Mediterranean Aquaculture Journal 2024 11 (1):21-33

Original Article

Evaluation of safety and quality indices of some shark species caught from Mediterranean Sea, Egypt

Aliaa Midhat A. El-Kasheif[1], Alaa Mahmoud El-Far[2], Medhat Helmy Hashem[3] and Sayed Mekawy Ibrahim[4]

[1]National Food Safety Authority (NFSA), Egypt.
[2] Fish Biology and Fisheries lab., National Institute of Oceanography and Fisheries (NIOF), Egypt .
[3] Animal Biotechnology Dept., Genetic Engineering and Biotechnology Research Institute, Sadat City Univ., Egypt.
[4] Fish Processing and Technology Lab., National Institute of Oceanography and Fisheries (NIOF), Egypt.

ABSTRACT: This study was performed to evaluate the safety and quality criteria of six raw sharks; *Centrophorus granulosus, Carcharhinus plumbeus, Heptanchias perio, Mustelus mosis, Mustelus mustelus*, and *Squalus blainvillei* samples caught from the Mediterranean Sea at Al-Anfoushi, Alexandria, Egypt during September 2021- Augustus, 2023. Some microbial and heavy metals pollutants as well as the nutritive value of sharks' samples were determined. The results obtained showed that the aerobic plate count (APC), coliform group and fungus count (FC) of raw samples recorded 7.90 - 9.12, 77.00 - 95.67, and 1.33 - 1.82×103 cfug-1, respectively. Other pollutants, levels of Pb, Cd, Hg and Me-Hg were within than the international maximum permissible limits (MPLs). The chemical composition of six shark species ranged of 74.62-75.52% moisture, 22.58-23.60 % crude protein, 0.50-0.64% fat, and 1.21-1.59% ash content. In addition, values of TVB and TMA content were increased in raw samples; due to shark's meat contains some non-nitrogenous compounds. The average of total essential amino acids (EAAs) was higher (57.41 - 58.61) than non-essential amino acids (NEAAs) content (39.70 - 42.19). in conclusion, this study based on results obtained recommends that raw sharks have high safety and quality as well as they one of solutions to overcome of fish gap in Egypt as a similar in other countries after their treatment to reduce the urea content that limit its use as food for human consumption.

Keywords: Cartilaginous fishes, sharks, pollutants, quality criteria, and nutritive value.

Received: April 25, 2024
Accepted: April 28, 2024

1. INTRODUCTION

The Mediterranean Sea is a semi-enclosed basin and it has a high heterogeneity in the physico-chemical and biological traits. Also, it is one of the most severe declines in elasmobranch, in particular large sharks (Ferretti *et al.*, 2008; Lotze *et al.*, 2011 and Barausse *et al*., 2014). Moreover, it has become threatened due to several pollutants such as heavy metals, pesticides, pharmaceuticals, microplastics and others.

Correspondence :
Aliaa Midhat A. El-Kasheif
National Food Safety Authority (NFSA), Egypt
Mail: aliaa.elkasheif@yahoo.com

The sharks are sensitive organisms to environmental changes and anthropogenic activities that affect their physiological states and fitness traits (Hazen *et al.,* 2019 and Wheeler *et al.,* 2020). Several works have been investigated some these pollutants; Kim *et al.,* (2019) concluded that shark meat can expose consumers to a high level of As and that copper sharks bigger than the predicted size should be avoided for excessive Hg. Souza-Araujo *et al.,* (2021) reported that the sale and consumption of shark meat will expose consumers to potentially harmful levels of As and Hg, as well as contributing to the population decline of species and also that are currently categorized as threatened. Shipley *et al.* (2021) found that the highest metal concentrations (i.e. pb, Cr, and Cu) exhibited of Caribbean reef shark (Carcharhinus perezi) and to a lesser extent of tiger shark (*Galeocerdo cuvier)* and when they reached the sexual maturity. Bregman *et al.* (2023) found that the most dominant microbial groups were Flavobacteria, Moraxellaceae, and Rhodobacteraceae for both sharks species;

Carcharhinus obscurus and Carcharhinus plumbeus. However, specific microbial biomarkers were identified for each shark too. So, the management of the Mediterranean Sea is considered a complex procedure, due to its high biodiversity and faces multiple threats; fisheries, eutrophication, pollution, transportation, habitat loss and degradation, climate changes and alien species too. These threats led to a decline in the elasmobranch as a result of anthropogenic activities (Bianchi and Morri, 2000; Fortibuoni *et al.,* 2010; Lotze *et al*., 2011; Coll *et al*., 2012; Sguotti *et al*., 2016; Dulvy *et al*., 2017 & 2021 and Pacoureau *et al.,* 2021).

With regard to quality of sharks, skates, rays, guitarfishes, sawfishes and chimaeras are rich in nutritional value. Also, consumption of shark fins is a source of luxury food in several countries (Fowler *et al.,* 2005). Although, sharks are caught for their fins, skins or oils, but their meats are often wasted.

On the other side, sharks meat is a rich source of protein however it contains some non-nitrogenous compounds that limit its use as food, but it being a safe for human consumption if treated. To confirm this true, shark meat has been used as a food source since the fourth century especially after the World War I (Vannuccini, 1999; Shipley *et al.,* 2021 and Hussey *et al.,* 2014).

Sharks are serving as an important protein source in developing countries and contributing also towards human food security. Shark fishing has intensified where sharks meat and their by-products are recorded high market values. Thus, both sharks protection action and human food security should be required. (Lack and Sant, 2009; Dulvy *et al*., 2014& 2017; Bornatowski *et al*., 2018). Nowadays, Bregman *et al*., (2023) showed that sharks are an essential ecological role in the food web and also maintaining balanced ecosystems although they are sensitive to challenges as pre-mentioned. Therefore, this study was carried to investigate the safety and quality criteria of six raw sharks; *Centrophorus granulosus, Carcharhinus plumbeus Heptanchias perio, Mustelus mosis, Mustelus mustelus, and Squalus blainvillei* samples caught from the Mediterranean Sea at Al-Anfoushi, Alexandria, Egypt during Septemper, 2021-August, 2022.

2. MATERIALS AND METHODS

2.1.Study site

Six raw shark species; *Centrophorus granulosus, Carcharhinus plumbeus Heptanchias perio, Mustelus mosis, Mustelus mustelus,* and *Squalus blainvillei* were purchased during September 2021 to August, 2022 from Al-Anfoushi fish market, Alexandria, Egypt (Fig. 1).

Fig. 1. Sampling site, Al-Anfoushi region.

All samples (Fig. 2) were packed in an insulated icebox and transported to the central Lab., Sadat Univ., and then properly labeled and kept frozen at -20˚C until analysis.

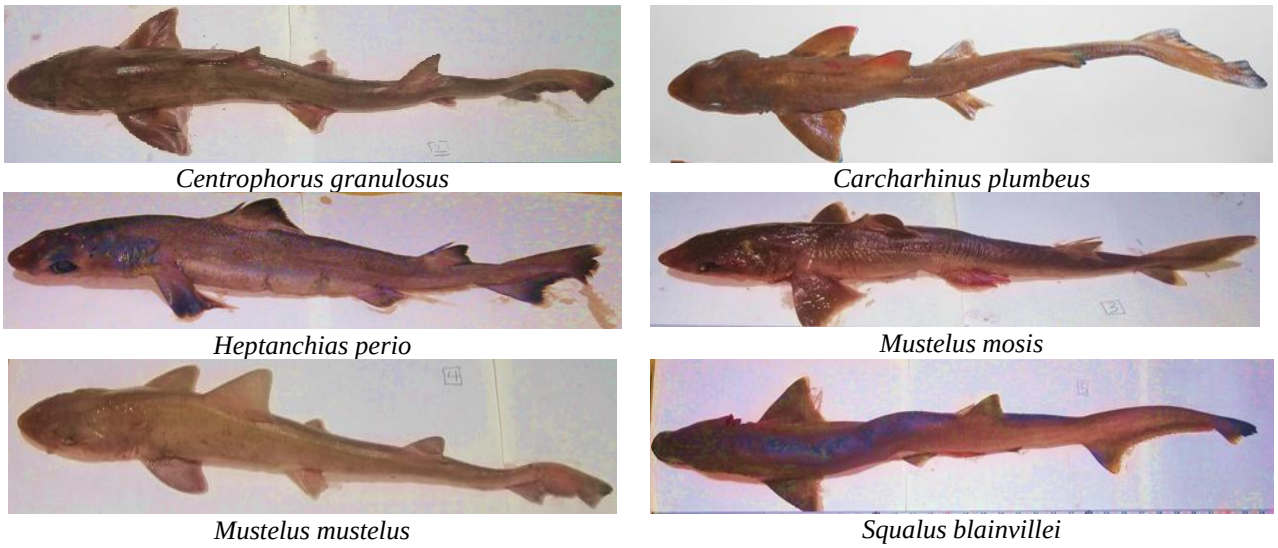

Fig. 2: Shark species samples.

2.2.Safety criteria

2.2.1. Heavy metals pollutants

The extracts of digested samples were filtered through disposable 0.2 µm PTFE syringe filters (DISMIC-25HP, Advantec, Tokyo, Japan). The metal concentrations (Pb, Cd, Hg and MeHg) were determined by inductively coupled plasma-mass spectroscopy (ICP-MS) (iCAP, Thermo, Germany) using certified reference chemicals (Merck, Germany). The recovery of metals was within the certified limits (APHA, 2005; Lambers *et al.*, 2008). The laboratory accredit from EGAC/ILAC under No. 217006. The results (ppm) were expressed average and SD.

2.2.2. Microbiological contaminants

10 g sample were aseptically removed from the shark's meats and homogenized with sterilized 90 ml 0.1% buffered peptone water (BPW) using a vortex for 30 se. Decimal serial dilutions using BPW were done. Aliquots of the dilutions were spread on the surface of specific media and incubated as follows: aerobic plate count (APC) on plate count agar (PCA) at 32ºC/48 h, coliform group bacteria on violet red bile agar (VRBA) (30±1°C/24 h), and fungus count (FC) were examined as described by (FDA, 1992).

2.3.*Quality criteria*

2.3.1. pH value

The pH values of raw shark's meats were measured using pH meter as the method described by Zaika *et al.*, (1976)

2.3.2. Total volatile basic nitrogen (TVB-N)

TVB-N content was determined according to the procedures of AMC (1979). The results were expressed as mg TVB-N/100g shark meat.

2.3.3. Trimethylamine nitrogen (TMA-N)

TMA-N was determined calorimetrically using the standard method as described in the (AOAC, 2005). The results were expressed as mg TMA-N/100g shark meat.

2.4.Nutritive value

2.4.1. Biochemical analysis

The chemical composition of sharks meat were determined (AOAC, 2005); moisture content using a dry oven at 105 ± 2ºC till a constant weight was obtained, crude protein using Micro-Kjeldahl method (TN×6.25); lipid by standard Soxhlet extraction method using petroleum ether (60-80ºC); and ash using Muffle Furnace at 550-600 ºC till a constant weight was obtained.

2.4.2. Amino acids composition

The amino acids (AAs) composition was determined by the method of Millipore Co-Operative (1987), tryptophan was not determined.

2.4.3. Major minerals

Some minerals content; K, Mg, Ca, and P were determined according to the methods described by APHA (2005) using an atomic absorption spectrophotometer.

2.5.Statistical analysis

The data (n=3) obtained were statistically analyzed using SPSS (Ver.16) and they expressed as mean and SD.

3.RESULTS AND DISCUSSION

The length and weight (Mean ± SD) of raw six shark species are presented in Table (1). The average of total length recorded 49.37 - 102.17 cm while total weight recorded 669.67 - 3907.33gm. *C. granulosus* recorded the lowest length and weight, *C. plumbeus* was more length while *M. mustelus* was more weight than others. This variation in sharks length and weight is due to species, sex, age, spawning period, season, fishing region, feeding behavior and others (Connell, 1976; Sadok *et al.*, 1996; Majumdar *et al.*, 2005; Reza *et al.*, 2009 and Chakma *et al.*, 2022).

Table 1. The length and weight (Mean ± SD) of raw six shark species.

Shark species	Length (cm)	Weight (g)
C. granulosus	49.37±15.19	669.67±557.90
C. plumbeus	77.40±15.40	2055.33±1139.99
H. perlo	70.30±17.53	1672.33±1107.58
M. mosis	68.70±7.96	1288.00±394.58
M. mustelus	102.17±25.85	3907.33±2243.37
S. blainvillei	55.33±6.51	821.33±365.16

3.1.Safety criteria

Some safety criteria; heavy metals (Pb, Cd, Hg and Me-Hg) and microbiological aspects (APC, coliform group bacteria and fungus count) of sharks investigated were determined.

3.1.1. Heavy metals pollutants

Table (2) shows the concentrations of Pb, Cd, Hg and Me-Hg accumulated in shark's meat. Concentrations of Pb ranged of 0.02 – 0.08, Cd 0.01-0.06, Hg 0.13 – 0.35 and Me-Hg 0.10 – 0.20 ppm in all sharks samples. The problems in hearing, vision, movement and coordination, drowsiness and headaches, organ dysfunction, cancers and impaired neurological development in fetuses are related to the harmful effects of

heavy metals concentration (Grandjean *et al.*, 2010; WHO, 2011; Guynup and Safina, 2012). The discharges of factories and farms can increase the concentration of heavy metals in coastal waters, low-trophic, and small-sized shark species, as found in smooth hound at South African (Bosch *et al*., 2016).

Table 2. Concentrations (mean ± SD) of heavy metals (ppm) of six shark species meat.

Species	Pb	Cd	Hg	Me-Hg
C. granulosus	0.05± 0.03	0.01±0.00	0.35±0.11	0.20±0.15
C. plumbeus	0.07±0.03	0.02±0.00	0.28±0.10	0.13±0.05
H. perio	0.03±0.01	0.02±0.03	0.16±0.09	0.17±0.07
M. mosis	0.08±0.04	0.06±0.04	0.26±0.03	0.16±0.02
M. mustelus	0.02±0.01	0.01±0.00	0.13±0.02	0.10±0.03
S. blainvillei	0.05±0.03	0.01±0.00	0.31±0.04	0.18±0.07
*MPLs				

*MPLs: Maximum Permissible limits.

The levels of pollutants and predatory behavior and also lifespan of the aquatic species are related to the bioaccumulation of Me-Hg (Mozaffarian, 2006). Larger species such as shark and swordfish are naturally bioaccumulated are higher Hg concentrations than other species (USEPA, 2013) and Cd levels in fish usually increase with age (WHO, 1992). The levels of Pb and Cd are usually present at significant levels in water systems and may possibly bioaccumulate in marine species (Zhou *et al*., 2008).

Storelli *et al.,* (2002) reported that hammer head sharks had the highest Hg level (18.29 ppm). They added that Gulper sharks (*Centrophorus granulosus*), longnose spurdog (*Squalus blainville*) and kitefin (*Dalatias licha*) sharks have 9.66, 4.53 and 4.38 ppm muscle, respectively whereas velvet belly (*Etmopterus spinax*) and smoothhound (*M. mustelus*) sharks caught from the Mediterranean Sea had the lowest levels of mercury (0.63 and 0.31 ppm, respectively).

According to previous studies, Kim *et al.,* (2019) reported that Hg and MeHg concentrations were significantly attributed with length, weight, and age, and they also added that Hg was expected to exceed the limit in *C. brachyurus* with a body length or weight of over 130 cm or 25 kg, respectively. So, shark meat can expose consumers to a high level of As and that copper sharks bigger than the predicted size should be avoided for excessive Hg. In other study, Hg level increased with size or trophic level whereas other metals may not (Tiktak *et al.,* 2020).

3.1.2. Microbiological contaminants

Data in table (3) show some microbiological aspects of shark's meats. APC ranged from 7.90×10^4 of *M. mustelus* to 9.12×10^4 cfu/g of *C. plumbeus*, coliform group bacteria 77.00 of *H. perio* to 93.67 cfu/g of *M. mustelus* meats and FC 1.33×10^3 of *S. blainvillei to* 1.82×10^3 cfu/g of *M. mosis*. Although data of microbial contaminants of raw sharks are rare but the most research had been basically done of dried sharks fins and meats. In general, the microbiological quality of fish and fishery products is very important for guarding consumer's health and hygiene (Lilabati *et al.,* 1999).

At the same time, it should be noticed that the human factor plays a significant role in cross contamination throughout processing steps (Chakma *et al.,* 2020). However, the improvement by using high quality raw to produce a desired quality of final product is still potential (Azam *et al.,* 2003). Based on these data, it could be noted sharks species exposed to

the unhygienic conditions as a result to cross contamination by fungal counts and coliform bacteria during supply chain.

Table 3. Some of microbiological quality (cfu/g) of six shark species meat.

Species	*APC $\times 10^4$	Coliform cfu/g	**FC $\times 10^3$
C. granulosus	8.49±1.03	95.67±8.62	1.34±0.08
C. plumbeus	9.12±0.59	89.00±12.77	1.46±0.07
H. perio	8.52±0.23	77.00±9.54	1.53±0.08
M. mosis	8.25±0.41	93.00±6.24	1.82±0.09
M. mustelus	7.90±0.38	93.67±9.07	1.35±0.08
S. blainvillei	9.02±0.53	81.00±3.61	1.33±0.08
***MPLs			

***APC: Aerobic plate count, **FC: Fungus count, ***MPLs: Maximum Permissible limits.**

Recently, Pratte *et al.,* (2022) concluded that the host identity, diet, and environmental exposure in structuring the shark microbiome are the complex roles and the intestinal microbial taxa as potential contributors to shark physiology.

Our data agree with the findings by Bregman *et al.,* (2023) the bacterial aspects were significantly different between the shark species and the most dominant groups were Flavobacteriaceae, Moraxellaceae, and Rhodobacteraceae. However, specific microbial biomarkers were also identified for each shark.

Montemagno *et al.,* (2024) suggested that the differences in sharks' microbiome can be controlled by shark species ecology.

3.2.Quality criteria

3.2.1. Physico-chemical quality

Table (4) exhibits some physico-chemical quality criteria (ww) of six shark's meat. The values of pH ranged of 7.47 – 8.01, TVB 57.23 – 60.35 mg/100 g sample, and TMA 40.19 – 43.54 mg/100 g sample. The pH values are not only a significant factor but can also be used as an index of quality (Huss, 1988; Ruiz-Capillas and Moral, 2001).

Both TVN and TMA compounds are used as spoilage indices to evaluate the degree of microbial spoilage of the fishery products. The variations in TVB-N compound in fishes varied by within the same species, intra-species, fishing time, region, age, gender and sex (Connell, 1976; Sadok *et al.,* 1996; Majumdar *et al.,* 2005; Reza *et al.,* 2009 and Chakma *et al.,* 2022).

Table 4: Some physico-chemical quality criteria of six shark species.

Species	pH	*TVB-N (mg/100g)	**TMA-N (mg/100g)
C. granulosus	7.67±0.18	59.48±0.93	41.39±0.70
C. plumbeus	7.47±0.13	60.35±2.01	40.19±1.25
H. perio	8.01±0.33	57.23±1.34	42.35±1.92
M. mosis	7.76±0.23	59.93±1.72	42.57±0.89
M. mustelus	7.76±0.20	59.69±1.08	42.99±1.55
S. blainvillei	7.82±0.09	59.23±2.27	43.54±0.87

***TVB-N: Total volatile basic nitrogen, **TMA-N: Trimethylamin nitrogen.**

Concerning high levels of TVN and TMA, the largest fraction of non-protein nitrogen (NPN) consists of urea, which occurs not only in the liver of land animals; however it retained in the body fluids. Urea is formed by arginine is converted to urea and ornithine by arginase. The

loss of urea through the gills is almost equivalent to its synthesis, causing its concentration become almost constant in the body (Hazon *et al.*, 2003). Although urea is non-toxic where converted to ammonia by bacterial action and can result in a strong ammonia taste and odour. So, sharks should be bled immediately after capture, dressed and iced as soon as possible to prevent the meat contamination with blood (Vannuccini, 1999).

Trimethylamine oxide (TMAO) is another NPN compound occurring in large quantities of elasmobranches meat, however urea and TMAO are compounds assist sharks to maintain their osmotic balance (Hazon *et al.*, 2003). In general, changes in quality attributes are depending mainly on species and other biological factors. In addition, these attributes are changed as resultant of protein breakdown by microorganisms (Chakma *et al.*, 2022).

32.2. Biochemical composition

The proximal analysis (%, ww) of six shark's meat is presented in Table (5). The range of moisture content recorded 74.62 - 75.52%, Crude protein 22.58 - 23.60%, fat 0.50 - 0.62%, and ash 1.21- 1.59% of all sharks meat. Also, it was found that all parameters are similar for all samples studied. The biochemical composition is an important aspect to indicate the nutritional value and affects keeping quality and physical traits of product (Sutharshiny and Sivashanthini, 2011).

With regard fat content, our data confirmed that sharks samples based on their weight are lean fish, whereas Jacquot (1961) classified shark as a semi-fatty fish with an average lipid content of 2.5%. Bousch (2012) found that *M. mustelus* meat has an average proximate composition of 75% moisture, 23% protein, 1.6% lipids and 1.4% ash content. Fofandi (2020) found that shark fish (*Scoliodon laticaudus*) contained moisture content 78.2%, length 42-48cm and weight 970g. Variation in these constituents is due to vary between individuals and different species, season, gender, size, life cycle stage and anatomical position (Huss, 1988 and Chakma *et al.*, 2022).

The correlations between metal concentrations were contributed with biological criteria; the same species, intra-species, fishing time, region, age, sex, size, and habitat (Sadok *et al.*, 1996; Majumdar *et al.*, 2005; Reza *et al.*, 2009; Kim *et al.*, 2019 and Chakma *et al.*, 2022).

Table 5. Biochemical analysis (%, ww) of six shark's meat.

Species	Moisture	Crude protein	Fat	Ash
C. granulosus	75.06±0.37	22.58±0.47	0.50±0.03	1.22±0.04
C. plumbeus	75.38±0.53	23.60±0.57	0.64±0.07	1.24±0.06
H. perio	75.50±0.38	23.18±0.45	0.62±0.03	1.25±0.07
M. mosis	74.62±0.69	23.04±0.37	0.55±0.07	1.21±0.09
M. mustelus	75.52±0.51	23.56±0.51	0.59±0.08	1.59±0.08
S. blainvillei	75.02±0.32	22.76±0.47	0.54±0.07	1.37±0.17

3.2.3.Amino acids composition

3.2.3.1. Essential amino acids (EAAs) content

The essential amino acids (EAAs) content (g/16g N) of six shark species is demonstrated in Table (6). Total EAAs content ranged 57.41 – 58.61 g/16g N of sharks meat studied.

The values of lysine, valine and arginine were 8.53-9.18, 8.23-8.64 and 8.12 – 8.40 g/16g N sample while sulfur-containing amino acids; methionine and cysteine were 2.73- 2.83 and 2.61- 3.70 g/16g N, respectively. Also, data showed that lysine, valine and arginine were the dominant EAAs.

3.2.3.2.Non-Essential amino acids (NEAAs) content

Table (6) shows the non-essential amino acids (NEAAs) content (g/16g N) of six shark species. Total range of NEAAs content ranged 39.70 – 42.19 g/16g N of sharks meat investigated. The values of aspartic, glutamic and alanine were 8.96-10.32, 8.85-9.63 and 8.70 – 9.84 g/16g N, respectively while serine, glycine and proline were 4.73 – 5.35, 4.45 – 4.62, 3.45 – 3.58 g/16g N Generally, the aspartic, glutamic and alanine were the dominant NEAAs in all sharks' meats. Numerous studies have confirmed that fish meat is a good source of nutrients, easily digestible proteins, excellent amino acids content and a desirable texture; fish have less connective tissue than terrestrial animals (Yáñez *et al.,* 1976 and Økland *et al.,* 2005). *M. mustelus* meat is a good source of some essential amino acids, especially lysine and threonine, but low in minerals. Although shark meat is considered to have a favorable nutritional value, but contains a high urea content (Bosch, 2012).

Table 6. Essential amino acids (EAAs) content (g/16g N) of six shark species.

EAAs	*C. granulosus*	*C. plumbeus*	*H. perio*	*M. mosis*	*M. mustelus*	*S. blainvillei*
Threonine	4.65	4.90	4.96	5.21	5.58	4.89
Cysteine	2.73	2.67	3.70	2.71	2.61	2.68
Valine	8.44	8.64	8.38	8.23	8.46	8.57
Iso-leucine	4.97	4.86	5.15	4.96	4.87	4.81
Leucine	5.92	5.86	6.00	5.91	5.84	5.77
Tyrosine	2.98	3.16	2.75	3.28	2.98	3.17
Phenyl-Alanine	4.00	4.00	3.88	4.19	4.04	4.08
Lysine	8.65	8.90	8.53	8.91	9.18	8.85
Histidine	4.14	4.01	3.97	3.87	3.94	3.93
Arginine	8.14	8.12	8.26	8.43	8.38	8.40
Methionine	2.79	2.83	2.73	2.76	2.73	2.74
Total	57.41	57.95	58.31	58.46	58.61	57.89

Table 7. Non-Essential amino acids (NEAAs) content (g/16g N) of six shark species.

NEAAs	*C. granulosus*	*C. plumbeus*	*H. perio*	*M. mosis*	*M. mustelus*	*S. blainvillei*
Aspartic Acid	8.96	9.49	9.18	9.73	10.32	9.92
Serine	4.80	4.79	4.73	4.88	5.26	5.35
Glutamic Acid	9.63	8.99	9.14	9.19	8.85	9.41
Proline	3.54	3.58	3.42	3.45	3.55	3.53
Glycine	4.46	4.53	4.45	4.58	4.48	4.62
Alanine	8.73	9.84	8.78	8.70	9.63	9.36
Total	40.12	41.22	39.7	40.53	42.09	42.19

3.2.3.3.Some major minerals

The values of some major minerals (i.e. K, Mg, P, and Ca) in sharks meats are tabulated in Table (8). The mean of K, Mg, P and Ca recorded 732.87 - 1589.43 ppm, 180.55 - 472.81ppm, 39.22 - 126.49ppm, 67.59 - 189.39ppm, respectively. *C. plumbeus* have the highest values of minerals than other ones.

Table 8. Some major minerals (mg/100gm) of six shark species meat.

Species	K	Mg	P	Ca
C. granulosus	950.81±0.52	219.02±0.22	39.22±0.11	74.55±0.26
C. plumbeus	1589.43±0.85	472.81±0.28	126.49±0.39	189.39±0.13
H. perio	880.58±0.27	185.52±0.17	36.16±0.10	73.33±0.26
M. mosis	832.31±0.26	180.55±0.61	40.35±0.13	58.97±0.20
M. mustelus	732.87±0.68	188.53±0.18	52.79±0.15	79.68±0.23
S. blainvillei	983.86±0.68	206.53±0.24	52.24±0.21	67.59±0.27

Bosch *et al.,* (2012) showed that *M. mustelus* meat was much lower in Cu, K, Mn and Na compared to the minerals content of Shortfin mako shark (Teeny *et al.,* 1984). The biological criteria including age, sex, total body length, body weight, and habitat in *C. brachyurus* were correlated with the minerals content (Kim *et al.,* 2019).

CONCLUSION

Although sharks' meats are a good source of protein and nutritional value, but contained high TVN and TMA values and also they exposed to the unhygienic conditions as a result to microbial contaminants throughout supply chain steps. Lysine, valine and arginine were the dominant essential amino acids whereas the aspartic, glutamic and alanine were the dominant non-essential amino acids in all sharks' meats. Besides, *C. plumbeus* have the highest values of minerals than other ones. Therefore, this work recommends that raw sharks has high safety and quality as well as they one of solutions to overcome of fish gap in Egypt as a similar in other countries after their treatment to reduce the urea content.

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