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Evaluation of Chemical Quality and Nutrition Value of Fresh Water Cray Fish (*Procambarus clarkii*)

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ABSTRACT: Crayfish are fresh water crustacean that look like a tiny lobster, fresh water crayfish (*Procombarus clarkii*) had been introduced accidentally and appear in the River Nile and its resource all over Egypt during the last years. The objective of this study was to evaluate the chemical quality and composition of crayfish as a human food, and to introduce a new high quality crustacean organism as a new cheap source of animal proteins. Total thirty crayfish samples collected from different markets in Alexandria were analyzed for total protein, amino acids using amino acids analyzer, fat, cholesterol, fatty acids (using GLC), ash, minerals, and some heavy metals (using atomic absorption spectrophotometer). Also, total volatile basic nitrogen, pH, free fatty acids, and thiobarbituric acid value as a chemical quality index were measured. The obtained results revealed good freshness and high chemical quality of crayfish, with regard to their ranges of total volatile basic nitrogen (28-35 mg/100g), pH (7.01-7.21), thiobarbituric acid (0.084-0.412) mg molanaldhyde/kg, and free fatty acids (0.56-0.93) mg/kg. Chemical composition and nutritive value of crayfish revealed mean values of total protein, fat, ash, and cholesterol contents 18.076%, 1.057, 1.48%, and 22.417 mg/100g, respectively. Minerals concentrations level were 0.61%, 443.22, and 356.45 μg/g for phosphorous, iron, and magnesium, respectively. Amino acid pattern showed that Glutamic, Aspartic, Arginine, and leucine were abundant essential amino acids and oleic acid represented 44% of total fatty acids. Crayfish evoked high nutritional value as respect to higher content of total unsaturated fatty acids (73.689%) with 56.56%, and 15.08% monoioneic and polyioneic (with ω_3 and ω_6), fatty acids also, high quality of protein which contain 55.703% essential amino acids. Also, this investigation showed that heavy metals concentration residue decreased in this order zinc > manganese > copper > chromium > cadmium > lead > nickel. Copper, chromium, and cadmium concentration were within the Egyptian permissible limits in all samples, but lead levels exceeded the maximum permissible limits in all samples and zinc exceeded this limit in 75% of examined samples. Information obtained in the present study can assist in developing quality standard for fresh crayfish in Egypt and recommended rules for its safety were mentioned.

INTRODUCTION

Crayfish, sometime called craw fish, are fresh water crustacean resembling small lobsters (*Procambarus clarkii*). The fresh water crayfish had been introduced to the Egyptian Nile water recently and widely dispersed in River Nile and its resources all over Egypt. It has been collected during fishing from many locations at the Northern parts of the river Nile. **Ibrahim *et al.*, (1996)**[1], **El-Mossalami & Emara, (1999)**[2], **Fisher, (2006)**[3] **Wikipedia, (2006)**[4]

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Crayfish is a cheap source of protein and sea food similar to the much expensive shrimp and lobster, but much less in price and relatively short life span and fact that they lose the accumulation of heavy metals and chemical when they moult. Crayfish are flavorful nutrition and valuable human food (similar to lobster) and are sold in fish market throughout the world, every year nearly 75.000 tons valued at over $50 millions are produced in U.S.A alone. **Helfrich, & Distelano (2003)[5], and Fisher, (2006)[3]**

Crayfish is also eaten in Europe and China but they are most popular in Louisiana. It is formed commercially and consumed in various dishes. They can be prepared like lobster, turn bright red when cooked. Some evidence shows that *Procambarus clarkii* feed well upon some benthic vector snails, so it might be used as a potential biological *Schistosoma* control agent. Also, for new medical problem in Egypt (*Faschiola* infection in human), Crayfish also are important indicator of water quality and environmental health, flourishing in clean water and perishing in polluted water. The flesh of *P. Clarkii* is recommended to stand as a source of animal protein for Egyptian Citizen and its carapace can be used as farage for animals. **Fisher,(2006)[3]** and **Baheyeldine, (2007)[6]**

As the crayfish has become important resident in River Nile, it is necessary to determine the chemical quality and composition of crayfish as a food. The aim of this study is to introduce a new high quality crustacean organism and thus adding a new cheap source of animal proteins which we badly needed.

MATERIAL AND METHODS

Sampling

Thirty samples (500 g of each) of fresh water crayfish (*Procombarus clarkii*) were collected alive from different markets in Alexandria. The samples were transported to the laboratory in isolated ice boxes for various examination. Crayfish are prepared by removing the cephalothorax and then removing the tail meat from the shell. Only the tail meat with the section of intestine passing through the tail muscles is retained for analysis.

Methods

I- Chemical quality and composition of proteins:

1- Total protein: According to **Pearson (1976)**[7], using macrokjeldahl apparatus.

2- Amino acids pattern and ammonia: according to **Peter *et al*. (1984)**[8], using amino acid analyzer Beckman, model (119 CL).

3- Total volatile basic nitrogen (TVB-N): Steam distillation methods as descried by **FAO (1986)**[9] using kjeldahl macro distillation units.

II- Chemical quality and composition of fat.

1- Total fat: total lipids were extracted with petrolium ether in a soxhlet apparatus according to **Pearson (1976)**[7]**.**

2- Free fatty acids: according to **AOCS, (1994)**[10].

3- Fatty acids profile: The methyl ester of extracted crayfish fat was prepared according to **Radwan, (1978)**[11] by using Gas liquid chromatography (GLC), GC made Shimadzu-4CM (PEE).

4- Cholesterol content: The level of cholesterol was determined colorimetrically according to **Bohac, *et al*. (1988)**[12]**.**

5- Thiobarbituric acid value (TBA): Determined calorimetrically according **to Pearson (1976)**[7]: The results were express as malonaldehyde mg/kg samples.

III- Hydrogen ion concentration pH:

According to **AOAC (1980)**[13]**,** it was estimated directly by using a digital genco pH meter (609).

IV- Determination of trace elements and heavy metals residues (Fe, Pb, Cu, Zn, Mn, Cr, Ni, and Cd)

Digestion: according to **Manson and Simikiss, (1983)**[14]**.**

Atomic absorption spectrophotometric method used for determination of heavy metals residues as described in Perkin

Elmer catalogue of atomic absorption model 2380, U.S.A. (1982).

2- Determination of phosphorous:

Were determined colorimetrically using phosphomolybadate method **AOAC, (1980)[13].**

3- Ash content: according to **Pearson (1976)[7].**

RESULTS AND DISCUSSION

Total volatile basic nitrogen, pH, Thiobarbituric acid (TBA) value, free fatty acid content, and polyen index are used as chemical quality and freshness index of fish and shell fish **Cobb & Vanderzant, (1975**)[15] and **Aubaurg *et al*., (1998)[16].**

Tables (1 and 2) showed that total volatile basic nitrogen, pH, thiobarbituric acid, and free fatty acids as indicators on chemical quality of protein and fat of fresh water cray fish were within **ES (2005)[17]**, with mean values of 31.92±1.204 (mg/100g), 7.14±0.037, 0.233±0.059, and 0.77±0.05 (mg malonaldehyde/kg), respectively.

Chemical composition and nutritive values of protein and fat of cray fish: Chemical composition and nutritive values of fish and shell fish are quite variable depending on several factors including species variety and nutritive degree, diet, season of harvesting, locality of catching and environmental condition **Tankol, *et al*., (1999)[18] and Berg *et al*, (2004)[19]**.

The percentage of the major biochemical constituents of muscles of Cray fish, total protein and fat were illustrated in Table (1) they ranged between (0.84 – 1.33) and (14.9 – 22.6) with mean value of 1.057 and 18.076, respectively. These results were in agreement with **Holland *et al*., (1993)[20]** and **El-Mossalami & Emara, (1999)[2]** they recorded that total protein percentage of Cray fish (*Procambarus clarkii*) (15.6, 14.9) and fat percentage (1.51, 0.8), respectively.

Mona, *et al*., (1999)[21] detected higher percentage of fat, 3.1% and protein 62.2%.

Cholesterol content of cray fish, table

(2a) revealed mean value of 22.417 mg/100 g in the context of recommended maximum cholesterol intake of 300-600 mg/day, this is insignificant. Cray fish contain lowest cholesterol content as compared with egg, prawns, shrimp, lobster, and atlantice salmon which contain 250, 195, 130, , 50-100, and 56 mg/100 g, respectively **(Fish foundation, (2007)[22], Island Sea Fare, (2007)[23]**, higher amounts of total cholesterol 47.0±1.5 mg/100 g. Detected by **Essien, (1995)**[24] in Cray fish *Palaemonets varians* .

Amino acids pattern of cray fish were obvious in table (1b) – amino acids analyzer pattern, detected nine essential, eight non-essential amino acid besides, and ammonia in Cray fish protein. Glutamic, aspartic, and arginine were the most abundant and the minor ones were theronine, serine, cystine, and tyrosine (less than 3%). Total essential amino acids represented 55.703% with respect to the ratio of EAA/NEAA (1.258). Cray fish protein has a high biological quality compared to ratio of farm, fresh and frozen shrimp recorded by **Thanae, *et al*., (2003)[25]** and other protein of high biological values such as beef, egg, and milk.

Fatty acids profiles have been determined using gas liquid chromatography (Table 2b), eleven fatty acids were identified. Unsaturated fatty acids were found in a very high proportion 73.689%., $C_{16}:_{0}$, $C_{18}:_{1}$, $C_{18}:_{2}$, and $C_{22}:_{1}$ were the predominant fatty acid; Oleic acid (18 : 1) represent alone 44.01%.

Biderre, *et al*., (2000)[26] revealed that oleic acid was prevalent in the fresh water fish and palmitic acid was prevalent in marine fish. Poly unsaturated fatty acids (omega – 6 and omega – 3) represent 12.5, and 2.58%, respectively. These fatty acids are essential for good health and normal growth **Sinclair, *et al*. (1998)[27], Bente, *et al*., (2004)[28]**. Nearly, the same pattern of different fatty acids showed by **Essien, (1995)[24].**

Calcium, phosphorus, and iron were the essential elements which were important for blood, protein, carbohydrates, and fat synthesizes and acts as coenzymes **Hays (1989)**[29] but if excesses causes toxic to biological system **Bathwell *et al*., (1979)**[30]. Table (3) showed that ash content of *P. clarkii* has 1.48%,phosphorous, iron, and magnesium concentration values were 0.61%, 443.22 and 356.45 μg/g, respectively. These results agree with **El-Mossalami & Emara, (1999)**[2] who detected ash content 1.51%. in contrast with results of **Mona, *et al*., (1999)**[21] who found higher ash content (10.2%) and higher phosphorus concentration (3436 μg/g) but detected lower iron concentrations (117 μg/g).

Heavy metals residues

Heavy metals represent the chemical residues which have a major role in animal and human health. These are cumulative poisons causing injury to health through progressive and irreversible accumulation as a result of ingestion of repeated small amounts . **Bathwell, *et al*., (1979)**[30] **& Ahlam El-laboudy *et al*., (1997)**[31].

Fresh water cray fish species are considered to be biological indicators of clean water because of their relatively lower locomotor activity in comparison with fish, they lose the accumulation of heavy metals and chemical when they moult. **Guda, (2002)**[32]**.**

In concerning the level of heavy metals residues in examined cray fish were illustrated in table (4). The results obtained revealed that heavy metals concentration level arranged in the following descending order: Zinc > Manganese > Copper > Chromium > Cadmium > Lead > Nikle. with mean values of 59.21±5.989, 22.545±5.399, 1.58±0.315, 0.708±0.317, 0.55±0.11, 0.936±0.0523, and 0.223±0.022 μg/g wet weight, respectively.

In respect to **ES (1993)**[33] all examined cray fish samples do not exceed these permissible limits of copper (20 μg/g). Chromium (5.5 μg/g) and Cadmium (0.1/μg/g) but zinc exceeded the maximum permissible limit (50 μg/g) in 75%

examined samples, and lead exceeded this limit (0.1 μg/g) 100%.

Results were nearly similar to the level reported by **Gouda, (2002)**[32] for Cu, Ni, and Cd but he recorded lower levels of Zn, Mn, and Cr.

In contrast **Abd-Allah & Abdallah (2006)**[34] they reported higher level of Cadmium, Lead, and Copper exceeding permissible limits and lower level of zinc in raw and cooked muscles of cray fish (*P. clarkii*) with nearly similar descending order of heavy metals concentrations. Also, **Mona *et al*., (1999)**[21] showed higher Zn concentration value 117mg/kg. Copper (Cu) and Zinc (Zn) are essential metals for Cray fish metabolism, always found in high concentrations independently with their environmental levels and longer exposure time, in contrast Pb, Ni, Cd, and Cr tend to increase with increasing concentration in surrounding environment and with longer exposure **Alcorolo, *et al*., (2006)**[35]. Generally, our results to somewhat over in accordance with those of **Young, (19991)**[36], **Holdich, (1999)**[37], **Fang *et al*., (2001)**[38]), **Blasco, *et al*., (2002)**[39], **Lopez *et al*., (2003)**[40], **Abdel Maged, (2004)**[41] . These differences may be due to Crustaceans accumulated some heavy metals directly proportional to the increase in bioavailability from water and trophic chain **Guoda, (2002)**[32]

In general, *P. clarkii* Cray fish revealed relatively higher or nearly the same level of nutritive value when compared with lobster, shrimp, and Atlantic salmon fish as regards to higher protein%, minerals contents low fat%, cholesterol, and heavy metals residues as discussed by **Holland *et al*.,(1993)**[20]**, Amine, *et al*., (2003)**[25] **and Amine, (2004)**[42]**.**

CONCLUSION

The obtained results revealed good freshness and high chemical quality of crayfish, with regard to their ranges of total volatile basic nitrogen, pH, thiobarbituric acid, and free fatty acids. Chemical composition and nutritive value of Cray fish revealed that Cray fish is a good source of protein and minerals with low content of fat and cholesterol. Crayfish evoked high nutritional value as respect to higher content of total unsaturated fatty acids (73.689%) with 15.08% and polyioneic (ω_3 and ω_6), fatty acids also, high quality of protein which contain 55.703% essential amino acids. Copper, chromium, and Cadmium concentration were within the Egyptian permissible limits in all samples, but lead levels exceeded the maximum permissible limits in all samples and zinc exceeded this limit in 75% of examined samples. Cray fish can provide a pollution-free source of protein, perfectly safe since all the poisonous material absorbed by Cray fish is stored in the shell, which it self sheded six times a year.

RECOMMENDATION

Information obtained in the present study can be assist in developing quality standard for fresh crayfish in Egypt and recommended rules for its safety were mentioned. The Cray fish meat industry is responsible for a $120 million/ year impact on Louisiana's economy, if Egypt can develop a similar industry, the profits could be considerable. We think that using Cray fish as a biological snail control agent will represent a much easier, cheaper,q and safer control than the chemical one. Also, Cray fish can be used as a fish meal. In addition, it is of great importance to complete this study by bacteriological and parasitical studies.

Table (1a): Statistical analysis of chemical quality and composition of protein of crayfish (n=30).

	Total protein %	T.V.N (mg/100g)	pH
Max	22.66	35	7.21
Min	14.9	28	7.01
Mean±S.E	18.076±1.477	31.92±1.204	7.14±0.037

Table (1b): Statistical analysis of amino acids pattern and ammonia in crayfish (n = 6).

Amino acids	Mean ± S.E
Aspartic acid	9.48±0.924
Threonine	1.494±0.231
Serine	2.891±0.389
Glutamic acid	11.207±1.120
Proline	4.006±0.223
Glycine	5.815±0.413
Alanine	5.59±0.521
Cystine	0.339±0.188
Valine	6.707±0.620
Methionine	5.928±0.296
Isoleucine	6.699±0.178
Leucine	9.831±6.057
Tyrosine	2.297±0.110
Phenylalanine	4.104±0.279
Histidine	3.0944±0.256
Lysine	6.462±0.627
Ammonia	2.639±0.313
Arginine	11.384±1.48
Essential aminoacids (EAA)	55.703
Non essential (NEAA)	44.297
EAA/NEAA (ratio)	1.258

Each reading represents mean value of 6 trial analysis for polling sample of 5 individual crayfish tissue samples

Table (2a): Statistical analysis of chemical quality and composition of fat of crayfish. (n = 30)

	Total fat%	Cholesterol mg/100ml	Thiobarbituric acid (mg malonaldehyde/kg)	F.F.A
Max	1.33	25	0.412	0.93
Min	0.84	20.91	0.084	0.56
Mean±S.E	1.057±0.082	22.417±0.781	0.2331±0.0599	0.77±0.05

Table (2b): Statistical analysis of fatty acid profile in crayfish, mg/kg (n=6).

Fatty acids	Mean ± S.E
Lauraic C12:0	0.531±0.322
Myristic C14:0	0.726±0.366
Palmitic C16:0	16.53±1.073
Palmitoletic C16:1	2.13±0.942
Stearic C18:0	5.005±1.097
Oleaic C18:1	44.01±1.853
Linoleaic C18:2	12.5±3.092
Linolenic C18:3	2.58±0.235
Arachidic C20:0	4.59±2.16
Behenic C22:0	0.913±561
Erucic C22:1	10.42±2.185
Monioneic fatty acids ($C_{16:1}$, $C_{18:1}$, $C_{22:1}$)	56.56±3.039
Polyioneic fatty acids ($C_{18:2}$, $C_{18:3}$)	15.08±3.196
Saturated fatty acid	26.311±1.091
Unsaturated fatty acid	73.689±5.438

Each reading represents mean value of 6 trial analysis for polling sample of 5 individual Cray fish tissues.

Table (3): Statistical analysis of minerals and total Ash in crayfish tissue (n=30).

	Phosphorous%	Iron µg/g	Magnesium µg/g	Total ash
Max	0.53	391.51	290.313	1.064
Min	0.72	490.96	410.992	1.80
Mean±S.E	0.61±0.034	443.22±18.2	356.45±20.94	1.48±0.133

Table (4): Statistical analysis of heavy metals residues in crayfish (µg/g wet weight) (n = 30)

	Min	Max	Mean±S.E
Pb	0.287	0.569	0.436±0.052
Cu	0.961	2.57	1.58±0.315
Zn	40.65	71.766	59.21±5.989
Mn	5.56	32.454	22.545±5.399
Cr	0.06	1.223	0.708±0.317
Ni	0.17	0.29	0.223±0.022
Cd	0.02	0.077	0.055±0.011

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