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Proximate Composition and Heavy Metals Bioaccumulative Capacity of Edible Muscles of Cuttlefish (*Sepia* spp) from Red Sea

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Abstract

The present study quantified the concentration of protein, protein electrophoretic bands, carbohydrates, lipids, amino acids (essential and non-essential amino acids), vitamins (B_1 and B_2,) and minerals (Na, K, Zn, Ca, P, S and Se) to evaluate the nutritional value of adult commercial cuttlefish *(Sepia)* to elucidate its real benefits as human diet. Additionally, the concentration of non-essential heavy metals Pb, Hg and Cd were determined to identify any potential public health risks that could be associated with dietary intakes of such seafood from the Red Sea. The present study revealed that edible muscles of *Sepia* contain nutrients for human good health. Furthermore, based on the bioaccumulative results, metal concentrations in the edible parts of the examined cuttlefish were in the safety permissible levels for human consumption. So, therefore, *Sepia* is considered to be very good cephalopod species for human consumption and could be employed as an alternative dietary supplement of protein, carbohydrate ,lipids amino acids ,vitamin and mineral matters in the body (food supplement).

Keywords: Edible muscles, *Sepia*, Proximate composition, Bioaccumulative, Non- essential heavy metals

1. Introduction

Seafood products have attracted considerable attention as important sources of nutrients in the human diet. Among seafood products cephalopod mollusk organisms that considered as important components of the aquatic fauna. Furthermore, cephalopod landings and consumption have been increasing worldwide during the past decades [1]. The main reason for this increasing demand is that cephalopods are a good protein and lipid source [2], thus a highly nutritious food that represents an alternative to over exploited fish resource[1]. Biochemical assays and nutrients play a vital role in physical growth, development, maintenance of normal body function of physical activity and health. The knowledge of the biochemical composition of any organisms is extremely important since the nutritive value is reflected in its biochemical contents [3] such as protein, amino acids, lipid, fatty acids carbohydrate, vitamins and minerals. Protein is essential for the sustenance of life and accordingly exists in the largest quantity of all nutrients as a component of the human body [4]. Moreover, the protein content is considered to be an important tool for the evaluation of physiological standards [5]. Additionally, protein is essential for normal function, growth and maintenance of body tissues [6]. Furthermore, any sort of cellular metabolism occurring in body involves one or many different proteins at cellular level. Proteins do play both structural and functional role. Being an integral part of the cell membrane, intracellular and extracellular passages are linked through it [7]. In general the occurrence of high protein and lipid contents in the tissues of mollusks reflects that the tissue is highly rich in energy containing substances [8]. According to [8], lipids are highly efficient as sources of energy and they contain twice the energy of carbohydrates and proteins. As a general rule, they act as major food reserve along with protein and are subject to periodic fluctuations influenced by environmental variables like temperature [9]. Regarding amino acids, amino acids are the building blocks of proteins and serve as body builders. The role played by amino acids in isosmotic intracellular regulation has been illustrated in several investigators [10, 11], and it can be an important source of energy producing compounds [12, 13]. In addition to, amino acids play important roles in physiological functions such as osmoregulation and buffer capacity in the tissues of aquatic animals [14] and some amino acids are involved in neurotransmission [15].

Minerals are particularly significant in the different biological functions. Such as Cu and Fe are oligoelements which play vital roles in the enzymatic and respiratory processes of marine organisms [16, 17]. However, Cu is the most toxic metal after mercury and silver [18] and considered as public health hazard if abnormal high level of Cu ingested. K and Na regulate the electrolyte and acid-alkali balances, the conductive capacity of the nerves, muscle contractions and the production of adrenaline and amino acids. Ca is considered that most important of the principal mineral element (macronutrients) which constitutes 60-80% of all the inorganic material in the human body. Furthermore, P is an essential mineral for cell function and it occupies key role in all reactions with Mg [19]. Furthermore, Co is readily absorbed from the gastro-intestinal tract and the surrounding water by aquatic organisms. It is an essential trace mineral that is a constituent of vitamin B_{12}. The main function is to prevent anemia. It works with vitamin B_{12} in the production of red blood cells and to ensure the health of the nervous system [20]. On the other hand, the heavy metals Hg, Pb and Cd which are non-essential trace metals and already toxic in very low concentrations. They cause metabolic anomalies due to their competition with the essential metals for binding sites and also their interference with sulfhydryl groups and structural protein [21-23].

Over the last few decades the marine environment has been contaminated by persistent pollutants of agriculture and industrial origin. Heavy metal contamination has been identified as a concern in coastal environment, due to discharges from industrial wastes, agricultural and urban sewage. Despite the low concentrations of heavy metals in the surrounding medium, marine organisms take them up and accumulate them in their tissues through a variety of pathways, including food or non-food particles, respiration and the skin to concentrations several folds higher than those of ambient levels [24] i.e., heavy metals in dissolved form are easily taken up by aquatic organisms, where they are strongly bound with sulfhydril groups of proteins and accumulate in their tissues [25]. Bioaccumulation means an increase in metals concentration in biological organisms compared to the concentration in the environment [26]. Metals accumulation in living things anytime they are taken up and stored faster than they are broken down So, marine organisms are considered as good indicators for the long term monitoring of metal accumulation in the marine environment [27]. A major hazard which may be associated with the use of dietary feed ingredients is the presence of potentially toxic mineral elements such as the accumulative elements copper, lead, cadmium and mercury [25]. Therefore, to ensure the safety of the selected marine organisms in this study for animal consumption, the accumulative capacity of this marine cephalopod organism with the heavy metal concentrations should be estimated in its edible muscles.

The objective of this study was to assess the nutritive value of edible muscles of *Sepia* which commonly consumed by the local communities in Saudi arabia to elucidate its real benefits as human diet through evaluation of the proximate composition of basic biochemical constituents, such as total protein ; its electrophoretic bands, lipids, carbohydrates, vitamins B_1, B_2, amino acid composition (essential and non-essential amino acids), minerals (Na, K, Zn, Ca, P, S and Se). Also, this study is important from the safety point of view of human health, through monitoring the percentages of non-essential heavy metals accumulation (Pb, Hg and Cd) in the edible muscles of this cephalopod mollusk.

2. Material and Methods

2.1. Collection of Samples

The study was carried out on the marine mollusks (*Sepia* spp) that were purchased from local fishermen at Jeddah, Saudi Arabia. Samples were put in crushed ice in insulated containers and brought to the laboratory for preservation prior to analysis. The internal shell of *Sepia* were removed from edible muscles (mantles). The mantle separated from the arms, skinned and rinsed in fresh water. Samples kept in ice until biochemical analysis

2.2. Biochemical Analysis

1- Homogenate mantle samples were analyzed for total proteins by [28], Carbohydrates [29] and total lipids [30]. All values were expressed on percentage dry weight basis.

2- Vitamin B contents: vitamin B_1(Thiamin), B_2 (Rioflavin) contents were analyzed using HPLC, the Varian 940-LC [31].

3- Amino acids measured by high performance liquid chromatography (HPLC); Beckman 6300 amino acid analyzer [32].

4- Polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis was carried out using silver stain protocol [33].

5- Mineral analysis: Analysis of 7 elements (Na, K, Zn, Ca, P ,S and Se) and 3 non-essential trace metals (Hg, Pb and Cd) were measured using Perkin Elmer Atomic (800) with flow injection analysis system(FIAS). Each mantle sample (2 g wet weight) was weighed. Twenty milliliters of concentrated nitric acid was added to each sample and the flask was left to stand overnight. Five milliliters of concentrated perchloric acid and 0.5 mL of concentrated sulfuric acid were added, and the flask was then heated until no white smoke was emitted. The samples were dissolved in 2% of hydrochloric acid and transferred into a volumetric flask, then analyzed using an atomic absorption spectrophotometer.

3. Results

3.1. Total Protein , Carbohydrates, Lipid Percentages and Vitamin B Analysis

The biochemical compositions ; total protein , carbohydrates , lipid and vitamins B of mantle samples (edible muscles of the studied *Sepia*) are illustrated in Figure 1& 2. The recorded data in Fig 1 showed that protein was the major constituent (46.77%±1.51) in edible muscles of *Sepia*, followed by lipids (8.53$^{\%}$±0.04**)**. While carbohydrates had the lowest percentage (1.95$^{\%}$±0.02). As shown in Fig. 2, the edible portion of *Sepia* had higher levels of vitamin B_2 (0.399 ±0.00 mg/ 100g) than vitamin B_1(0.313 ±0.00 mg/ 100g).

3.2. Amino Acids Analysis

The results obtained from this study indicated the presence of 9 essential amino acids (EAAS) that are represented by arginine, histidine, lysine, threonine, methionine, leucine, isoleucine, valine and phenylalanine (Fig. 3). The highest average concentration of arginine(4.08 ±0.02mg/ 100g) was recorded in edible muscles compared to other essential amino acids. On the other hand, histidine (0.953±0.012 mg/ 100g) had the lowest level. The arrangement of EAAS was recorded according to the following order: arginine > leucine > methionine > isoleucine > lysine > histidine. Furthermore, the edible portion of cuttlefish accumulated 7 non-essential amino acids (NEAAS) (proline, tyrosine, glycine, alanine, serine, glutamic acid and aspartic acid). Aspartic acid (4.77±0.01 mg/ 100g) and glutamic acid (3.58 ±0.01mg/ 100g) had the highest concentrations compared to other non-essential amino acids, while the lowest level of NEAAS was tyrosine (1.45±0.03 mg/ 100g). The arrangement of NEAAS was aspartic acid > glutamic acid > proline > serine > glycine > alanine > tyrosine.

3.3. SDS-gel Electrophoresis

The electrophoretic analysis of edible muscle proteins of *Sepia* was illustrated in Photo 1 and Table 1. The total number of protein electrophoretic bands of mantle muscles of *Sepia* from Red Sea was 13 bands around molecular weight 13.67 to 133.01 KD.

3.4. Mineral Analysis

The results of the mineral analysis are recorded in Figs 4, 5 and 6. The data showed that great variation in mineral levels was observed in edible muscles of *Sepia.* The muscles accumulated higher concentrations of Zn (352.6±1.45 mg/ 100g), S (88.34±0.17 mg/ 100g), P (67.28± 1.64 mg/ 100g), Fe(46.39±0.87 mg/ 100g) and Ca (50.49±1.17 mg/ 100g). On the other hand, lower levels of Na, K, Cu, Co and Se (17.16± 0.45, 12.92±0.42, 1.9±0.01, 0.092±0.01 and 0.173±0.01 mg/ 100g, respectively) were recorded in edible portion of *Sepia.* Regarding with the contents of non-essential heavy metals, the present data in Fig . 7 recorded this descending order Pb > Hg > Cd (4.07±0.01, 2.52±0.01 and 0.144±0.00mg / 100g, respectively)

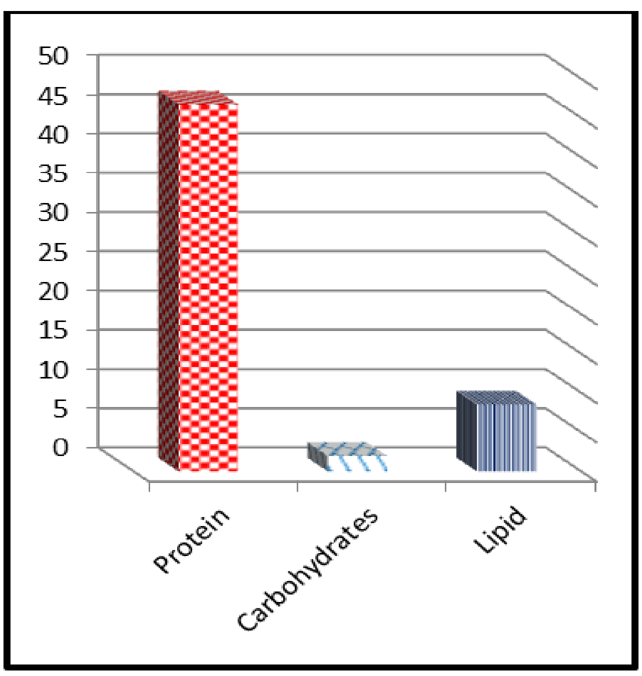

Fig-1. Protein**,** carbohydrates, and lipid percentages in mantles of *Sepia*

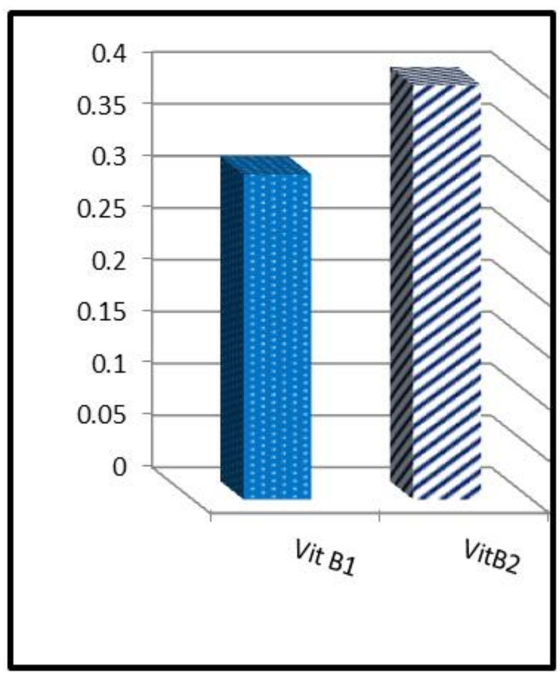

Fig-2. Vitamin B1 and B2 contents(mg/100g) in mantles of *Sepia*

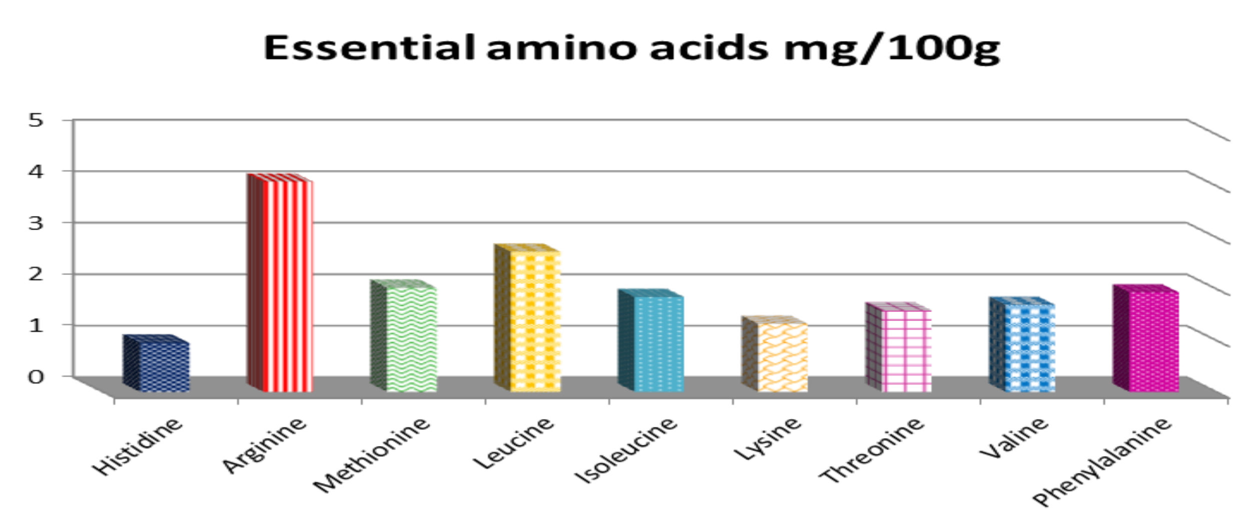

Fig-3. Essential amino acids(EAAS) concentration in edible muscles tissue of *Sepia*

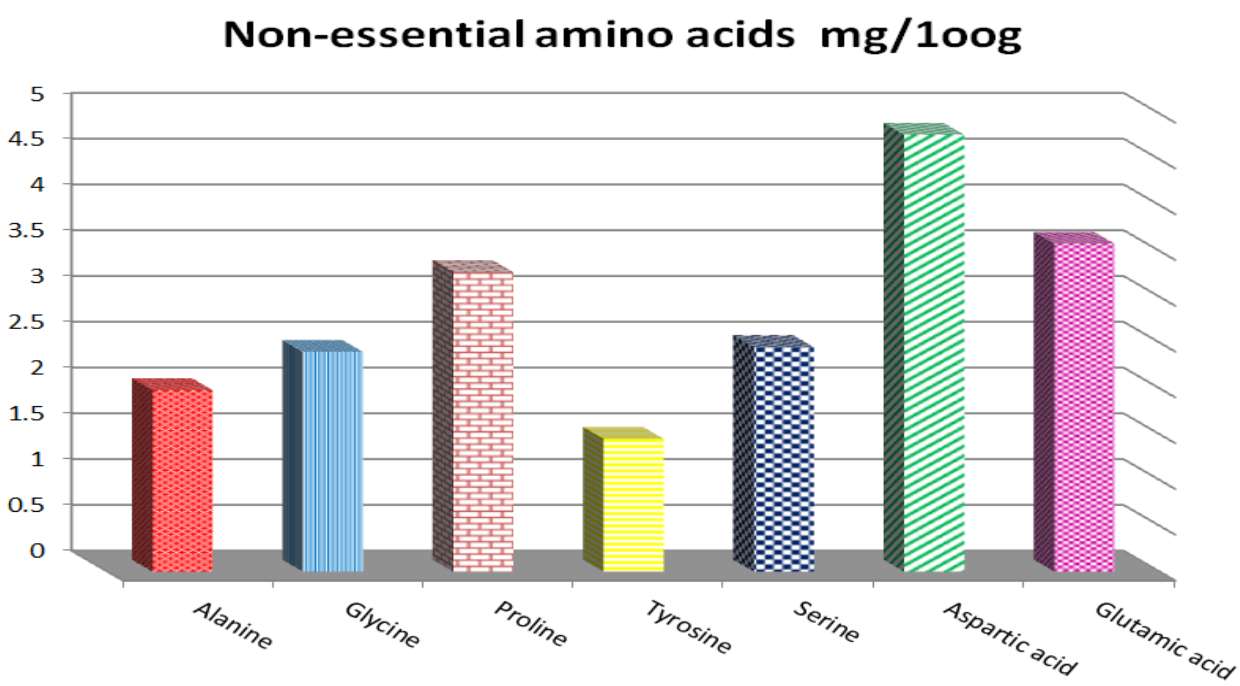

Fig-4. Non- essential amino acids(NEAAS) concentration in edible muscles tissue of *Sepia*

Table-1. SDS-PAGE gel electrophoresis of muscles of Sepia

M		*Sepia* Red Sea	
KD	%	KD	%
260	2.98	133.01	10.20
135	2.42	114.66	5.36
95	3.17	70.31	1.23
72	13.20	59.54	11.10
52	12.50	52.75	3.06
42	5.70	43.60	11.10
34	4.99	41.09	5.34
26	22.70	38.40	3.02
17	12.30	32.16	13.50
10	19.90	29.77	6.97
11		27.29	10.10
12		15.88	8.60
13		13.67	10.10
Sum	100		
In lane	100		

M: Protein marker; Mol. Wt.: Molecular weight in KD

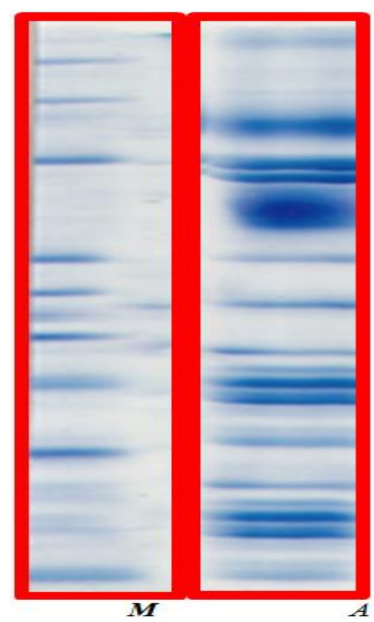

Photo-1. electrophoretic pattern of muscle proteins of Sepia. M: marker; A: Sepia

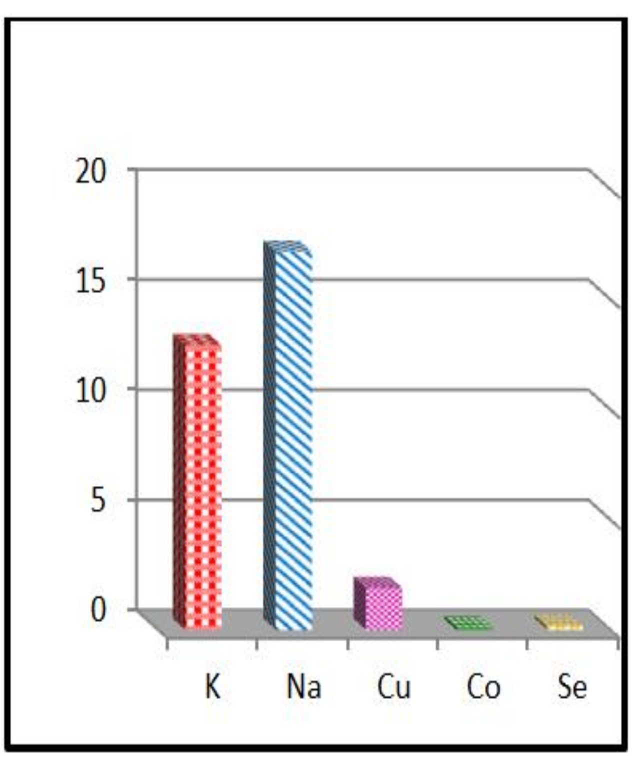

Fig-5. K, Na, Cu, Co and Se(mg/ 100g) levels in mantle of *Sepia*

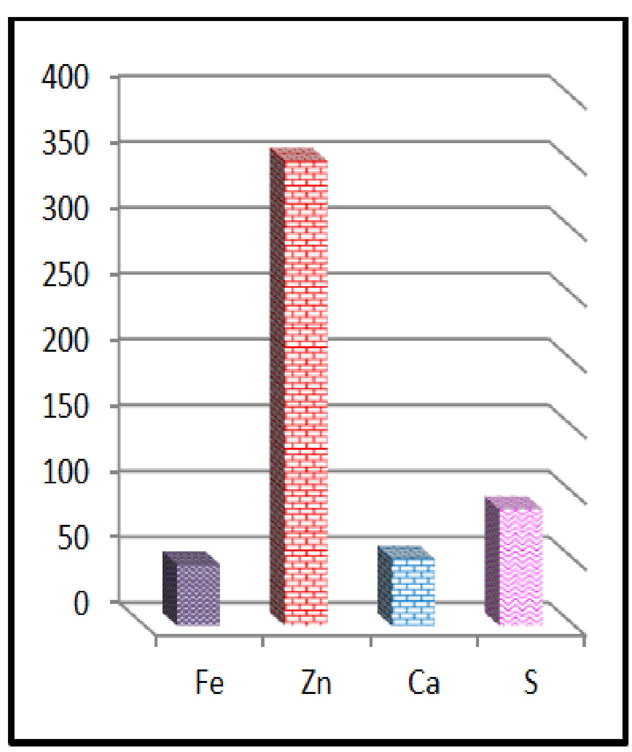

Fig-6. Fe, Zn, Ca and S(mg/100g) in mantle of *Sepia*

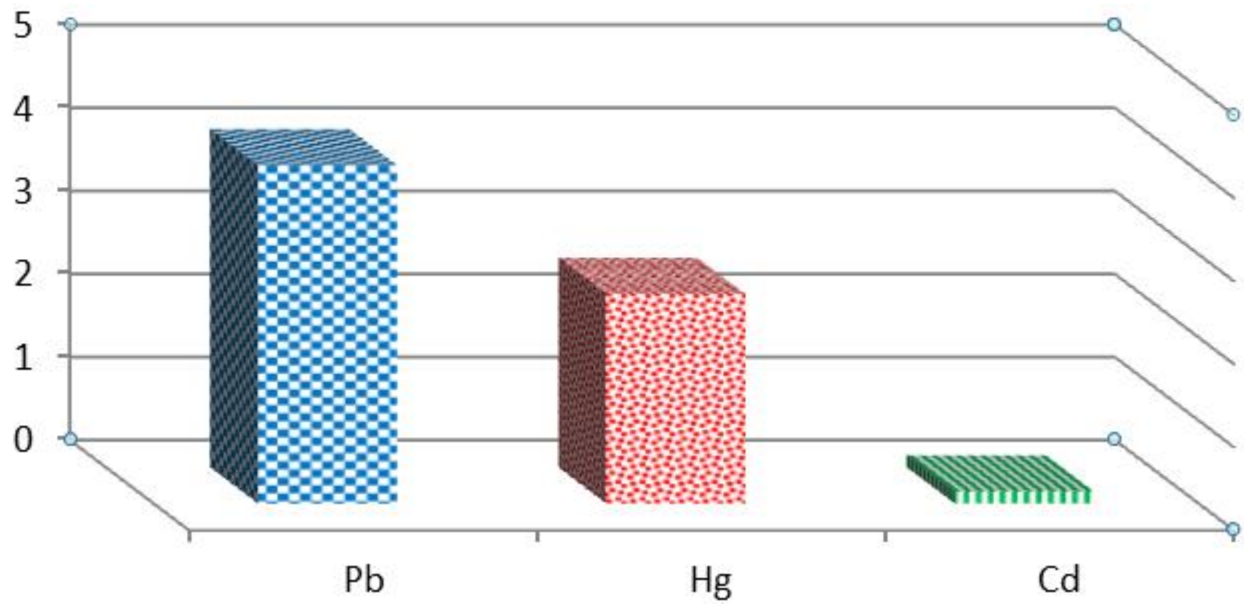

Fig-7. Non-essential heavy metal levels (mg/ 100g) in mantle of *Sepia*.

4. Discussion

Over the last decades, there is a growing concern of the value of biochemical estimations of marine organisms for two reasons , the first is for understanding the process of their process of their metabolism and physiological adaptations , while the second for determining the possibility of using them as an alternative source of food. Furthermore, studies on the biochemical components of economic cephalopod mollusks species (*Sepia*) as well as, its metals uptake are carried to assess the nutritive quality and safety of this species for human consumption [34- 36].

In the present study, it was observed that the major constituent in the muscle of the mantle; the main edible portion of *Sepia* was protein (46.77%%) that followed by lipid (8.53%) then carbohydrates (1.95%). This result agreed with other studies which stated that protein is the most prominent biochemical component of *Sepia* [35-38]. However protein percentage in the present study is still higher compared to the protein level which was recorded in their studies. These higher amounts of protein content in edible portion of *Sepia* indicate that this cephalopod species is one of the good nutritive food items for human. Lipids are considered very important during gametogenesis for gonad maturation, especially in females to provide energy for subsequent embryo development [39]. [40] reported the total lipid content as ranging from 6.88% (head) to 11.50% (mantle) of *Speia inermis*. While , [36] recorded that the lipid content of head, arm, mantle and tentacles of *Speia. kobiensis* are found to be 2.11, 2.46, 1.44 and 1.19 %, respectively. This difference in the lipid content may be due to species, gender, geographical origin and season [41] apart from the abundance of food [42]. Regarding carbohydrates concentrations, the results of [40] recorded that the carbohydrate content of *S. inermis* was found to be varying between 3.31 and 5.63% in the tentacle and mantle, respectively. In the another study[36], the carbohydrate content in head, arm, tentacle and mantle was found to be 11.70, 11.85, 9.18 and 9.33 respectively. The carbohydrate content of mollusks is mainly com-posed of glycogen, and the change in the carbohydrate level may be due to the accumulation of glycogen at different stages of gametogenesis and spawning [43].

Vitamins have important roles in human metabolism, immune system and digestive system. Regarding vitamins evaluation in aquatic organisms, no extensive studies have been published on the vitamin contents in the muscle of various species of marine invertebrates. Vitamins have a nutritional value and play important roles in cell metabolism. The B vitamins often work together to deliver a number of health benefits to the body[19]. In the present study , the mantle of *sepia* had higher vitamin B_2 (Riboflavin) (399mg/100g) than B_1 (Thamin) (0.313 mg/100g). These values are higher than the recorded values in edible muscles of shrimps *(Penaeus semisulcatus*) and crabs (*Portunus pelagicus*) from Red Sea in Saudi Arabia [19]. Generally, the high level of vitamin B_1 and B_2 in edible muscles of *Sepia* indicates its high nutritive value.

Amino acids are the building blocks of proteins and serve as body builders. They are utilized to form various cell structures, of which they are key components and they serve as source of energy [12,13]. The recorded data in the present study indicate the presence of 9 essential amino acids (arginine, histidine, lysine, threonine, methionine, leucine, isoleucine, valine and phenylalanine) and 7 non-essential amino acids (proline, tyrosine, glycine, alanine, serine, glutamic acid and aspartic acid). The arrangement of EAAS was recorded according to the following order: arginine > leucine > methionine > isoleucine > lysine > histidine, while, the arrangement of non-essential amino acids (NEAAS) is proline, tyrosine, glycine, alanine, serine, glutamic acid and aspartic acid. Arginine and proline are served as the main substrates amino acid catabolism for energy in the cephalopods [44]. The availability of arginine tend to increase during anaerobic work. Arginine phosphate is hydrolyzed and the arginine available is condensed with glucose-derived pyruvate to form octopine, the main anaerobic end product that accumulates in adult cephalopods during periods of exercise and stress. In addition, arginine with glutamic acid, alanine and glycine are free amino acids responsible for the formation of flavor [35] In mantle of *S. recurvirostra*, arginine, leucine and lysine represented 49% of the essential amino acids, while glutamatic acid, glycine and alanine represented 56% of the non-essential Amino Acids (NEAA) [36]. Furthermore, arginine, leucine and lysine were among the highest component of EAAS in *S. officinalis* [45]. The amino acids content could vary among organisms due to geographical differences, species, age and physiological condition [35].Electrophoretic analysis of edible muscle proteins indicated the presence of 13 bands around molecular weight 13.67 to 133.01 KD. Also, 13 protein bands around molecular weight 10.10 to 154.20 KD in edible muscles of *Sepia* from Arabian Gulf in Saudi Arabia[50]. While in bivalve mollusks from Red Sea in Saudi Arabia, 14 protein bands were detected with molecular weight from 154.43 to 13.80 KD and from 160 to 14.80 KD from Arabian Gulf [46]. The variation in protein bands might be due to sex variation or to physiological factors such as size, molting cycle, season, nutritional state etc…Another suggestion was in accordance with that of [46] who reported the differences in electrophoretic protein bands might be to an increased synthesis of acute phase proteins (new polypeptide chain) which act as buffer or as protective protein against toxicity with heavy metals.

Cephalopods are known as carnivorous and active predators. As they have very high feeding rates, most part of the elements can be assumed to be incorporated by the diet. The absorption of minerals from seawater also could take place by osmotic uptake through the gills and the body surface as the cephalopods live in hypoosmotic environment. Minerals also absorbed by digestive gland as they swallow massive quantities of sea water during and after feeding [35]. Minerals are required for the maintenance of normal metabolic and physiological functions of living organisms. The main functions of essential elements in the body include the formation of skeletal structure, maintenance of colloidal systems, as well as regulation of acid base equilibrium. They are important components of hormones, enzymes They are important components of hormones, enzymes and structural proteins[47]. The present study revealed that mineral contents of edible muscles of *Sepia* indicate this decreasing arrangement Zn > S > P > Fe> Ca> Na> K> Cu> Se > Co. While, heavy metals levels had this decreasing order Pb > Hg > Cd. Furthermore, the present data declare that the bioaccumulative capacity of essential metals in edible muscles of *Sepia* was higher than that of non-essential trace metals Pb , Hg and Cd. That could be explained because of these essential metals play a role in the enzymatic and respiratory processes of in aquatic animals] and relatively high level of these metals is necessary to carry out these biological functions, while Cd, Pb and Hg were among elements that could be harmful for organism. Comparing the present data with guidelines and limits of non-essential heavy metals Pb, Hg and Cd as recommended by [48]. The accumulation of these metals in edible muscles of *Sepia* were below the maximum limit. So, their contribution to the body burden can be therefore considered negligible, and the edible muscles of *Sepia* seem to be safe when incorporated into human consumption. In edible muscles (mantle) of *Sepia* from Arabian Gulf [48], the levels of Pb, Hg and Cd (7.62, 6.03 and 0.183 mg/100g respectively) were higher than the recorded contents in the present study. The variation in metal bioaccumulation in marine organisms might be due to variation of species, sex, season and species location.

5. Conclusion

In the present work, nutritional analysis of muscle of *Sepia* indicate the presence of high amount of protein, , moderate content of lipids and low level of carbohydrates. In addition to, considerable amount of vitamins B_1 and B_2, nine essential amino acids and seven non-essential amino acids which have benefit for man health. Furthermore, from the present electrophoretic protein analysis of muscles of *Sepia* detected the presence of 13 protein bands. Additionally, the edible portion of *Sepia* form a good source of minerals where nine important minerals ; Zn, S, P, Fe, Ca, Na, K, Cu, Co, Se are detected with different concentrations that serve a variety of functions for man. Furthermore, *Sepia* (cephalopod mollusk) accumulated the lowest levels of essential and non-essential heavy metals which in turn indicate the safety of consumption of this marine organism as human food. In conclusion, this cephalopod mollusk used in this study could be a balanced human diet and could be employed as an alternative dietary supplement of protein, carbohydrate ,lipids ,vitamin and mineral matters in the body(food supplement). Moreover, further studies on the mode of action and characterization of the active components in this mollusks must be done to open the door in the future to use this marine cephalopod specific health foods (functional supplements as antioxidant agents) and to play an important role in some pharmaceutical industries.

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