility is 47.69 %.

The presented data on the rate of protein digestibility of cricket flour compared to the control (wheat flour) in the proteinase system "pepsin-trypsin" (in vitro) with an increase in nitrogen-containing compounds in the dialysate indicate the adjacent nature of the cleavage of raw materials. Therefore, the digestibility of cricket meal proteins is characterized by a pronounced two-stage type. Cricket flour proteins are rapidly digested by pepsin, which is characterized by a rapid rise in the digestibility curve. At the first stage of protein digestibility under the influence of pepsin, a steep rise in the curve can be noted. However, by 3.5 hours of digestion, a slowdown in protein hydrolysis is noted. Further addition of trypsin to the system leads to a new acceleration of the digestion process, causing the curve to rise sharply upward again at the beginning of 4 hours, in contrast to wheat flour proteins. Considering the digestibility curve of cricket flour proteins, we note that the total duration of digestion is about 7 hours. The recorded course of protein digestion of both types of flour indicates that CF protein is of high quality, easily digestible by the human body and contains a significant amount of essential amino acids.

## Conclusions

According to the results of the study, a comparison of the amino acid composition of wheat flour (WF) and cricket flour (CF) revealed significant differences in their nutritional properties, which may have implications for the food industry.

Cricket flour contains significantly more protein – 5.76 times more than wheat flour. This indicates that CF is an important source of protein and can be used to increase the nutritional value of products, especially for people who require high-protein diets, such as athletes, vegans or people with increased physiological protein needs.

Cricket flour was found to contain a higher content of essential amino acids, such as lysine, methionine, valine, isoleucine and threonine. In particular, the lysine content in CF exceeds the lysine content in wheat flour by 3.13 %, which is important because lysine is necessary for protein synthesis, normal functioning of the immune system and calcium absorption.

CF also has a higher content of methionine, which is an essential amino acid for the synthesis of other important compounds in the body, such as cysteine. This confirms that CF has a more complete amino acid profile, which can provide a high biological value for products made from this flour.

Regarding non-essential amino acids, cricket flour has a higher content of non-essential amino acids such as aspartic acid, serine, glutamic acid, cysteine and tyrosine. At the same time, wheat flour has a slightly higher level of proline, which is important for collagen formation. However, this parameter does not compensate for the absence of other key amino acids in wheat flour.

Chromatographic analysis of CF confirmed the results of the chemical composition. It was found that wheat flour exhibits higher peak voltage values on the chromatogram, which may indicate higher electrochemical activity of its protein molecules. This may be due to the specific

structure of wheat flour proteins and the interaction with solvents, which affects the change in their electrical properties.

In summary, cricket flour is a promising source of high-quality protein, which has a high degree of digestibility and is easily absorbed by the human body. The high content of essential amino acids in CF makes it particularly useful for meeting the needs for amino acids that are lacking in traditional plant products. The use of cricket flour in the food industry can significantly increase the nutritional value of products, especially for people with increased physiological needs for protein, and also become a new and valuable raw material in the food industry, which meets the principles of sustainable development.

Despite its obvious benefits, challenges to the introduction of cricket flour include regulatory issues, consumer bias, and the need to develop processes to effectively integrate this raw material into a variety of food products. However, given the growing interest in sustainable and innovative food products, cricket flour has significant future potential to shape a sustainable food system.

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