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Chemical Composition and Analysis of Some Mineral Elements in the Cuttlefish *Acanthosepion pharaonic* (Ehrenberg 1831(Cephalopoda: Sepiidae) from Southern Iraqi Coastal Waters

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Abstract - The Chemical composition of protein%, fats%, Total Moisture%, Total Ash weight (gm), Total organic matter (gm) and minerals of the *Acanthosepion pharaonic.* was analyzed. The individuals were divided into three categories which are (650-750, 1000-1900 and 2000-3000) (gm). The Sepia was separated into two parts, which are the head and mantle. The aim of the present study is to explore the possibilities for future applications in the health and food sectors. The total average Wet weight (gm) was 700±50, 1566. 7±410.9 and 2441.7±401.9 respectively. Total Dry weight (gm) was151.155±43.38, 236.807±39.9, 423.875±112.9 respectively. Total organic matter where 140.8±42.6,225.3±44.1, 396.5±131.5, and Total Protein %39.45±0.33, 39.75±0.77, 41.4±0.50, and Total Fat % 10.96±0.23,11.24±0.7, 12.36±0.23. The percentage of mercury (Hg) in 700±50(gm). the category was very high in the head, higher than in the mantle, reaching (38.9±0.046%), while it was low in the mantle 5.63± 0.011 %. The percentage of potassium (K) was lower than that of the other minerals, reaching (0.8± 0.003) %, while the remaining minerals: Fe, Zn, Pb, Mg, Na, Mn, Ca, and Cu were low percentages. Metals (Hg, Fe, Zn, Pb, Mg, Na, Mn, Ca, Cu, and K) in head, mantle and total was low in small dry weights and began to increase in medium and large weights. The results of the statistical analysis found a strong positive relationship between the protein percentages and the total dry weight of the three groups (r=0.91), while a very weak positive relationship was found between the fat percentages and the total dry weight of the three groups in different parts of the body (r=0.31).

التركيب الكيميائي وتحليل المعادن للحبار(*Acanthosepion pharaonic* (Ehrenberg 1831 في المياه الساحلية البحرية / الفاو جنوب العراق

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المستخلص – تم تحليل التركيب الكيميائي للبروتين (٪) والدهون (٪) والرطوبة الكلية (٪) ووزن الرماد الكلي (غم) والمادة العضوية الكلية (غم) والمعادن للحبار *Acanthosepion pharaonic* تم تقسيم الأفراد إلى ثلاث فئات وزنية وهي (650 – 750 ، 1000 – 1900 و 2000 - 3000) غم. كذلك تم فصل كل فرد إلى جزئين، الرأس والجسم (الجبة). هدف الدراسة الحالية هو استكشاف الإمكانيات التطبيقية المستقبلية للحبار في قطاعات الصحة والغذاء. كان متوسط الوزن الرطب الكلي (غم) 700± 50، 1566.7± 410.9 ، 2441.7 ± 401.9 على التوالي. والوزن الجاف الكلي (غم) كان 151.155± 43.38 ، 236.807±39.9، 423.875 ± 112.9على التوالي. وبلغت المادة العضوية الكلية 14.08 ± 42.6، 225.3 ± 44.1، 396.5 ± 131.5 ، ونسبة البروتين الكلي٪ 39.45 ± 0.33 ، 39.75 ± 0.77 ، 41.4 ± 0.50، ونسبة الدهون الكلية٪ 10.96 ± 0.23 ، 11.24 ± 0.7 ، 12.36 ± 0.23. كانت نسبة الزئبق (Hg) في فئة 700 ± 50 (غم) عالية جدًا في الرأس، أعلى من الجسم، حيث بلغت (38.9 ± 0.046 %) ،

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بينما كانت منخفضة في الجسم (5.63 ± 0.011 %). كانت نسبة البوتاسيوم (K) أدنى من تلك الموجودة في المعادن الأخرى، حيث بلغت (0.8 ± 0.003 %) ، بينما كانت نسب المعادن المتبقية منخفضة في الرأس والجبة والمجموع الكلي في الأوزان الجافة الصغيرة وبدأت في الزيادة في الأوزان المتوسطة والكبيرة. وأظهرت نتائج التحليل الإحصائي وجود علاقة إيجابية قوية بين نسب البروتين والوزن الجاف الكلي للمجموعات الثلاث(r=0.91) ، في حين وُجدت علاقة إيجابية ضعيفة جدًا بين نسب الدهون والوزن الجاف الكلي للمجموعات الثلاث في أجزاء الجسم المختلفة (r=0.31).

الكلمات المفتاحية: التركيب الكيمياوي، الدهون، المعادن، البروتين.

Introduction:

Marine organisms are important as a significant food source for humans by analyzing the muscles of various species of crustaceans belonging to the class Malacostraca represented by shrimps, crabs and mollusks (Senadheera *et al.*, 2023). The seafood is widely used as food and feed supplement around the world and may be used in the enrichment of diet and new value-added products to decrease processing waste and increase profits (Obatolu *et al*., 2005); (Tibbetts *et al*, 2011); (Velu and Munuswamy, 2007). The pharaoh cuttlefish *Sepia pharaonis* is widely distributed in the Indo-West Pacific region ranging from the Red Sea to Japan and Australia and forms a dominant species in the commercial fisheries. (Mehanna *et al.*,2014). The *Acanthosepion pharaonis* is a large cuttlefish species, growing to 42 cm in mantle length and 5 kg in weight (Pal *et al.*, 2022).

The chemical composition is defined as the percentage ratios of the main components represented by proteins, fats, carbohydrates, water content, and ash. Marine organisms contain high levels of energy sources, and there are many factors affecting the ratios of meat components in organisms, including variations in environments, species differences, diet, water temperature, and seasonal changes (Skonberg and Perkins, 2002).

Meats are composed of several components, including proteins, fats, vitamins, minerals, and other compounds. Proteins are one of the most important components, and they are considered one of the three major nutrients used by the body to build cells and tissues and to produce energy; they are the basic unit for building muscles. (Santi *et al*., 2017). High levels of proteins raise the nutritional value of species and make them among the most sought-after food types in many countries, especially Asian ones. Therefore, invertebrates are considered some of the most delicious foods in China and are cultivated at high densities (Yang *et al*., 2014).

Fats are the second most important part of macronutrients and are one of the essential energy sources for the body. They play a role in building membranes and regulating vital functions, consisting of chains of fatty acids that may be saturated or unsaturated. (Kummerow , 2014). Zlatanos *et al.*, (2006) stated that the high protein content of cephalopods and their low fat make them an important and healthy element in human diet either as fresh food or processed products. There are a lot of studies which included: (Gabr *et al*.,1998, Al-Nahdi *et al*,2009, Al-Farraj *et al*,2011, Ghazvineh *et al*,2012, Riad *et al*., 2015, Al-Khafaji, *et al*., 2017; Al-Maliky, *et al*., 2017; Al-Khafaji *et al*., 2019; Al-Maliky *et al*., 2021; Al-Maliky *et al*., 2024; Asvad *et al*., 2024). The aim of the present study is to determine the chemical composition of *Acanthosepion pharaonic* in terms of total proteins, total fat, ash, Total Moisture, and mineral analysis to explore possibilities for future applications in the health and food sectors.

Materials and Methods:

The samples of *A. pharaonic* were collected and preserved in plastic bags at a temperature of 20 C° before their transportation to the laboratory. These samples were categorized into three distinct groups based on their weight, which ranged from (650 – 3000) gm. All specimens were obtained from the coastal marine environment of Al-Faw, located in southern Iraq. In the laboratory, the internal organs of the specimens were systematically dissected and removed, while the muscular part was retained. Each specimen was anatomically divided into two sections: the head (which encompasses the eyes, mouth, and tentacles) and the mantle (which constitutes the primary body of the squid) using dissection instruments. Subsequently, the wet and dry weights were recorded using a sensitive balance scale for each part.

The samples were dried in a BINDER type drying oven of American origin at a temperature of 60 °C for 24 hours to obtain the dry weight then the dried samples were burned in a furnace at a temperature of 550°C for one hour, after which the ash was weighed using a sensitive scale. Total moisture was measured based on the following: Moisture% = (W1 - W2) / W1 * 100 Where: W1 = weight (gm) of sample before drying. W_2 = weight (g) of sample after drying. Determine the weight of the organic matter by calculating the difference between the dry weight and the ash weight, and express the result in grams. The Kjeldahl method was used to estimate the protein percentage in samples based on the procedure mentioned by Van Dijk, (2000) and others. The protein percentage is calculated according to the following equation: Protein % = (Volume of HCl consumed x Normality x 0.014 x 6.25) / Weight×100. The fat was estimated based on the method (AOAC 1995) where a weight of (10 g) of the dried sample was taken and placed in a filter paper and rolled up and placed in the extraction device (Soxhlet), and the percent fat was extracted according to the following equation: Fat percentage (%) = (Weight of the flask before extraction - Weight of the flask after extraction) / Weight of the sample x 100. The minerals were extracted and estimated according to the method (Özkan and Akbaba, 2013).

Statistical analysis:

Three replications were used to obtain average values and standard deviations for proximate biochemical properties and results are given as averages ± SD. Pearson correlation coefficient (r) was used to find the correlation between body parts, the head, the mantle, as well as the whole organism for the three categories and the percentage of protein and fat.

Results:

Table 1 shows the mean of Total Wet weight and proximate composition of *A. pharaonic* were (700±50-2441.7±401.9) gm. The mean Total Dry weight was (151.155±43.38-423.875±112.9) gm and the mean Total Moisture were (78.42±85.36 -84.9±374.7) % ،while the mean of Total Ash weight (10.364±2.71-27.308±5.5) gm also the mean Total organic matter was (140.8±42.6-396.5±131.5) g. in addition Total Protein% and Total Fat% were (39.45±0.33-41.4±0.50) % and (10.96±0.23-12.36±0.23) %.

Table 1. The mean of total weight and Parts Measurements of the *A. pharaonic*

weight classes / Parameters	650-750(gm)	1000-1900(gm)	2000-3000(gm)
Total average Wet weight (g)	700±50	1566.7±410.9	2441.7±401.9
Total Dry weight (g)	151.155±43.38	236.807±39.9	423.875±112.9
Total Moisture%	78.42±85.36	82.63±289.9	84.9±374.7
Total Ash weight (g)	10.364±2.71	11.495±6.31	27.308±5.5
Total organic matter (g)	140.8±42.6	225.3±44.1	396.5±131.5
Total Protein%	39.45±0.33	39.75±0.77	41.4±0.50
Total Fat%	10.96±0.23	11.24±0.7	12.36±0.23

Table 2 gives the total average of the Head wet weight, head dry weight and the Chemical composition of the head of *A. pharaonic*, (163.067±24.26-676.567±79.9) gm to (34.271±6.42-142.967±14.3) gm and Total Head Moisture% between (78.9±15.8-80.4±91.13) % while the Head Ash weight 3.66±1.97-10.744±1.07 gm and the Total Head Protein% and Total Head fat% were (17.5±0.16-18.34±0.12) %, (8.48±0.14-9.3±0.15) %.

Table 2. The mean weight and Parts Measurements of the head of *A. pharaonic*

Parameters \ weight classes	650-750(gm)	1000-1900(gm)	2000-3000(gm)
Total average Head wet weight (gm)	163.067±24.26	409.305±122.3	676.567±79.9
Total average (gm)	34.271±6.42	80.269±31.5	142.967±14.3
Total head Moisture %	78.9±15.8	78.9±65.9	80.4±91.13
Total head Ash weight (gm)	3.66±1.97	5.108±3.67	10.744±1.07
Total Protein %	17.5±0.16	17.66±0.5	18.34±0.12
Total Fat %	8.48±0.14	8.7±0.46	9.3±0.15

The Total mean weight and Parts Measurements of the Chemical composition (Table 3) in the mantle of *A. pharaonic* mantle wet weight and mantle dry weight were (415.821±92.83-1239.5±398.3) gm by (80.529±35.3-267.482±72.8) gm. Total Mantle Moisture% was (80.6±57.7-83.5±157.4) % then average of mantle Ash weight between (6.704±0.77-16.564±4.51) gm. The Mantle Total Protein% was (21.95±0.21 to 23.057±0.45) % whilst Mantle Total fat% (2.46± 0.14-3.1 ± 0.12) %.

Table 3. The mean weight and Parts Measurements of the mantle of *A. pharaonic*

Parameters \ weight classes	650-750(gm)	1000-1900(gm)	2000-3000(gm)
Total Average Mantle wet weight (gm)	415.821±92.83	780.98±152.9	1239.5±398.3
Total Average Mantle dry weight(gm)	80.529±35.3	128.572±24.6	267.482±72.8
Total Mantle Moisture%	80.6±57.7	78.4±399	83.5±157.4
Total Mantle Ash weight (gm)	6.704±0.77	6.39±2.18	16.564±4.51
Total Protein%	21.95±0.21	22.09±0.45	23.057±0.45
Total Fat%	2.46± 0.14	2.6 ± 0.39	3.1 ± 0.12

(Figuer 1) illustrates the percentage of some mineral levels in *A. pharaonic* for the category (650- 750) gm. The figure shows that the total mercury content in this category is high, reaching (44.5± 0.06) % compared to other elements. The percentage of mercury was very high in the head, higher than in the mantle, reaching (38.9±0.046%), while it was low in the mantle (5.63± 0.011) %. The percentage of potassium was lower than that of the other minerals, reaching (0.8± 0.003) %, while the remaining minerals Fe, Zn, Pb, Mg, Na, Mn, Ca and Cu also had low percentages, reaching, (1.7±0.004) %, (1.43± 0.004) %, (2.7± 0.016) %, (1.6±0.005) %, (1.4± 0.003) %, (1.37± 0.004) %, (2.4± 0.002) % and (1.33± 0.005) % respectively.

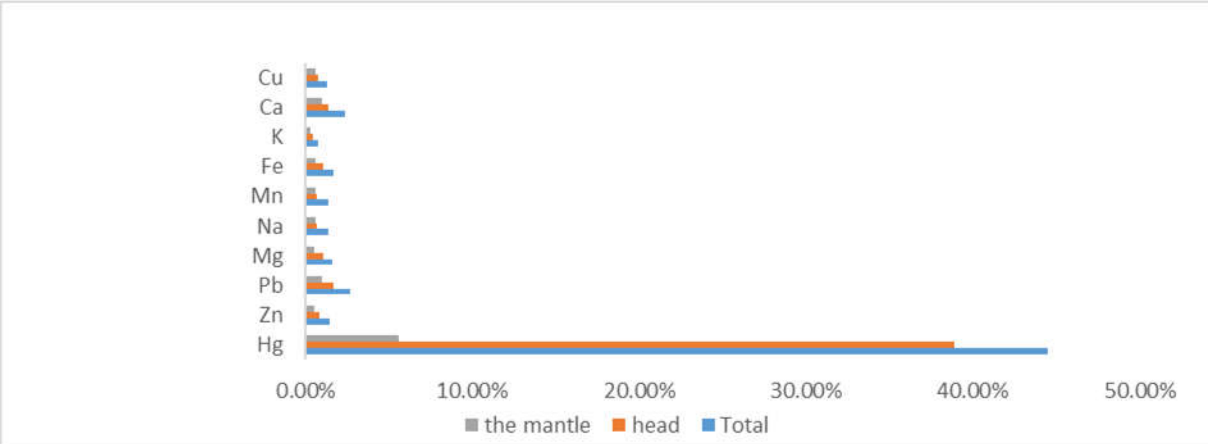

Figure 1. The average of the Mineral Compositions (Total, head and mantle) for dry weight (700±50) g of the *A. pharaonic*

In Figure (2), an increase in the percentages of the elements in category (1000-1900) gm is observed, and it is also noted that in the overall percentage of the mercury element in the category which reached (13.37±0.11) %. The percentage in the head also rose compared to the mantle, where it reached (9.9±0.09) % and (3.4±0.016) % respectively. As for the other elements (Fe, Zn, Pb, Mg, Na, Mn, Ca and Cu), their percentages were low, reaching (1.4±0.001%, 1.3±0.004%, 3.034±0.004%, 1.3±0.004%,1.4±0.007%, 1.4±0.005%, 1.8±0.004% and 1.97±0.004%) respectively. Regarding the lowest percentage, it belonged to the potassium element (0.9±0.003%).

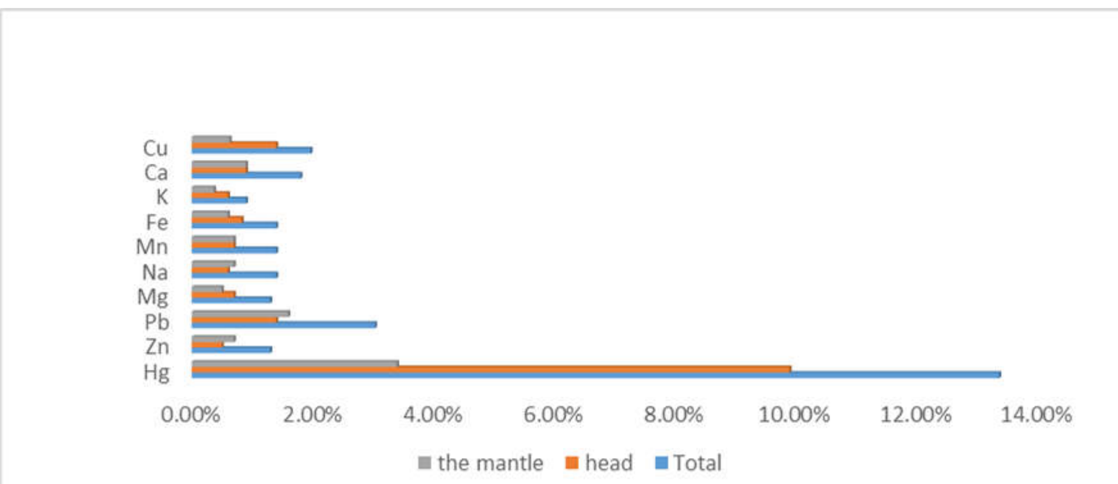

Figure 2. The average of the Mineral Compositions (Total, head and mantle) for weight (1000-1900) g of the *A. pharaonic*

Realized increase is observed in the overall percentages of elements, especially Mercury, Lead, and Copper, with the values reaching (6.63±0.03%, 6.03±0.033% and 2.03±0.009%) respectively. It is also noted that the percentages are lower in the head compared to the mantle, which recorded the highest values for all elements, while the lowest values were for Potassium (0.9±0.004%). Fig. (3).

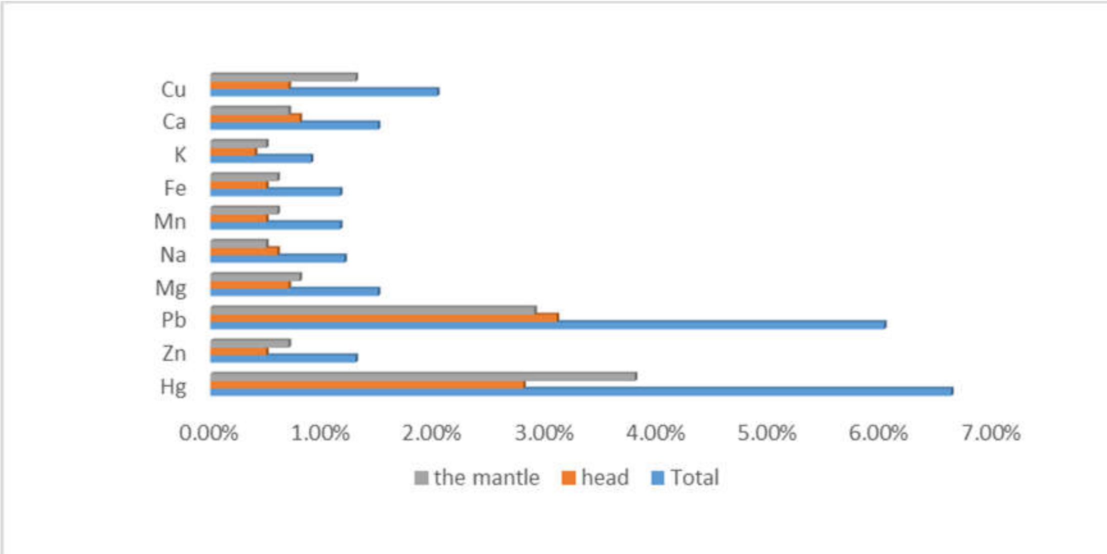

Figure 3. The average of the Mineral Compositions (Total, head and mantle) for weight (2000-3000) g of the *A. pharaonic*

Figure 4 illustrates the relationship between the dry weights of the head, the mantle and the total weight for the three categories, along with the percentages of protein and fat. From the figure, it can be observed that the protein percentages recorded the lowest values in the head for the three categories, with the highest values reaching (18.34±0.12) %, while the lowest percentages were (17.5±0.16) %. As for the mantle, the highest protein value reached (23.057±0.45) % and the lowest was (21.95±0.21) % for the three categories. In general, it can be noted that the percentage of protein increased in both the head and the mantle for the three categories, with the highest values recorded (41.4±0.50) % in the large weights, while the lowest percentages were recorded in the small weights, reaching (39.45±0.33) %. As for the fat percentages, the lowest values were recorded in the dry weights of the three categories in the mantle region, reaching (2.46 ± 0.14%). The highest values were recorded in the head compared to the mantle reaching (3.1 ± 0.12%). As for the total weight in the three categories, fat percentages were higher in the larger weights, with the highest percentage recorded at (12.36 ± 0.23%), while the lowest reached (10.96 ± 0.23%) compared to the smaller weights. A strong positive correlation was recorded between the percentages of protein in the head, mantle and the whole body and the different sizes of squid for the three categories with a value of r = 0.91. While a very weak positive correlation was recorded between the percentages of fat, with a value of r = 0.31 in the head, mantle, and body for the three categories.

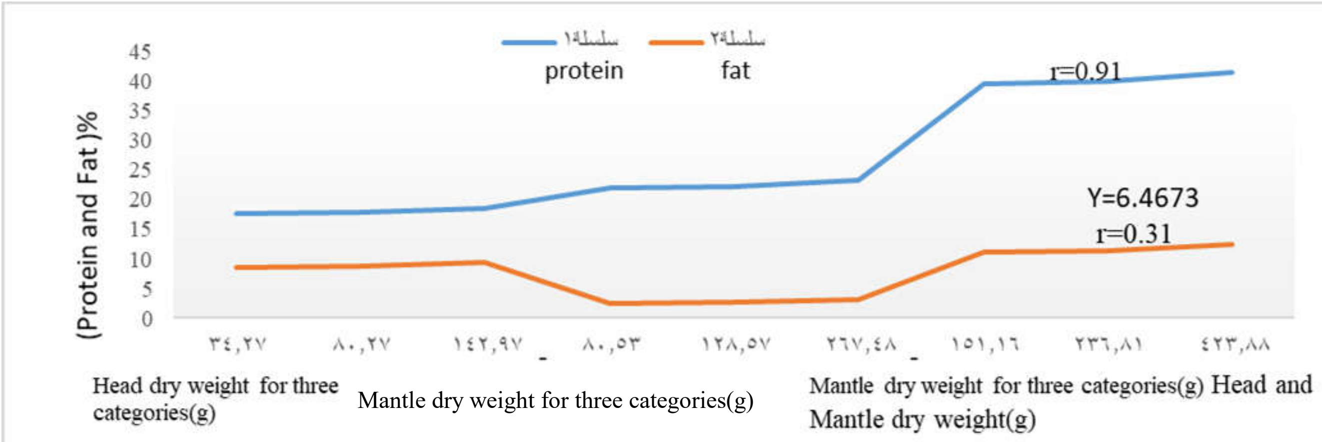

Figure 4. The relationship between dry weight for three categories and the percentage of protein and fat

Discussion:

The relationship between wet weight and dry weight is very important, as the difference between them is crucial in calculating the net organic matter, including protein, fat, and others. In relation to the texts, the chemical composition of discarded shells from various crustaceans could be used in diets for human consumption and fish as an alternative animal protein or in other feeds (Gyeong Bong, 2007; Mach *et al*., 2010).

From the results, it is observed that the percentage of moisture in small sizes is higher compared to large sizes. This is because as the mass increases, the amount of water also increases (Talal *et al*., 2024), in addition to the fact that the environment itself is aquatic and the looseness of its tissues is rich in water. Moreover, it is not exposed to evaporation compared to terrestrial organisms, and it possesses a high physiological regulation that maintains water and salt balance. All of this is consistent with (Zambrano *et al*., 2019).

Ash is defined as the inorganic mineral content remaining after burning a biological sample at high temperatures, and it includes elements such as calcium, phosphorus, magnesium, potassium, iron, and so on. In many aquatic organisms, especially mollusks, the ash content increases as the organism grows. This is due to the skeletal tissues, which become more calcified with age and increased size. From a physiological perspective, small individuals grow rapidly, so their mineral content is low compared to larger individuals, whose growth is slower, leading to the accumulation of minerals and ash (Nigam and Kumari, 2018).

Organic matter refers to the portion of organic carbon (proteins, fats, carbohydrates, and living tissues) in the dry mass, while ash represents inorganic materials (minerals). In invertebrates, there are mineral structures and shells that increase ash content and reduce organic matter. In the present study, it is noted that the organic matter content in squid is high, which is due to the low ash content, and this is consistent with the study of (Spitz *et al*., 2010; Santi *et al*., 2019). Ahmed *et al*., (2023) found in a study on three types of Cephalopods that the ash content was very low, which is consistent with the current study, as the muscle tissues of squid contain a low percentage of minerals. It is also noted that the muscle tissue that is being pickled is completely free of shell.

Many recent studies focus not only on toxic metals such as Pb, Cd, Hg, and As, but also on the levels of essential mineral nutrients like Fe, Zn, Cu, Mn, Ca, and P, as they affect nutritional and biological value. (Younis *et al*., 2024). The current study observes a higher concentration of mercury in the head of the *A. pharaonic* compared to its mantle. It also notes that the mercury level is higher compared to other elements, while the lowest concentration was found for potassium in category (700±50) g. A significant increase in the levels of elements in the third category was observed particularly in the mantle area particularly the elements mercury, lead, and copper. (Mok, *et al*,2014). The increase of certain minerals is essential for their functions, such as copper, lead and zinc, while some elements like cadmium and lead may accumulate due to environmental pollution or from food, which agrees with (Briffa *et al*., 2020; Li *et al*., 2024; Yang *et al*.,2024).

The current results are compatible with Ahmed, *et al*. (2022) who evaluate the level of some heavy metals in two cephalopods, as recorded through their study of the metal pollution index in Sepia, the lowest values were found in the head and mantle. The rise in lead levels is a warning of pollution. This agrees with (Asvad, *et al*.,2024) study which found that the elements zinc and copper recorded the highest values in the muscles of two types of squids in the Arabian Gulf. The increase in mercury and lead levels was consistent with (El Gammal *et al.*,2016) which reported in their study of three types of invertebrates. Proteins represent the main organic component in invertebrates, as they constitute a large proportion of the dry weight.

Many minerals are associated with proteins either as trace minerals or as part of enzymes containing metals, such as hemocyanin, which contains copper as a key element for oxygen transport (Pajarillo *et al*., 2021). An increase in protein content reflects high metabolic activity and consequently an increase in some essential mineral elements (Rosa and Brad, 2010). It is noted in the current study that the protein percentage ranges between (39.45±0.33-41.4±0.50), which corresponds with the study of (Al-Subiai *et al*., 2012), as it indicated that squid has a high protein content (13-22) % of wet weight, reflecting high nutritional efficiency.

The results show an inverse relationship between ash content and protein, as when the ash percentage is high, protein decreases. This was found in the studies by Nigam and Kamari (2018). Fats play an important role as they are one of the main sources of energy and in regulating metabolic processes and the absorption of minerals. In many studies, it has been found that an increase in fat content is accompanied by a decrease in the concentration of certain elements such as Cu, Zn, Fe, and Cd. The reason is that minerals bind more to proteins than to fats, so they are concentrated more in muscle and liver tissues than in fatty tissues Bustamante *et al*., (2008).

These results are consistent with what the current study recorded regarding the low-fat percentages in the head and mantle, which reached (10.96±0.23-12.36±0.23) %. It is also noticeable that fat percentages are higher in larger weights than in smaller ones, which may be due to physiological changes, sexual maturation, and changes in metabolic activities, in addition to the effects of the environment and diet. This was confirmed by Aubourg *et al.*, (2021), in their study on the Chemical Constituents Obtained from Patagonian Squid. The relationship between head and mantle weights and the total weight of the three categories, along with the percentages of protein and fat can be observed that the first category with small sizes has low percentages of protein and fat. This may be that smaller individuals are in a growth and tissue-building phase, which requires a large amount of energy. Many studies have documented this situation, including Seasonal and dietary changes, as well as body composition, which also play a role (Angilletta *et al*., 2004; Clarke and Fraser, 2004; Yan *et al*., 2009).

Conclusions:

The results of the study show that *Acanthosepion pharaonic* possesses a high nutritional chemical composition, with protein levels increasing as body size increases, reaching about 41.4% in the largest size category, while fat content remains low especially in the mantle compared to the head. The findings also indicate high moisture and organic matter percentages, whereas ash content remains low reflecting muscle tissues that are rich in organic components and relatively poor in minerals.

The study further reveals those mineral concentrations—particularly mercury, lead, and copper—rise with increasing body size, with the highest levels found in the head compared to the mantle, highlighting physiological differences in bioaccumulation between body parts. Smaller individuals showed lower mineral concentrations, indicating faster tissue growth and higher metabolic activity that prioritize muscle development over mineral deposition. Overall, the results support the classification of this cuttlefish species as a nutrient-dense food source, rich in protein and low in fat, while emphasizing the need to monitor heavy metal concentrations, particularly in larger specimens. The study provides valuable insights for nutritional and health related applications and contributes to assessing marine environmental quality in southern Iraq.

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