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Assessment of Protein Content and Amino Acid Composition in Pharaoh Cuttlefish (Sepia pharaonis) Ehrenberg 1831 from North-Western Arabian Gulf Waters

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Abstract
The pharaoh cuttlefish, Sepia pharaonis, is a cephalopod species of economic and nutritional importance in the northwestern Arabian Gulf. However, the information on its protein quality and amino acid composition is still limited, especially in different body tissues and growth stages. The present study was carried out to determine the protein content and amino acid profiles of S. pharaonis collected from the north western Arabian Gulf and to examine the differences between the three weight categories and between the head and mantle tissues. Protein content was determined by the Kjeldahl method and amino acid composition was determined by high-performance liquid chromatography (HPLC). The results showed significant differences in the distribution of proteins in the tissues. In all weight categories the mantle always had higher protein levels than the head. The total protein content ranged from 19.7% to 20.3%, with the highest values observed in medium-sized specimens. Amino acid analysis showed a balanced ratio of essential and non-essential amino acids. Methionine, glycine, lysine, arginine and histidine were among the common amino acids while glutamic and aspartic acids were the predominant non-essential amino acids identified. The total concentrations of non-essential and essential amino acids declined gradually with increasing body weight, but the ratio of essential to non-essential amino acids was relatively stable (0.93-0.95), indicating that protein quality was high throughout growth. Chromatographic analysis also revealed significant quantitative differences between tissues, with mantle samples containing approximately twice the total amino acid concentration recorded in head tissues. The present findings indicate the high nutritional value of S. pharaonis and emphasise its potential as a valuable seafood resource in the Arabian Gulf region. The study adds baseline biochemical information that may aid future investigations on seafood quality, nutritional assessment, and sustainable employment of this commercially important cephalopod species.
Keywords: Sepion pharaonis, EPA, DHA, Cuttlefish, Protein, Amino acids.
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1.Introduction

Cephalopods are considered as most valuable groups of marine invertebrates, supporting global fisheries and human nutrition. Of special interest among them are the pharaoh cuttlefish (*Sepia pharaonis*) because of its high-quality edible muscle with a rich protein content and a well-balanced amino acid composition. These nutritional properties have increased its commercial value and attracted more interest from researchers in seafood quality and functional food studies (Paulose and Chakraborty, 2024). Proteins and amino acids are crucial to the biological performance of cephalopods besides their nutritional significance. They are actively involved in muscle growth, tissue maintenance, metabolism, and cell regulation. Free amino acids are also involved in osmoregulation and cephalopods can use them to maintain physiologic stability under changing environmental conditions, such as variations in salinity and temperature (Fabri *et al*., 2024). Consequently, the biochemical composition of cephalopod tissues provides valuable insight into both their nutritional value and their physiological adaptation. Recent advances in analytical techniques have considerably improved the characterization of cephalopod muscle composition. Comparative studies have shown that amino acid profiles differ not only among species but also between tissues and according to body size, habitat, and post-harvest handling. For example, Jiang *et al*. (2024) demonstrated that variations in amino acid composition are closely associated with tissue type and storage conditions, while Silva *et al*. (2025) highlighted the importance of non-essential amino acids in maintaining osmotic balance in marine organisms inhabiting hypersaline environments. These results suggest that local environmental conditions may heavily influence the biochemical characteristics of cephalopods. Amino acid composition is also closely related to seafood quality. The metabolism of amino acids is altered by microbial activity during storage and biogenic amines, compounds commonly used as indicators of freshness deterioration, are favoured. As noted by Wang *et al.* (2024), monitoring the changes in amino acids is an effective way of evaluating seafood quality during storage and processing. The results suggest that the biochemical properties of cephalopods are strongly influenced by the local environment. Amino acid composition is also closely related to seafood quality. The metabolism of amino acids is altered by microbial activity during storage and biogenic amines, compounds commonly used as indicators of freshness deterioration, are favoured. Wang et al. (2024) found that monitoring amino acids changes is an effective method for evaluating the quality of seafood during storage and processing. The northwestern Arabian Gulf is one of the most extreme marine environments in the world where organisms are exposed to chronically high salinity and pronounced seasonal temperature fluctuations . These conditions are expected to impact biochemical processes, potentially leading to tissue compositions that differ from those reported for cephalopods in other marine environments. High protein levels have been detected in several cephalopod species (Cameselle *et al.,* 2026), but little is known about *S. pharaonis* from the Arabian Gulf. This knowledge gap has become more relevant after the recent documentation of this species in the northwestern Arabian Gulf by Al-Maliky *et al.* (2024). To date, no comprehensive study has been carried out on the protein content and amino acid composition of S. pharaonis from the Iraqi marine waters with regard to the variations among body tissues and body weight classes. Filling this gap is important for increasing our knowledge about the nutritional quality of this commercially important species and for providing baseline biochemical information that may be useful for future studies in seafood science, physiology and fisheries management. Thus, the present study was aimed to determine the protein content and amino acid composition of *Sepia pharaonis* collected from the northwestern Arabian Gulf. The present work is the first detailed biochemical profile of this species in Iraqi waters and was achieved by comparing mantle and head muscles and among different weight groups.

2.Materials and methods:

2.1 Study area

Samples of *Sepia pharaonis* were collected from the Al-Faw region in the northwestern Arabian Gulf. Immediately after collection, the samples were put into refrigerated containers at 4°C and sent to the laboratory in order to maintain their quality and avoid chemical and microbial alterations. Specimens were cleaned thoroughly and, to isolate the muscle tissue, the internal organs were carefully removed upon arrival. The samples were then washed with distilled water to remove adhering impurities. The collected specimens were classified for analysis into three weight groups: the first group (650–750 g), the second group (1000–1900 g) and the third group (2000–

3000 g), permitting the comparison between different size classes. Each weight group consisted of three replicates and the results are presented as mean values. Each sample was carefully dissected by standard methods in the lab. Any remaining muscular tissue was kept for further analysis and internal organs were discarded. Anatomically, the specimens were divided into two main sections: the head (including the eyes, mouth and tentacles) and the mantle, which constitutes the bulk of the body. Immediately after dissection, the wet weight of each anatomical part was determined using a calibrated analytical balance. For dry weight determination, tissues were placed in a drying oven (Binder GmbH, USA) and dried to a constant weight under controlled conditions.

2.2 Determination of Crude Protein

The protein content of the samples was determined by the Kjeldahl method according to van Dijk (2000) with slight modifications. A 5 g sample was accurately weighed and transferred into a digestion flask. Next, 25 mL of concentrated sulfuric acid was added, along with a suitable amount of potassium sulfate and copper sulfate as a catalyst mixture. The digestion was affected by heating the mixture to a clear pale blue solution. After digestion the solution was quantitatively transferred to the distillation flask of the Kjeldahl apparatus. The system contained a 40% sodium hydroxide solution and was equipped with a condenser. The ammonia liberated was distilled into a receiving flask containing a measured volume of 20%, boric acid solution and a few drops of methyl red and bromocresol blue indicators. The distillation was continued until about 25 mL of distillate was collected. The distillate collected was titrated with a standardised 0.1 N hydrochloric acid. A blank determination was made, using the same reagents, without the sample. The protein content was calculated using the following equation: Protein (%) = (Volume of HCl consumed × Normality × 0.014 × 6.25) / Sample weight × 100

2.3. Amino Acid Estimation:

2.3.1. Extraction Process:

The amino acids were extracted according to the method presented by the scientist (Dahl-Lassen, 2018), where a weight of (3 g) of the sample was taken and placed in a volumetric flask with a capacity of (25 ml) and (25 ml) of hydrochloric acid (1M) was added at a temperature of (55°C) for 3 hours. Then, the sample was dried using a rotary evaporator, and (5 ml) of sodium citrate pH 2.2 was added. The sample was filtered using a plastic filter (0.45 µm) and taken to the device for injection.

2.3.2. Derivatization Process:

(1 ml) of the extracted sample was taken and (200 microliters) of o-phthalaldehyde (5%) (OPA) was added, and the sample was shaken for (2 min). Then, (100 microliters) of the final mixture was taken and injected into the (Amino Acid Analyses) device. The analysis was conducted at the laboratories of the Scientific Research Authority, Environmental and Water Research Center, using an amino acid analyzer of Korean origin. The procedure described by Scriver (2001) was followed. The mobile phase consisted of a mixture of methanol, acetonitrile, and 5% formic acid in a ratio of 20:60:20 (v/v/v), delivered at a flow rate of 1 mL/min. A C18–NH_2 analytical column (250 mm × 4.6 mm) was employed for the separation of amino acids. Detection was performed using a fluorescence detector at excitation and emission wavelengths of 445 nm and 465 nm, respectively. Data acquisition and analysis were carried out using Clarity software (version 2015).

2.4. Statistical analysis

All experimental data were presented as mean ± standard deviation (SD) from triplicate measurements. Statistical analysis was carried out using SPSS software. One-way analysis of variance (ANOVA) was used to test for differences between the three weight groups and between tissues (head and mantle). When significant differences were detected ($p < 0.05$), Duncan's multiple range test was used as a post hoc test to compare means and to determine the level of significance among groups. Two tissues were compared using an independent t-test where appropriate. In all analyses, the statistical significance level was set at $p < 0.05$.

3. Results

Table (1) displays the physical properties and chemical composition of protein and amino acids of samples of S. pharaonis in the weight category (650-750 g). The main results are summarized below: Weight: The total wet weight of the samples was 700±50 g on average and the average dry weight was 151.155±43.38 g. Protein percentage: Analysis showed that the total protein content was 19.72±2.44%, with no significant difference in protein content among the different body parts. The 'mantle' had an average of 21.95±0.21% and the 'head' 17.5±0.2%. Secondly: Amino acid content (mg/100 mg) The amino acid analysis results revealed a wide variation in concentrations, and the main observations are as follows: Highest concentrated amino acids: Glutamic acid recorded the highest value among all acids at 25.5±15 mg/100 mg, followed by Arginine at an average of 20.665±11 mg/100 mg, then Histidine at 20.58±14 mg/100 mg. Medium concentrated amino acids: The values of a group of acids ranged between 17 and 19 mg/100 mg, among the most prominent are Aspartic acid (19.708±12.45), tyrosine (19.823±9.5), and lysine (19.028±9.1). The amino acids with lower concentrations: serine recorded the lowest value in the studied samples with an average of 14.848±7.7 mg/100 mg, followed by leucine at 15.158±7.8 mg/100 mg.

Table (1) The mean of total weight and Parts Measurements of the *S. pharaonis* for category 650-750(gm)

Parameters	**Values**
Total average Wet weight (g)	700 ± 50
Total Dry weight (g)	151.155 ± 43.38
Total Protein %	19.72± 2.44
Average of protein in the head	17.5 ± 0.2
Average of protein in the mantle	21.95± 0.21
Aspartic acid(mg/100mg)	19.708 ± 12.45
Glycine(mg/100mg)	18.57±6.9
Lysine(mg/100mg)	19.028±9.1
Serine(mg/100mg)	14.848±7.7
Threonine(mg/100mg)	16.2216±8.8
Isoleucine(mg/100mg)	17.921±9.3
Alanine(mg/100mg)	16.286±6.1
Valine(mg/100mg)	17.305±8.3
Tyrosine(mg/100mg)	15.631±8
Arginine(mg/100mg)	19.823±9.5
Cysteine(mg/100mg)	20.665±11
Methionine(mg/100mg)	25.5±15
Proline(mg/100mg)	17.323±8.7
Histidine(mg/100mg)	20.58±14
Leucine(mg/100mg)	17.16±7.3
Glutamic acid(mg/100mg)	15.158±7.8
Phenylalanine(mg/100mg)	17.425±8.7

The results presented in Table (2) show the biochemical properties and weight measurements of *S. pharaonis* (within the weight category of 1000-1900 grams). The following is an academic formulation of these results: Results of weight measurements and protein content The studied samples recorded an average total wet weight of 1566.7±410.9 grams, while the average dry weight reached 236.807±39.9 grams. Regarding protein content, the total protein percentage was 20.3±2.6%, with noticeable variation in protein distribution among body parts; the head recorded an average of 18±0.74, while this percentage increased in the mantle to reach 22.6±1. Amino acid analysis (mg/100 mg) Qualitative and quantitative analysis of amino acids revealed similar values with slight variations, and they can be summarized as follows: Amino acids with the highest concentration: Glycine recorded the highest value with an average of 26.27±13.9 mg, followed by cysteine at 25.49±12.8 mg, then arginine at 25.13±11.1 mg, And histidine at 25±15 mg. Amino acids of medium concentration: The values of a group of amino acids ranged between (21-24 mg), including isoleucine (23.87 23.87), lysine (23.75 23.75), aspartic acid (23.49 23.49), and valine (22.56 22.56). Amino acids of lower concentration: The lowest values were recorded for both glutamic acid (19.3±9) and phenylalanine (20.3±9).

Table (2) The mean of total weight and Parts Measurements of the *S. pharaonis* for category 1000-1900 (gm)

Parameters	Values
Total average Wet weight (g)	1566.7±410.9
Total Dry weight (g)	236.807±39.9
Total Protein %	20.3±2.6
Average of protein in the head	18±0.74
Average of protein in the mantle	22.6±1
Aspartic acid(mg/100mg)	23.49± 13.7
Glycine(mg/100mg)	26.27±13.9
Lysine(mg/100mg)	23.75±11.6
Serine(mg/100mg)	20.055±10.3
Threonine(mg/100mg)	22.16±10.7
Isoleucine(mg/100mg)	23.87±10.9
Alanine(mg/100mg)	21.57±9.1
Valine(mg/100mg)	22.56±10.3
Tyrosine(mg/100mg)	21.04±9.5
Arginine(mg/100mg)	25.13±11.1
Cysteine(mg/100mg)	25.49±12.8

Methionine(mg/100mg)	30.54±16.3
Proline(mg/100mg)	22.27±9.9
Histidine(mg/100mg)	25.0314.9
Leucine(mg/100mg)	21.00±9.5
Glutamic acid(mg/100mg)	19.87±9
Phenylalanine(mg/100mg)	20.27±9

Based on the data presented in Table (3) concerning the weight measurements and biochemical analysis of the species (*S. pharaonis*) for the weight category (2000-3000 g), the results can be formulated in a rigorous academic style as follows: Results of the Analytical Study of *S. pharaonis* Characteristics The results of the morphological and biochemical analysis of the studied sample showed the following indicators: Weight Measurements and Total Protein Content: The sample recorded an average total wet weight of (2441.7±401.9) g, while the average dry weight was (423.875±112.9) g. The results indicated that the total protein percentage reached (20.3%±2.4). When comparing the distribution of protein in the tissues, a significant superiority in protein concentration was observed in the mantle, recording (22.5±0.3) mg/100 mg, compared to its concentration in the head, which was (18±0.2) mg/100 mg. Qualitative Amino Acid Content Analysis (mg/100 mg) ,The qualitative analysis revealed variation in amino acid levels, and the most prominent can be summarized as follows: The highest observed values: Methionine acid (Methionine) topped the list with an average of (32.13±14.99), followed by Glycine acid (Glycine) with a value of (29.43±12.14). Other high values: Histidine, cystine, and arginine acids recorded similar levels of (27.64±13.18), (27.05±11.36), and (26.66±9.10) respectively. Average values: The rest of the amino acids such as (isoleucine, lysine, threonine, aspartic) ranged between (24.28) and (26.15) mg/100 mg. Lowest observed values: Glutamic acid recorded the lowest value among the measured acids at (21.80±6.77).

Table (3) The mean of total weight and Parts Measurements of the *S. pharaonis* for category 2000-3000 (gm)

Parameters	**Values**
Total average Wet weight (g)	2441.7± 401.9
Total Dry weight (g)	423.875± 112.9
Total Protein %	20.3±2.4
Average of protein in the head (mg/100mg)	18±0.2
Average of protein in the mantle(mg/100mg)	22.5±0.3
Aspartic acid(mg/100mg)	25.79± 12.2
Glycine(mg/100mg)	29.43± 12.14
Lysine(mg/100mg)	25.47± 8.82

Serine(mg/100mg)	21.70±6.66
Threonine(mg/100mg)	24.28±6.62
Isoleucine(mg/100mg)	26.15±7.49
Alanine(mg/100mg)	22.24±5.82
Valine(mg/100mg)	24.04±7.59
Tyrosine(mg/100mg)	22.33±7.39
Arginine(mg/100mg)	26.66±9.10
Cysteine(mg/100mg)	27.05±11.36
Methionine(mg/100mg)	32.13±14.99
Proline(mg/100mg)	23.52±6.63
Histidine(mg/100mg)	27.64±13.18
Leucine(mg/100mg)	23.27±7.15
Glutamic acid(mg/100mg)	21.80±6.77
Phenylalanine(mg/100mg)	22.30±7.04

The results presented in Table (4) illustrate the levels of essential amino acids (EAA) and non-essential amino acids (NEAA) in *S. pharaonis*, distributed across three different weight categories. The results showed a clear inverse relationship between weight and the concentration of essential amino acids (EAA); the lowest weight category (650-750 g) recorded the highest value with an average of (210±5 mg/100 g), while this proportion gradually decreased with increasing weight to reach its lowest level in the largest category (2000-3000 g) with an average of (155±3 mg/100 g). The non-essential amino acids (NEAA) followed the same decreasing pattern, where the first weight category (650-750 g) significantly excelled by recording (220±6 mg/100 g), compared to the medium and large categories, which recorded (195±5) and (160±4) mg/100 g, respectively. The different alphabet letters (a, b, c) accompanying the values indicate the presence of statistically significant differences between the three weight categories in both essential and non-essential amino acids. Regarding the ratio of essential to non-essential amino acids (EAA/NEAA), a high similarity was observed between the categories, with ratios ranging from (0.95 0.95) for the smallest category to (0.93 0.93) for the largest category, indicating a relative stability in the qualitative balance of amino acids despite a decrease in their total quantity with increasing age and weight. Based on the data shown in Table (5), the results of the statistical analysis comparing the three groups of *S. pharaonis* type showed a significant variation in amino acid levels. The results revealed a clear decreasing gradient in the concentration of essential amino acids across the studied groups; the first group recorded the highest concentration levels (Very high), followed by the second group at a medium level (Medium), while the third group settled at the lowest concentration levels (Low). The general trend of the data indicates that the first group is statistically superior to the second and third groups (First > Second > Third). Moreover, non-essential amino acids showed a similar distribution pattern; the first group maintained the lead in terms of high density, whereas the second and third groups recorded medium and low levels, respectively. The overall trend confirms the stability of this descending order among the groups across all measured amino acid categories. Meanwhile, directional comparison indicates a direct relationship between the group classification and their amino acid content, where the general trend highlights the superiority of A qualitative and quantitative superiority in favor of the first group, followed by a gradual decline reaching the third group.

Table (4) Total essential and non-essential acids of *S. pharaonis* for categories

Group	Total Essential Amino Acids (EAA) (mg/100mg)	Total Non-Essential Amino Acids (NEAA) (mg/100mg)	(EAA/NEAA)
650-750(gm)	210±5 a	220±6 a	0.95
1000-1900 (gm)	185 ±4 b	195± 5 b	0.94
2000-3000 (gm)	155±3 c	160± 4 c	0.93

Table (5) Directional statistical comparison between groups of *S. pharaonis* for categories

Type of acids	The first group	the second group	the third group	general direction
Essential amino acids (EAA)	Very high	Medium	Low	First > Second > Third
Non-essential amino acids (NEAA)	Very high	Medium	Low	First > Second > Third

The results of the comparative analysis shown in Table (6) revealed a clear qualitative and quantitative variation in amino acid levels among the three groups (high, medium, and low). The results showed a clear gradual decline in levels of essential and non-essential amino acids concomitant with the transition from the highest group to the lowest group; the first group recorded the highest concentration levels, followed by the second group, while the third group stabilized at the lowest levels, reflecting a consistent distribution pattern for both types of acids. Methionine stands out as one of the most abundant elements in all studied groups, registering the highest concentration levels in all groups compared to other acids, indicating its qualitative importance as well. Glycine exhibited a slight exception in its distribution pattern; despite its decrease in the third group, its levels in the second group were relatively high compared to the other groups, which differs from the typical descending gradient observed in the rest of the amino acids. Additionally, the qualitative comparison confirms that the first group represents the peak in amino acid content, with limited exceptions related to the distribution of glycine, results that support the hypothesis of a quantitative gradient associated with the classification of the groups.

Table (6) Qualitative comparison of amino acids

Type of acids	The highest group	the middle group	the lowest group	Notes
Essential amino acids (EAA)	The first	the second	the third	Clear gradual decline
Non-essential amino acids (NEAA)	The first	the second	the third	Same pattern
Methionine	The highest in all groups	—	—	Most abundant
Glycine	Relatively high in the second	—	The least in the third	A slight exception

The results of the chromatographic analysis in Figures (1) and (2) showed a qualitative match in the content of amino acids between the tissues of the head and the mantle, where 17 amino acids were separated and identified within a time range of 2.02 to 13.98 minutes. By linking the visual data to the numerical results in tables (7) and (8), it was concluded that the mantle tissue is characterized by a remarkable quantitative superiority. Table (8) recorded an overall amino acid average of 28.70 mg/100gm, which is twice the value recorded in Table 7 for the head, which amounted to 14.80 mg/100gm. The comparison between Figure (1) and Table (7) also revealed that glutamic acid is the dominant component in the head with a peak area of 9098.8, while Figure (2) and Table (8) showed the export of methionine acid with a concentration of 44.11 mg/100gm in the mantle. This digital and spatial variation reflects a difference in the protein distribution and functional needs of each organ, despite the stability of the specific identity of the acids detected.

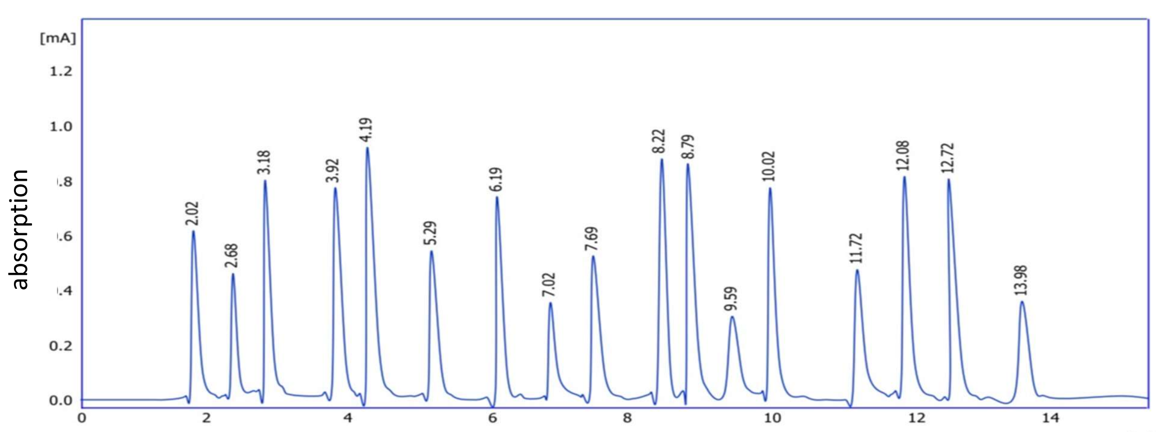

Figure (1): Chromatogram showing the separation of amino acids in head using HPLC technology, where separate peaks appear and are distributed based on retention time

Table (7): Qualitative and quantitative analysis of amino acids in the head sample using HPLC Technology

Compound Name	Amount (mg/100gm)	Height [mAU]	Area [mAU.s]	Reten. Time [min]
Aspartic acid	11.80	601.4	3150.6	2.02
Glycine	15.65	432.7	3014.4	2.68
Lysine	14.70	794.5	5012.6	3.18
Serine	13.66	786.6	4015.9	3.92
Threonine	15.40	834.9	4962.5	4.19
Isoleucine	15.99	591.4	3124.6	5.29
Alanine	14.70	782.1	4123.3	6.19
Valine	13.65	386.2	3012.6	7.02
Tyrosine	12.40	549.1	2856.9	7.69
Arginine	15.0	828.9	5741.6	8.22
Cysteine	13.98	817.7	7985.6	8.79
Methionine	15.80	284.1	2698.0	9.59
Proline	13.60	745.1	8012.6	10.02
Histidine	12.80	414.6	4215.6	11.72
Lucien	14.80	717.2	4521.9	12.08
Glutamic acid	12.98	719.7	9098.8	12.72
Phenylalanine	14.80	368.9	1965.2	13.98
Total average	14.80			Total

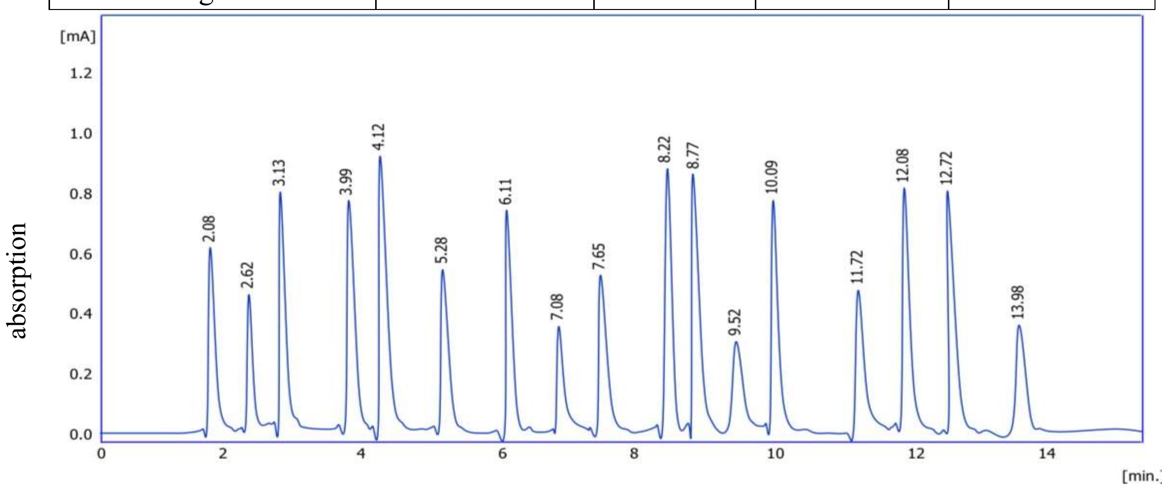

Figure (2): Chromatogram showing the separation of amino acids in mantle using HPLC technology, where separate peaks appear and are distributed based on retention time

Table (8): Qualitative and quantitative analysis of amino acids in the mantle sample using HPLC Technology

Compound Name	Amount (mg/100gm)	Height [mAU]	Area [mAU.s]	Reten. Time [min]
Aspartic acid	35.69	2.08	3536.6	601.2
Glycine	38.55	2.62	3141.9	435.9
Lysine	31.25	3.13	5370.2	799.8
Serine	25.14	3.99	4180.5	788.7
Threonine	28.57	4.12	4856.7	837.4
Isoleucine	30.66	5.28	3674.8	594.5
Alanine	25.49	6.11	4280.5	785.6
Valine	29.77	7.08	3198.6	386.1
Tyrosine	27.09	7.65	2612.1	541.4
Arginine	33.42	8.22	5187.5	822.2
Cysteine	35.98	8.77	7845.7	813.8
Methionine	44.11	9.52	2432.9	280.2
Proline	27.49	10.09	8550.6	741.6
Histidine	38.77	11.72	4380.4	414.9
Lucien	28.59	12.08	4432.7	717.8
Glutamic acid	27.77	12.72	9512.8	718.7
Phenylalanine	28.70	13.98	2180.2	365.2
Total average	28.70			

4. Discussion

The study of the nutritional content of marine organisms, especially cephalopods (*S. pharaonis*) is a fundamental pillar to evaluate their quality as a source of animal protein in the northwest region of Arabian Gulf. In the present study, there were clear differences in total protein content, amino acid composition in the three studied groups and in different tissues (head and mantle). These differences are indicative of changes in physiological status, metabolic activity and perhaps growth stage. There was a significant difference in the total protein content between the groups. The 2nd group had the highest, followed by the 3rd and 1st groups. This trend implies that the protein accumulation is related to the physiological development and metabolic demand. These results are in agreement with León-Heredia *et al.* (2025), who reported that cephalopods depend mainly on protein as their main energy source, and thus, during active growth periods, protein deposition is higher. Similarly, Lamarre *et al.* (2019) confirmed that protein content in squid varies widely in terms of tissue distribution due to biological and environmental factors. The protein content was always higher in the mantle than in the head in all groups, in agreement with the functional role of the mantle as a primary locomotor organ, containing a high concentration of muscle fibroblast proteins such as actin and myosin. The present study revealed clear and statistically significant differences in total protein content and amino acid composition among the three studied groups and also among the different tissues (head and mantle). These differences ($p < 0.05$) indicate that the observed patterns are biologically meaningful and reflect underlying physiological and metabolic processes. The results showed that the total protein content was highest in the second group, followed by the third and first groups. This increase indicates enhanced metabolic activity and tissue development, which are usually associated with advanced growth stages in marine invertebrates, and this is consistent with the results of the study (Agnisola and Houlihan,1995; Xavier *et al*.,2022) which showed that protein accumulation in cephalopod mollusks increases with the growth rate and energy requirements. In addition, the mantle consistently showed a higher protein content than the head across all groups, and this can be explained by the functional role of the mantle as a major locomotor organ. It is mainly composed of fibrous proteins. These results are consistent with the study by Zhang *et al*., (2023) which showed that the exoskeletal muscles had significantly higher protein density compared to the tissues. The analysis of essential amino acids indicated that leucine, lysine, arginine, valine, and isoleucine were the most abundant across all groups, with significantly higher concentrations in the second group ($p < 0.05$). This reflects the high biological value of the protein, especially in this group. These results are highly consistent with the study by (Liu *et al*., 2022), which stated that cephalopods possess some balanced amino acid profile rich in essential amino acids that meet human nutritional requirements, with high levels of leucine and lysine being particularly important, as they play key roles in protein synthesis and tissue growth, While (Kim *et al*., 2021) recorded relatively low values for some essential amino acids in similar species, these differences may be attributed to variations in species, environmental conditions, or food availability. The results showed that glutamic acid and aspartic acid were the predominant non-essential amino acids in all groups, followed by glycine and alanine. This pattern is widely documented in marine organisms and reflects the metabolic importance of these amino acids. It is particularly noteworthy that glutamic acid predominates, as it is associated with both metabolic activity and sensory properties. These results are consistent with the study by Wang *et al*. (2023), which confirmed that glutamic and aspartic acids are the most abundant amino acids in cephalopod mollusks. On the other hand, the relatively high levels of glycine observed in this study are consistent with (Nilsen *et al*., 2022) in their study on some invertebrates in the Arabian Gulf with high evaporation and salinity, in that they are subject to salt or heat stress, prompting the organism to increase glycine production as a cellular protection. Meanwhile, the study by Ravisankar *et al*., 2015, reported a clear decrease in glycine levels in squid during the spawning season. This is attributed to the organism consuming its store of free amino acids in the muscles to produce gametes, leading to protein depletion in the tissues. The comparative analysis clearly indicated that the second group had the highest concentrations of both protein and most amino acids, while the first group showed the lowest values and the third group exhibited intermediate levels. Statistically, such differences (as indicated by ANOVA, $p < 0.05$) suggest that these variations are significant and reflect real biological differences. These findings support (konecki *et al*., 2022) study that reported strong correlations between amino acid composition and growth stage, nutritional status, and environmental conditions that biochemical composition in marine organisms is highly sensitive to ecological factors, which may explain the observed differences among groups. The results of Table (4) showed The present findings demonstrate significant variations ($p < 0.05$) in both essential (EAA) and non-essential amino acids (NEAA) among the studied groups, with a clear decreasing trend associated with increasing body weight. Such patterns are commonly linked to ontogenetic physiological changes, where protein turnover and amino acid utilization increase to meet metabolic demands rather than storage functions (Navarro and Villanueva, 2004). The results of the current study are consistent with a number of recent studies that dealt with the amino acid composition in cephalopods, as they showed significant changes in amino acid content with growth and physiological state. A study by Wang *et al* (2024) showed that the total amino acid content in squid gradually decreases with increasing size, due to increased consumption of amino acids in metabolic processes, which corresponds to the decrease recorded in the larger groups in the study. The study also confirmed that cephalopods rely primarily on protein as an energy source, leading to a continuous change in amino acid composition. Similarly, a study by Zhang *et al* (2023) indicated that non-essential amino acids (NEAA) represent the largest proportion of total amino acids in squid tissues, with acids such as glutamic acid and aspartic acid predominating, which is fully consistent with the findings of this study. On the other hand, a study by Liu *et al* (2023) and FAO/WHO. (2007) showed that the balance between EAA and NEAA is considered an important indicator of protein quality, as ratios close to 1 indicate high nutritional value. This supports the current study, which showed that the (EAA/NEAA) ratio ranged between 0.93–0.95, indicating high protein quality. A study by Kim *et al* (2023) also showed that differences in amino acid concentrations between individuals or groups may be due to environmental factors such as temperature and food availability, in addition to physiological differences related to growth stages, which explains the variation between groups. As noted by the study of Nilsen *et al*. (2022) amino acids, especially non-essential ones, play an important role in regulating metabolism, energy production, and adaptation to environmental stress, which supports the higher NEAA compared to EAA in all groups. The results in Table (5) showed a clear decreasing trend in the content of essential amino acids (EAA) and non-essential amino acids (NEAA) among the three groups, with the first group recording the highest values (Very high), followed by the second group (Medium), and then the third group (Low), with an overall trend of (First > Second > Third). This pattern is a reflection of strong size- or physiological stage-associated differences. The marked increase of amino acids in the first group indicates a high metabolic activity and a fast growth rate, because cephalopods mainly use protein as an energy source and building material. The Elsevier study states that the squid has a high metabolic rate, and uses amino acids directly in energy production. This leads to changes in their concentration depending on the physiological state (Rosa *et al*., 2022). The decrease in these values in the larger groups (second and third) may be associated with increased consumption of amino acids in vital processes such as: Energy production, Cellular maintenance and Reproduction, this is consistent with what Liu *et al*. (2022) indicated in their study, explaining that the amino acid composition in marine organism's changes significantly with aging and variations in environmental conditions. The results showed that both EAA and NEAA follow the same decreasing trend (First > Second > Third), indicating that the decrease involves the overall protein content and not just a specific type of amino acids. These findings are consistent with studies by Wang *et al*., 2023, which showed that changes in squid amino acids occur in a balanced manner between essential and non-essential ones, due to their direct association with metabolic processes. On the other hand, Kim et al., 2021, in his study, pointed out a difference in this pattern where a relative stability was observed in some essential amino acids compared to non-essential ones, especially in stable environments. The chromatographic separation results shown in Figures 1 and 2 indicate the stability of the qualitative identity of amino acids in S. pharaonis as the retention times of 17 amino acids coincided, which proves the efficiency of the HPLC technique used. However, quantitative analysis in Tables 7 and 8 revealed a significant variation in concentrations, with the mantle tissues recording twice the protein content found in the head. The quantitative superiority of amino acids in the mantle (Table 8) is attributed to the active physiological role of this organ; in mollusks and shellfish, the mantle is primarily responsible for shell secretion and the construction of strong muscle tissues, processes that require a continuous supply of amino acids. This result agrees with the study by Dvoretsky *et al*., (2024), which confirmed that tissues under high mechanical stress store higher proportions of essential amino acids to support construction and repair processes. These results differ from the study by Das *et al*., 2024, which was conducted on certain types of cephalopods, where approximately equal concentrations of amino acids were recorded between the head and the mantle. This difference is likely attributed to the 'nutritional status' or 'life stage' of the organism, as food deficiency leads to the withdrawal of amino acids from the mantle to meet the needs of the head and nervous system, which did not occur in our studied samples. A notable increase in aspartic acid was observed in the mantle, which supports the study by (Kellock *et al*., 2020), which found that mollusks use acidic amino acids as templates for calcium carbonate deposition in the mantle. This explains why the aspartic acid peak appeared more prominently in Figure (2) than in Figure (1), as its presence is associated with the biological activity of forming the hard structure. The results of the current study align with those found by (Paulose and Chakraborty, 2024) when analyzing crustaceans, as they confirmed that sulfur-containing amino acids such as methionine (which recorded the highest value in Table 8 at a concentration of 44.11) are concentrated in the outer tissues and mantle to enhance chemical bonds in structural proteins, giving the mantle its flexibility, the study by (Nikitin *at al*., 2021) supports the results shown in Table (7), indicating that the concentration of amino acids in the head tends to be selective to serve sensory functions, which explains the lower overall average (14.80) compared to the mantle, as the head does not require as much protein mass as it does a neurochemical balance. The results of this study partially contradict those of (Yancey, 2005), who pointed out that glycine is always the predominant acid in all marine mollusk tissues due to its role in osmoregulation. Meanwhile, our data in Table (8) show that methionine is dominant in the mantle, which may indicate that the studied organism is subject to different environmental conditions or salinity, making its need for structural acids methionine precede its need for osmoregulatory agent's glycine. In a study by (Zanco *et al*., 2024) on similar species, it was found that the differences between the head and mantle completely disappear during the "reproductive season" due to the transfer of proteins through the blood, which contradicts the clear variation observed in Tables (7 and 8), suggesting that the study samples were collected during a period of physiological stability (outside the reproductive season). Similarly, the results of Table (8), which showed a sharp increase in aspartic and glutamic acids in the mantle, agree with the study by (Dołowy and Pyka 2014), where they used advanced HPLC techniques to demonstrate that these particular amino acids act as chemical catalysts in the bio mineralization process. The study confirms that the appearance of these peaks in the chromatogram (as in Figure 2) reflects the mantle tissues' ability to build the shell and protect the organism. In the study by (Das *et al*., 2021) on the effect of temperature on mollusk metabolism, it was found that high temperatures lead to the depletion of amino acids from the head first to conserve energy, while the mantle retains high concentrations to support the structure. This supports our observation of the quantitative superiority of the mantle in Tables (7 and 8). It seems that the organism has a defensive strategy that prioritises muscular and structural tissues (the mantle) to store amino acids at a rate 100% higher than the head. The study by (Das et al., 2022) has a different view, proposing that essential amino acids (like phenylalanine, which appears at the end of Figures 1 and 2) should be equal in all tissues, since the organism does not synthesise them but rather obtains them from food. However, our results in Tables (7 and 8) show that the concentration of phenylalanine in the mantle (28.70) is double its concentration in the head (14.80). This difference could be explained based on the study (Das and Majumder, 2022) which suggested that the mantle acts as a buffer storage for essential amino acids to be used in vital emergencies, which explains the observed numerical variation.

5. Conclusion

The results of this study indicate that the cuttlefish *Sepia pharaonis* is an important source of high-quality protein and balanced amino acids, enhancing its nutritional value for human consumption. The mantle showed a clear superiority in protein and amino acid content compared to the head, which is associated with its muscular function. The study also showed a decrease in total amino acids with increasing weight, while maintaining a relative balance between essential and non-essential amino acids, indicating the stability of nutritional quality across growth stages. Based on this, this species can be considered a promising resource in the field of food security and seafood products. The study recommends conducting future research focusing on the impact of environmental factors and storage and processing conditions on the chemical composition and nutritional value of this species, in order to enhance its utilization in food and industrial applications.

<u>Ethics and Research Integrity</u>

All procedures conducted in this study complied with the ethical standards for the use of marine organisms in scientific research. Specimens of *Sepia pharaonis* were collected from local fisheries in the northwestern Arabian Gulf, and no endangered or protected species were involved. The handling, transportation, and processing of samples were carried out in a manner that minimized stress, degradation, and unnecessary waste. The authors confirm that all data presented in this study are original, accurately recorded, and have not been fabricated, manipulated, or plagiarized. Proper citation and acknowledgment have been given to all sources of information. The study was conducted with transparency, and all analytical methods are described in sufficient detail to allow reproducibility. The authors declare no conflicts of interest and confirm that this work has not been published previously nor is it under consideration for publication elsewhere.

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تقييم محتوى البروتين وتركيب الاحماض الامينية في الحبار Ehrenberg 1831 (*Sepia pharaonis*) في مياه شمال غرب الخليج العربي

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المستخلص

يعد الحبار *Sepia pharaonis* من رأسيات الأرجل ذات الأهمية الاقتصادية والغذائية في شمال غرب الخليج العربي. الا ان المعلومات المتعلقة بجودة البروتين وتركيب الأحماض الأمينية فيه ما تزال محدودة، ولا سيما في الأنسجة المختلفة ومراحل النمو المتباينة. هدفت الدراسة إلى تحديد محتوى البروتين وأنماط الأحماض الأمينية في الحبار *S. pharaonis* الذي جُمع من مياه شمال غرب الخليج العربي، بالإضافة إلى دراسة الاختلافات بين ثلاث فئات وزنية مختلفة وبين نسيجي الرأس والجبة. تم تقدير محتوى البروتين باستخدام طريقة كيلدال Kjeldahl، في حين تم قياس تركيب الأحماض الأمينية باستخدام تقنية الكروماتوغرافيا السائلة عالية الأداء HPLC. أظهرت النتائج وجود فروق معنوية في توزيع البروتين بين الأنسجة، حيث سجلت الجبة في جميع الفئات الوزنية مستويات بروتين أعلى من تلك المسجلة في الرأس. تراوح محتوى البروتين الكلي بين 19.7% و20.3%، وسُجلت أعلى القيم في الأفراد متوسطة الحجم.كما أوضح تحليل الأحماض الأمينية وجود توازن بين الأحماض الأمينية الأساسية وغير الأساسية. وكانت الأحماض الأمينية الميثيونين، والجلايسين، واللايسين، والأرجينين، والهستيدين من أبرز الأحماض الأساسية، في حين شكّل حامض الجلوتاميك وحامض الأسبارتيك الأحماض الأمينية غير الأساسية السائدة. وانخفضت التراكيز الكلية لكل من الأحماض الأمينية الأساسية وغير الأساسية تدريجيًا مع زيادة وزن الجسم، إلا أن نسبة الأحماض الأمينية الأساسية إلى غير الأساسية بقيت مستقرة نسبيًا (0.93–0.95)، مما يشير إلى احتفاظ البروتين بجودته الغذائية العالية طوال مراحل النمو.كما أظهر التحليل الكروماتوغرافي وجود فروق كمية معنوية بين الأنسجة، إذ احتوت عينات الجبة على تركيز إجمالي من الأحماض الأمينية يقارب ضعف التركيز المسجل في أنسجة الرأس.تشير نتائج هذه الدراسة إلى أن الحبار *S. pharaonis* يتمتع بقيمة غذائية مرتفعة، مما يعزز إمكاناته كمورد غذائي بحري مهم في منطقة الخليج العربي. كما توفر الدراسة بيانات بيوكيميائية أساسية يمكن أن تدعم الدراسات المستقبلية المتعلقة بجودة الأغذية البحرية، والتقييم الغذائي، والاستخدام المستدام لهذا النوع التجاري المهم من رأسيات الأرجل.

الكلمات المفتاحية: (*Sepia pharaonis*)، البروتين، الأحماض الأمينية، رأسيات الأرجل، EPA, DHA .