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Analysis of amino acid composition in sheep by-products by High-Performance Liquid Chromatography

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Abstract
The study aimed to investigate the amino acid composition of sheep by-products (kidney, liver, heart, spleen, blood, and lung) using High-Performance Liquid Chromatography (HPLC). The results showed that 17 amino acids in the samples were successfully detected by HPLC, and the chromatographic peak shape was sharp without interference from extraneous peaks, demonstrating good separation. The amino acid content and composition in by-products serve as key factors in evaluating protein nutritional value and directly influence this value in by-products. Among the six types of samples, there is a significant difference in the content of total amino acids; among them, liver and spleen exhibit the highest total amino acid content, 21.335 g/100 g and 19.325 g/100 g respectively, while blood exhibited the lowest value of 12.49 g/100 g.

Keywords
Kidney, liver, heart, spleen, blood, lung, essential amino acids, non-essential amino acids

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Анализ аминокислотного состава бараньих субпродуктов с помощью высокоэффективной жидкостной хроматографии

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Аннотация
Целью настоящего исследования было изучение аминокислотного состава бараньих субпродуктов, таких как почки, печень, сердце, селезенка, кровь и легкие, с помощью высокоэффективной жидкостной хроматографии (ВЭЖХ). Результаты показали, что с помощью ВЭЖХ удалось четко определить 17 видов аминокислот в субпродуктах; форма хроматографических пиков была четкой, без посторонних шумов, а разделение – хорошим. Содержание и состав аминокислот в субпродуктах являются основными факторами при оценке пищевой ценности белка и напрямую влияют на пищевую ценность белка в субпродуктах. Среди 6 типов образцов наблюдается значительная разница в содержании общего количества аминокислот; среди них печень и селезенка имеют самое высокое общее содержание аминокислот – 21.335 г/100 г и 19.325 г/100 г соответственно, а кровь – самое низкое – 12.49 г/100г.

Ключевые слова
Почки, печень, сердце, селезенка, кровь, легкие, незаменимые аминокислоты, заменимые аминокислоты

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Introduction

By-products are of high value as a source of cheap protein. Offal is quite varied regarding composition and functionality, most of it contains a good amount of nutrients such as essential amino acids, minerals, and vitamins [1, 2]. Most by-products are characterized by good digestibility of proteins. Spleen, kidney, lungs, and tripe proteins have the highest rate of digestibility (in vitro) [3]. Offal with a high content of connective tissue proteins is promising for producing hydrolysates of these proteins and compositions for the production of the antioxidant peptides [1, 4, 5]. Protein hydrolysates from meat by-products are an interesting alternative to soy products due to the lack of allergenic proteins and the presence of large amounts of all essential amino acids [6]. It has been proven that offal is a good source of essential and limiting amino acids.

Currently, High-Performance Liquid Chromatography (HPLC) [7] and amino acid analyzers [8] are advanced methods for detecting amino acids in food products. Compared to amino acid analyzers, HPLC offers advantages such as lower detection limits, higher sensitivity, and greater stability of derivatization products [9]. In Mongolia, there is limited quantitative information on the amino acid composition of offal, and the existing detection methods have certain limitations. Therefore, conducting studies using High-Performance Liquid Chromatography holds significant scientific importance. In this study, 6 types of offal samples were collected and analyzed to detect and compare their amino acid profiles.

Research Methods

Sample collection. In this study, we collected (Tsaluut Impex LLC Animal slaughtering house) various by-products, including kidney, liver, heart, spleen, blood, and lung, from 11 sheep and performed subsequent analysis on the samples. After sample collection, the samples were placed in refrigerated containers and transported to the laboratory.

Sample preparation. Before analysis, all meat by-products were chopped into small pieces. Samples were subjected to acid hydrolysis using 6 N HCl with added phenol at 120°C for 22 h. Hydrolysates were diluted to 100 mL with 0.1 N HCl and filtered through 0.45 µm membrane filters prior to analysis. Subsequently, 500 µL of the sample solution was accurately measured and placed into a 2 mL sample vial. Then, 50 µL of internal standard solution was accurately added, mixed thoroughly, and used as the sample solution for derivatization.

Accurately measured 100 µL of the amino acid standard solution from the amino acid analysis method component package, placed it into a 2 mL sample vial, then accurately added 50 µL of internal standard solution and 400 µL of 0.1 M HCl solution. (Table 1).

Chromatographic Conditions. Amino acid analysis column (Shimadzu AJS-02, C18, 3 µm, 4.6 × 150 mm) was used. The RF20A fluorescence detector was set at an excitation wavelength of 340 nm and an emission wavelength of 450 nm, with the column temperature maintained at 50°C. The mobile phase gradient and flow rate for the amino acid composition analysis of the sample are shown in Table 2.

To detect Pro in the sample, the excitation and emission wavelengths were adjusted to 266 nm and 305 nm, respectively, after a 27-minute reaction.

Data Processing. All the analyses were repeated three times and the data are expressed as mean ± SD. Statistical analysis of amino acid data was performed using SPSS27.0. Differences among organs were evaluated by one-way ANOVA followed by Tukey's HSD test at $p < 0.05$.

Results and Discussion

Amino acid standard solutions were analyzed using the HPLC FR detector (Figure 1). Figure 1 demonstrates that all amino acids exhibited sharp peaks without interference from other peaks, indicating excellent resolution. The FR detector demonstrated excellent response for all 17 amino acids (Trp was excluded as it was not detected in the total hydrolyzed sample), demonstrating effective separation [10]. Three replicate analyses were performed, and the results were reproducible, demonstrating the high performance capability of the FR detector for amino acid detection. Furthermore, the FR detector was used to analyze the amino acid content in sheep by-products, with quantification conducted using the internal standard method.

The total amino acid content in by-products and their average values are reported in Table 3. As shown in Table 3, there are significant differences in the composition and relative content of amino acids among the kidney, liver, heart, spleen, blood and lung. The total amino acid content of the samples is significantly different ($p < 0.05$); the essential amino acid content (Val, Met, Ile, Phe, Lys, Leu, and Thr) in liver is the highest, at 7.988 g/100 g, and the content in blood is the lowest, at 4.501 g/100 g. Leu is the essential amino acid with the highest content in samples. Leu can promote the synthesis of muscle protein in the body [11]. The second most abundant essential amino acid in by-products is Lys. Lys, as the first limiting amino acid in the human body, plays an important role in regulating human metabolism and enhancing disease resistance. Particularly, the high content of Lys in the hydrolyzed offal protein could potentially provide good protein supplementation for vegetable foods, in which Lys is commonly a limiting amino acid [12, 13].

Table 1. Standard concentration of amino acids.

Таблица 1. Стандартная концентрация аминокислот.

Amino acids ***Аминокислоты***	**Molecular mass** ***Молекулярная масса***	**Concentration (μg/mL)** ***Концентрация, μг/мл***	**Amino acids** ***Аминокислоты***	**Molecular mass** ***Молекулярная масса***	**Concentration (μg/mL)** ***Концентрация, μг/мл***
Histidine *Гистидин*	155.15	387.9	*Cysteine* *Цистеин*	240.30	600.8
Serine *Серин*	105.09	262.7	*Lysine* *Лизин*	146.19	365.5
Arginine *Аргинин*	174.20	435.5	*Tyrosine* *Тирозин*	181.19	453.0
Glycine *Глицин*	75.07	187.7	*Metionine* *Метионин*	149.21	373.0
Aspartic acid *Аспартановая кислота*	133.10	332.8	*Valine* *Валин*	117.15	292.9
Glutamic acid *Глутаминовая кислота*	147.13	367.8	*Isoleucine* *Изолейцин*	131.17	327.9
Threonine *Треонин*	119.12	297.8	*Leucine* *Лейцин*	131.17	327.9
Alanine *Аланин*	89.09	222.7	*Phenylalanine* *Фенилаланин*	165.19	413.0
Proline *Пролин*	115.13	287.8			

Jukniené I. et al., researchers from Lithuania, also conducted an analysis of the amino acid composition in sheep by-products and compared the results. It was found that in Mongolian sheep, the content of essential amino acids such as Ile, Phe, Lys, and Leu was significantly higher across all by-product types than in Lithuanian sheep, with the exception of Met. In addition, the non-essential amino acids such as Arg, Tyr, Pro, Gly, Ser, and Asp were found to be higher in all by-product samples, while the levels of the remaining amino acids were similar in both studies [14]. The ratio of essential to non-essential amino acids (EAA/NEAA) is commonly used to evaluate protein nutritional quality. Our results showed that the EAA/NEAA ratios were comparable to values reported in the literature and consistent with the amino acid pattern recommended by FAO/WHO, indicating that sheep by-products represent an excellent source of high-quality protein [15]. Amino acid profiles of by-products exhibit significant variation among livestock species, affecting their nutritional quality.

Table 2. Mobile phase gradient and flow rate.

Таблица 2. Градиент мобильной фазы и скорость потока.

Time (min) *Время, мин*	**Mobile phase A** *Мобильная фаза А*	**Mobile phase B** *Мобильная фаза Б*	**Flow rate (ml/min)** *Скорость потока, мл/мин*
0	95	5	1.6
6	90	10	1.6
8	90	10	1.6
10	84	16	1.3
23	60	40	1.0
30	50	50	1.6
31	0	100	1.6
34	0	100	1.6
35	95	5	1.6
38	95	5	1.6

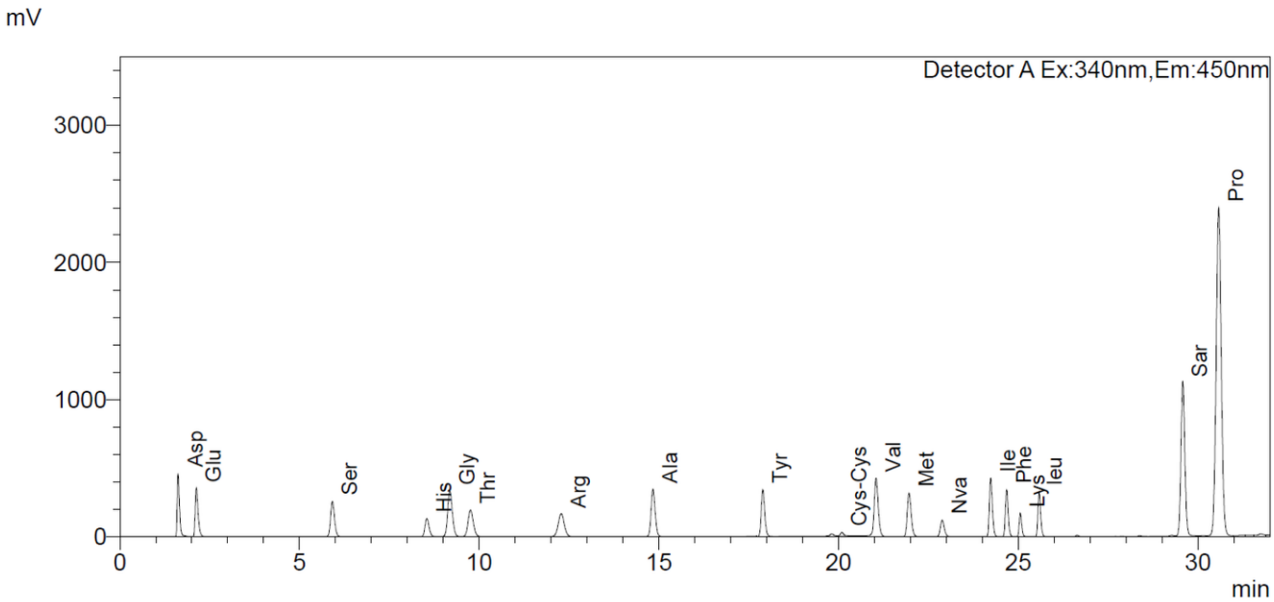

Figure 1. High-Performance Liquid Chromatography chromatogram of the amino acid standard.

Рисунок 1. Хроматограмма стандартного раствора аминокислот, полученная методом высокоэффективной жидкостной хроматографии.

Table 3. Determination of amino acid composition in sheep by-products (g/100 g).

Таблица 3. Определение аминокислотного состава бараньих субпродуктов (г/100 г).

Amino acids ***Аминокислоты***	**Kidney** ***Почки***	**Liver** ***Печень***	**Heart** ***Сердце***	**Spleen** ***Селезенка***	**Blood** ***Кровь***	**Lung** ***Легкие***
Asp	1.131±0.012[ab]	1.114±0.091[ab]	1.187±0.053[b]	1.206±0.05[b]	1.012±0.02[a]	1.081±0.072[ab]
Glu	1.973±0.052[b]	2.08±0.12[b]	2.377±0.136[c]	2.006±0.174[b]	1.009±0.028[a]	1.92±0.133[b]
Ser	0.886±0.036[bc]	1.085±0.042[d]	0.865±0.027[bc]	0.962±0.091[c]	0.559±0.021[a]	0.824±0.063[b]
His	0.522±0.04[ab]	0.855±0.053[bc]	0.387±0.079[a]	0.91±0.326[c]	0.558±0.004[abc]	0.661±0.069[abc]
Gly	1.434±0.089[b]	2.428±0.173[c]	1.533±0.022[b]	2.114±0.281[c]	0.532±0.028[a]	2.129±0.213[c]
Thr	0.626±0.018[b]	0.601±0.046[b]	0.501±0.084[ab]	0.516±0.083[ab]	0.442±0.021[a]	0.435±0.074[a]
Arg	1.204±0.062[b]	1.626±0.012[c]	1.403±0.135[bc]	1.422±0.256[bc]	0.474±0.02[a]	1.379±0.158[bc]
Ala	1.076±0.043[b]	1.286±0.037[c]	1.25±0.022[c]	1.53±0.133[d]	0.813±0.032[a]	1.266±0.072[c]
Tyr	0.636±0.023[b]	0.837±0.028[c]	0.602±0.056[b]	0.582±0.106[b]	0.359±0.011[a]	0.557±0.061[b]
Cys	0.516±0.024[b]	0.867±0.05[c]	0.168±0.036[a]	0.515±0.244[b]	0.373±0.003[b]	0.427±0.035[b]
Val	0.779±0.018[a]	0.962±0.032[b]	0.804±0.012[a]	1.024±0.04[b]	0.763±0.026[a]	0.83±0.047[a]
Met	0.396±0.012[bc]	0.582±0.005[d]	0.465±0.051[c]	0.434±0.071[bc]	0.122±0.005[a]	0.339±0.04[b]
Ile	0.629±0.017[c]	0.739±0.045[d]	0.639±0.055[c]	0.5±0.048[b]	0.138±0.003[a]	0.51±0.044[b]
Phe	0.825±0.033[a]	1.127±0.047[b]	0.787±0.078[a]	1.003±0.129[b]	0.728±0.023[a]	0.805±0.082[a]
Lys	1.465±0.076[b]	1.955±0.029[d]	1.745±0.059[cd]	1.95±0.2[d]	1.013±0.032[a]	1.519±0.119[bc]
Leu	1.551±0.065[b]	2.023±0.052[c]	1.73±0.05[b]	1.986±0.131[c]	1.297±0.053[a]	1.578±0.121[b]
Pro	1.914±0.084[c]	1.168±0.073[b]	0.667±0.041[a]	0.665±0.013[a]	2.299±0.22[d]	1.097±0.058[b]
EAA	6.271±0.239	7.988±0.255	6.671±0.39	7.413±0.703	4.501±0.162	6.015±0.528
NEAA	11.292±0.466	13.346±0.679	10.438±0.608	11.912±1.674	7.989±0.386	11.342±0.933
TAA	17.564±0.704	21.335±0.934	17.109±0.997	19.325±2.377	12.49±0.548	17.356±1.461
EAA/ NEAA	55.535	59.853	63.911	62.231	56.340	53.033

Note. TAA (Total amino acids); EAA (Total essential amino acids); NEAA (Total non-essential amino acids); $p < 0.05$. Values are expressed as mean ± SD (n = 3). Different superscript letters within the same row indicate significant differences among organs ($p < 0.05$) according to one-way ANOVA followed by Tukey's HSD test. The full names of the amino acids are given in Table 1.

Примечание. TAA (общее количество аминокислот); EAA (общее количество незаменимых аминокислот); NEAA (общее количество заменимых аминокислот); $p < 0,05$. Значения представлены в виде среднего значения ± стандартное отклонение (n = 3). Различные буквы-индексы в одной строке указывают на значимые различия между органами ($p < 0,05$) по результатам однофакторного дисперсионного анализа (ANOVA) с апостериорным HSD-тестом Тьюки. Полное наименование аминокислот приведено в таблице 1.

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Conclusions

In recent decades, analytical methods for determining amino acid composition have been further optimized and are determined using highly sensitive analytical techniques. This study, for the first time using HPLC technology, compared the amino acid compositions in by-products from Mongolian sheep. The analysis of amino acid compositions revealed significant differences in the amino acid composition. Liver has the highest TAA and EAA contents, while blood has the lowest. Compared with the amino acid profiles recommended by FAO/WHO [15], the protein quality of the hydrolyzed offal protein was fairly high, due to their high contents of essential amino acids, including Ile, Leu, Lys and Val. The nutritional, functional, bioactive, and potential flavoring aspects of sheep by-products are remarkable. Extraction and isolation of bioactive compounds from sheep by-products should receive special attention and further study.

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Conflict of interests

The authors declare no relevant conflict of interests.

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