Evaluation of chemical and Nutrition Value of Fresh Water Crayfish (*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 tributaries all over Egypt during the last years. Therefore, the objective of this study is 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. Thirty crayfish samples were collected from different markets in Alexandria Governorates, and were analysed for total protein, amino acids, total fat, total cholesterol, fatty acids, ash and minerals. Total volatile basic nitrogen, pH, free fatty acids and thiobarbituric acid value as a chemical quality index, were also determined. The obtained results revealed good freshness, with regard to their ranges of total volatile basic nitrogen (49-58mg/100g), pH (5.9-6.2), thiobarbituric acid (0.123-0.637) mg molanaldhyde/kg and free fatty acids (0.93-1.56). Chemical composition and nutritive value of crayfish possessed mean values of total protein, total fat, ash and total cholesterol 13.88%, 1.76%, 1.52% and 13.575mg/100g respectively. Minerals concentration levels were 1.32%, 506.33 and 415.63 ug/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 showed 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 w3 and w6) fatty acids, and also, high quality of protein which contain 55.703% essential amino acids. Also, the present study showed that heavy metals concentration residue copper and chromium concentration were within Egyptian permissible limits in all samples but lead and cadmium levels exceeded the maximum permissible limits in all samples, while zinc exceeded this limit in 75% of examined samples. Results obtained in the present study can be concluded that most crayfish marketed in Alexandria were contaminated with higher limits of cadmium, lead and zinc considered as unsafe for human consumption.

Key words: *Cray fish, chemical quality, nutritive value.*

Introduction

Crayfish, sometimes 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 all River Nile and its tributaries all over Egypt. It has been collected during fishing from many location at the Northern parts of the river Nile **(Ibrahim *et al.*, 1996; El Mossalami and Emara, 1999; Fisher, 2006 and Wikipedia, 2006)**. 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. Crayfish are flavorful nutrition and valuable human food (similar to lobster) and are sold in fish market throughout the world. Every year 75.000 tons valued at over 50 millions $ are produced in U.S.A alone **(Helfrich, and Distelano, 2003** and **Fisher, 2006)**. Crayfish is also consumed in Europe and China but they are most popular in Louisiana, where it is consumed in various dishes. They can be prepared like lobster, turn bright red when cooked. Some evidence shows that *Procambarus clarkia* feed well upon some benthic vector snails, so it might be used as a potential biological *Schistosoma* control agent

Wikipedia (2006).

Crayfish also is an important indicator of water quality and environmental health, flourishing in clean water and perishing in polluted water **Fisher (2006)**. The flesh of *Procambarus clarkii* is recommended as a source of animal protein for Egyptian Citizen and its carapace can be used as farage for animals **(Fisher, 2006** and **Baheyeldine, 2007)**.

Thereby crayfish became very important resident in the River Nile areas so, it is necessary to determine the chemical quality and composition of crayfish as a human food, in order to introduce a new high quality crustacean organism and thus adding a new cheap source of animal proteins which we need.

Materials and Methods

Sampling

Thirty samples (500 gm of each) of fresh water crayfish (*Procombarus clarkii*) were collected from different markets in Alexandria. The samples were transported to the laboratory Of Arabian and Academic Science and Techenology in isolated ice boxes for various examination. Crayfish are prepared by removing the cephalothorax and the intestine. Only the tail muscles were retained for analysis.

Methods

I. Chemical quality and composition of proteins parameters were determined:

1. Total protein: according to **Pearson (1976),** using macrokjeldahl apparatus.
2. Amino acids pattern and ammonia: according to **Peter (1984)**, using amino acid analyzer Beckman, model (119 CL).
3. Total volatile basic nitrogen (TVB-N): steam distillation methods as descried by **FAO (1986)** using Kjeldahl macro distillation units

II. Chemical quality and composition of fat parameters were also determined.

1. Total fat: total lipids were extracted with petroleum ether in asoxhlet apparatus according to **Pearson (1976)**.
2. Free fatty acids: according to **AOCS, (1994)**.
3. Fatty acids profile: The methyl ester of extracted crayfish fat were prepared according to **Radwan, (1978)** by using Gas liquid chromatography (GLC), GC made shimadzy-4CM (PEE).
4. Total Cholesterol : The level of cholesterol was determined colorimetrically according to **Bohac *et al*. (1988)**.
5. Thiabarbturic acid value (TBA): Determined calorimetrically according to Pearson (1976), the results were expressed as malonaldehyde mg/kg samples.

III. Hydrogen ion concentration pH was determined according to AOAC (1980), 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 Simkiss, (1983).**

Determination of heavy metals residues

By using Perkin Elmer atomic absorption model 2380, U.S.A.

Determination of phosphorous

Phosphorous contents were determined colorimetically using phosphomolybadate method **AOAC, (1980)**.

Determination of ash content

Ash content was determined according to **Pearson (1976)**.

Results

Table (1). The chemical quality and composition of protein of crayfish (n=30).

	Total protein %	T.V.N (mg/100g)	pH
Max	18.23	58	6.2
Min	9.53	49	5.9
Mean±S.E	13.88±1.477	53.5±1.204	6.05±0.037

Table (2). The amino acids pattern and ammonia in crayfish (n = 6).

Amino acids	Mean% ± S.E
Aspartic acid	9.48±0.924
Theronine	1.494±0.231
Serine	2.891±0.389
Glutamic acid	11.207±1.120
Praline	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
Hidstiadine	3.0944±0.256
Lysine	6.462±0.627
Ammonia	2.639±0.313
Arginine	11.384±1.48
Essential aminoacids	55.703
Non essential	44.297
EAA/NEAA (ratio)	1.258

Each reading represent mean value of 6 trial analysis for pooling sample of 5 individual crayfish tissue samples.

Table (3). The chemical quality and composition of fat of crayfish. (n = 30)

	Total fat%	Total Cholesterol mg/100ml	Thiabarbiturc acid (mg malonaldehyde/kg)	F.F.A%
Max	2.45	16.12	0.637	1.56
Min	1.13	11.03	0.123	0.93
Mean ± S.E	1.76±0.082	13.575±0.781	0.380±0.0599	1.245±0.05

Table (4). The fatty acid profile in crayfish (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
Palmitaletic 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 (C16:1, C18:1, C22:1)	56.56±3.039
Polyioneic fatty acids (C18:2, C18:3)	15.08±3.196
Saturated fatty acid	26.311±1.091
Unsaturated fatty acid	73.689±5.438

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

Table (5). The of minerals and total ash in crayfish. (n = 30)

	Phosphorus %	Iron µg/g	Magnesium µg/g	Total ash mg/g
Max	1.68	605.33	469.15	1.46
Min	0.96	407.23	362.11	0.58
Mean ± S.E	1.32±0.21	506.33±20.21	415.63±18.24	1.52±0.138

Table (6). The heavy metals residues in crayfish (µg/g wet weight) (n = 30)

	Min	Max	Mean±S.E
Pb	1.216	2.680	1.948±0.192
Cu	3.088	4.354	3.721±0.241
Zn	51.139	78.325	64.732±5.689
Mn	23.881	46.471	35.176±4.724
Cr	3.02	4.34	3.68±0.721
Ni	2.56	3.68	3.12±0.656
Cd	5.05	7.67	6.37±0.731

Egyptian Organization for Standardization and Quality Control (ES 2360/1993-1796/1996) stated that heavy metals residues in fish should not exceed the maximum permissible limits (MPL) of 0.1 and 0.1 ppm for cadmium and lead and respectively.

Discussion

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

Table (1, 2 & 3) showed that total volatile basic nitrogen, pH, thiobarbituric acid and free fatty acids as indicator on chemical quality of protein and fat of fresh water crayfish were within ES (2005) ,with mean values of 53.50 ± 1.204 (mg / 100g), 6.05 ± 0.037,0.380 ± 0.059, 1.245 ± 0.05 (mg malonaldehyde / kg), respectively.

Chemical composition and nutritive values of protein and fat of Crayfish

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, local of catching and environmental condition **(Tankol, *et al*., 1999 and Berge *et al*, 2004)**. The percentage of the major biochemical constituents of muscles of Crayfish, total protein and fat were illustrated in Tables (1, 2, 3 & 4) and ranged between 1.13 – 2.45% for total fat and 9.53 – 18.23% for total protein with mean values of 1.76 and 13.88 respectively. These results were in agreement with **Holland *et al*., (1993) and El-Mossalami and Emara, (1999)** they recorded that total protein percentage of Crayfish (*Procambarus clarkii*) (15.6, 14.9) and fat percentage (1.51, 0.8) respectively.

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

Total cholesterol content of crayfish

Table (3) revealed that the mean value of total cholesterol content of crayfish 13.575 mg/100 g. Crayfish contain lowest cholesterol content in compared with egg, prawns, shrimp, lobster, atlantice salmon which contain 250, 195, 130, , 50-100, 56 mg/100 g, respectively (Fish foundation, 2007, Island sea fare, 2007), higher amounts of total cholesterol 47.0±1.5 mg/100 g. detected by **Essien (1995)** in Crayfish *Palaemonets varians*.

Amino acids pattern of crayfish were obvious in Table (2) – amino acids analyzer pattern, detected nine essential and eight non essential amino acid besides 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).

Crayfish protein of a high biological quality in compared to ratio of farm, fresh and frozen shrimp recorded by **Thanae, *et al*., (2003)** and other protein of high biological values such as beef, egg, and milk. Fatty acids profiles has been determined using gas liquid chromatography (Table 4), eleven fatty acids were identified, unsaturated fatty acids were found in a very high proportion 73.689%., C16 : 0, C18 : 1, C18 : 2, C22 : 1 were the predominant fatty acid; Oleic acid (18 : 1) represent alone 44.01%.

Biderre *et al*. (2000) revealed that oleic acid was prevalent in the freshwater fish and palmitic acid was prevalent in marine fish, Poly unsaturated fatty acids (omega – 6 and omega – 3) represent 12.5, 2.58% respectively. These fatty acids are essential for good health and normal growth **(Sinclair, *et al*. 1998, Bente, *et al*., 2004)**. Nearly, the same trends of different fatty acids were shown by **Essien, (1995)**.

Calcium, phosphorus and iron were the essential elements important for blood, protein, carbohydrates and fat synthesized and act as coenzymes **(Hays 1989)** but if excesses caused toxic to biological system **(Bathwell *et al*., 1979)**. Table (5) showed that ash content of *Procambarus clarkii* were 1.52%, phosphorous, iron and magnesium concentrations were 1.32%, 506.33 and 415.63 ug/g respectively.

These results agree with **Elmossalami and Emara, (1999)** who detected ash content 1.51%. In contrast with results of **Mona (1999)** 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 and Ahlam Elleboudy *et al*., 1997)**.

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

Concerning the levels of heavy metals residues in examined crayfish were illustrated in Table (6). The results obtained revealed that the mean values of heavy metals concentration level of: Zinc , Manganese , Cadmium , Copper ,Chromium , Nickel and Lead were 64.732 ± 5.689, 35.176 ± 4.724, 6.37 ± 0.731, 3.721 ± 0.241, 3.68 ±0.721, 3.12 ± 0.656 and 1.948 ± 0.192 µg/g wet weight respectively.

In respect to **ES (1993)** all examined crayfish samples not exceed these permissible limits of copper (20 ug/g), Chromium (5.5 ug/g) and Cadmium (0.1/ug/g) but zinc exceeded the maximum permissible limit (50 ug/g) in 75% of lead and cadmium exceeded this limit (0.1 ug/g) 100%.

Results were nearly similar to the level reported by **Gouda, (2002)** for Cu, Ni and Cd but he recorded lower levels of Zn, Mn and Cr. In contrast **Abd-Allah and Abd- Allah (2006)** they reported higher levels of Cadmium, Lead and Copper exceeding permissible limits and lower level of zinc in raw and cooked muscles of crayfish (*Procambarus. clarkii*) with nearly similar descending order of heavy metals concentrations. Also, **Mona Hegazy *et al*., (1999)** showed higher Zn concentration value 117mg/kg. Copper (Cu) and Zinc (Zn) are essential metals for Crayfish metabolism, always found in high concentrations independently with their environmental levels and longer exposure time, in contrast Pb, Ni, Cd, Cr tend to increase with increasing concentration in surrounding environment and with longer exposure **(Alcarolo, *et al*., 2006)**. Generally our results were somewhat nearly to those of **Bendell young, (1991), Holdich,(1999), Fang *et al*. (2001), Blasco, *et al*. (2002), Lopez *et al* (2003), Abdel Maged, (2004)**. These differences may be due to that Crustaceans accumulated some heavy metals directly proportional with the increase in bioavailability from water and trophic chain **(Guoda, 2002).** In general, *Procambarus. clarkii* Crayfish revealed relatively higher or nearly the same level of nutritive value when compared with lobster, shrimp and Atlantic salmon fish due to their higher protein%, minerals contents and low fat%, cholesterol and heavy metals residues as discussed by **Holland *et al*. (1993), Thanae, *et al*. (2003) and Thanae, (2004)**.

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 Crayfish revealed that Crayfish is a good source of protein, and minerals with low content of total fat and total cholesterol. Crayfish evoked high nutritional value as respect to higher content of total unsaturated fatty acids (73.689%) with 15.08% and polyioneic (w3 and w6), fatty acids also, high quality of protein which contain 55.703% essential amino acids. Copper and chromium concentrations were within the Egyptian permissible limits in all samples, but lead and Cadmium levels exceeded the maximum permissible limits in all samples and zinc exceeded this limit in 75% of examined samples. as stated by Egyptian standard (ES2360/1993-1796/1996).So we conclude that most cray fish marketed in Alexandria were contaminated with higher limits of cadmium, lead and zinc are considered as unsafe for human consumption.

Recommendation

Results obtained during the present study that most cray fish marketed in Alexandria were contaminated with higher limits of cadmium, lead and zinc and are considered as unsafe for human consumption We think that using Cray fish as a biological snail control agent will represent a much easier and cheaper than the chemical one. In addition, it is of great importance to complete this study by bacteriological and parasitical studies.

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دراسة للتقييم الكيميائى والقيمة الغذائية لاستاكوزا المياة العذبة

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الملخص العربى

الاستاكوزا نوع من أنواع قشريات المياه العذبة ويطلق عليها استاكوزا المياه العذبة حيث دخلت بالصدفة بطريقة الخطأ لنهر النيل وفروعة على مستوى مصر فى السنوات الأخيرة .

الهدف من هذه الدراسة هو عمل تقييم كيميائى لمكونات وجودة استاكوزا المياه العذبة كغذاء للانسان ومن أجل إدخال أنواع من القشريات عالية الجودة وكمصدر جديد ورخيص للبروتين الحيوانى .حوالى 30عينة من استاكوزا المياه العذبة تم تجميعها من اسواق مختلفة من محافظة الإسكندرية وتم تحليل البروتين الكلى والأحماض الأمينية والدهون الكلية والكولوستيرول الكلي والأحماض الدهنية والرماد والمعادن باستخدام الاسبكتروفوتوميتر .

أيضا تم تقديرالنيتروجين المتطاير والأس الهيدروجينى والأحماض الدهنية الحرة وحمض الثيوباربتيوريك كمقياس للجودة الكيميائية والنتائج المتحصل عليها أظهرت طزاجة عالية وكذلك قيمة كيميائية عالية للاستاكوزا .

حيث كانت نسبة النيتروجين المتطاير تتراوح من 49 58جم 100 /جرام ودرجة الأس الهيدروجينى من 5.9 6.2وحمض الثيوباربتيوريك 0.123إلى 0.637 .مجم مولا ألدهيد /كجم وقيمة الأحماض الدهنية الحرة من 0.93 1.56

التركيب الكيميائى والقيمة الغذائية للاستاكوزا وضحت ان متوسط البروتين الكلى والدهون الكلية والرماد والكوليسترول الكلي كانت % 13.88و % 1.76و % 1.52و 13.575%مجم 100 /جرام .أما المعادن فكانت النسبة % 1.32و % 56.33و 415.63ميكروجرام /جرام للفوسفور والحديد والماغنسيوم .أما بالنسبة للأحماض الأمينية :فكانت نسبة حمض الجلوتامك وأسبارتك والأرجنين والليوسين تعتبر من الأحماض الأمينية الأساسية والأوليك أسيد وصلت النسبة إلى % 44من الأحماض الدهنية الكلية .

مما سبق يتضح ان :الاستاكوزا تعد ذو قيمة غذائية عالية وبها نسبة عالية من الأحماض الدهنية الغير مشبعة 73.689منها 56.56 %و % 15.08من أوميجا 3و 6أحادية ومتعددة الأيونات للأحماض الدهنية كانت تحتوى أيضا على نسبة عالية من البروتين عالى الجودة % 55.703)من الأحماضالأمينية الأساسية . ايضا نسبة المعادن الثقلة كانت قليلة حيث كانت تلك المعادن فى الحدود المصربة المسموح بها فى كل المعادن لكن الرصاص و الكادميوم زاد عن ذلك فى كل العينات اما الزنك زاد فقط فى 75% من العينات التى تم فحصها .من هذه الدراسة نستخلص ان استاكوزا المياة العذبة تعتبر من الكائنات المائية ذو الجودة الغذائية العالية ولكن ارتفاع نسبة الرصاص والكادميوم والزنك تجعلها غير امنة للاستهلاك الادمى.