

Heavy Metals Pollution Level in Marine Hammour Fish and the Effect of Popular Cooking Methods and Freezing Process on These Pollutants

Hend Hassan Ali Ganbi

Nutrition and Food Science Department, Faculty of Education for Home Economics and Art Education,
King Abd-Elaziz University, Jeddah, Saudi Arabia

Abstract: This study was carried out to throw lights on the pollution level of marine hammour (*Epinephelus areolatus*) fish at Saudi Arabia and the effect of the popular cooking methods and freezing process on these pollutants. Results showed that all determined heavy metals (Fe, Pb, Cr, Cu, Mn, As, Cd, Co, Zn and Hg) were detected in the different hammour fish organs. The lowest accumulation rate of all tested heavy metals was observed in the fish muscles, except Pb and Mn which were found at the lowest levels in fish gills and viscera respectively. In addition that hammour fish flesh contained all determined heavy metals at lower level than the maximum permissible limits (MPLs) of these pollutants except Pb and As which were found at level of 2.105 and 0.218 ppm, on wet weight basis, higher than their MPLs being 2 and 0.2 ppm respectively. Cooking operations (roasting, deep-fat frying and broiling) exhibited variable effect on the tested heavy metals' level in marine hammour fish flesh. Most tested heavy metals in fish exhibited a highly stability during freezing process and frozen storage for 6 months.

Key words: Heavy metals pollution · Hammour fish · Pollutants · Cooking · Freezing process · Marine fish · Chemical contaminants · Food processing.

INTRODUCTION

The beneficial health effect due to providing fish in a diet is well known and has been documented in several studies [1-3]. Fish is a good source of many important nutrients such as proteins, vitamins, minerals and ω -3-poly enoic fatty acids [3-5]. Fish intake is associated with improving cardiovascular health and other related health conditions [6]. Fish has long been a favorite meal of people living around the Arabian Gulf and has been a major source of food for people living in this region [3, 7]. A part from the beneficial effects, there are also some factors that contribute to risk from fish consumption, mainly due to the potential adverse effects of heavy metals [8, 9]. Increasing pollution in water bodies is directly or indirectly related to increasing urbanization and indiscriminate disposal of agrochemical and industrial effluents [10, 11]. Heavy metals are well known environment pollutions that cause serious health hazards to humans; their effects are not immediate and show up after many years [12, 13]. Studies to determine the heavy

metals concentrations in fish in Saudi Arabia indicated the safety of fish for consumption [14] and similar results from studies along the Arabian Gulf have been reported [15]. Increasing interest in the health gains obtained by regular fish intake has put emphasis on the need for documentation of both nutrients and contaminants in fish and seafood, with a balanced risk assessment [5].

Marine organisms, in general, accumulate contaminants from the environment and therefore have been extensively used in marine pollution monitoring programmes [16]. In many countries, significant alterations in industrial development lead to an increased discharge of chemical effluents into the ecosystem, leading to damage of marine habitats. Heavy metal discharged into the marine environment can damage both marine species diversity and ecosystems, due to their toxicity and accumulative behavior [17, 18].

Environmental pollution represents a major problem in both developed and undeveloped countries. Saudi Arabia is among countries which suffering from high biosphere pollution (air, soil and water). Many ecological

changes occur in water as a result of human activities, including agricultural, industrial and municipal wastes. Cadmium, copper, lead and zinc salts are usually found in agricultural and industrial liquid wastes [19-21] which are discharged into water resources. These metals are toxic to aquatic life at low concentration, particularly in soft-water interments. Such metals may be accumulated from water to higher levels in fish tissues [18, 22-24]. Evidently these metals accumulated frequently in fish flesh and in internal organs [25, 26].

The excess of heavy metals intake, especially mercury and lead, causes many harmful and neurotoxic effects to the human health. Hg is the most toxic heavy metals that affects the human brain causing the syndromes of nerve disturbances and insomnia, in addition to its harmful effects on inhibiting growth and the activity of some enzymes [27-29], implements specifically related to Pb toxicity in humans include abnormal size and hemoglobin contents of the erythrocytes, hyper stimulation of erythropoiesis inhibition of both haeme synthesis and some enzymes activity, catecholainergic and cholinergic nervous system, anemia and permanent damage of brain, liver and central nervous system [21, 30]. In general, heavy metals are interfering with cellular biochemistry, mainly by disruption of a variety of enzyme systems. Though disruption of specific enzymes, metal can alter the normal patterns of growth, reproductive function, immune function and general metabolism [29]. Secondly, toxic metals interfere with the normal metabolism and functions of required metals (i.e. Ca and Mg). In extreme condition some metals are known to be mutagenic or teratogenic [25, 29, 31, 32]. Therefore, it is essential to know the level of heavy metals in both cooked and processed Hammour fish which are approximately consumed by most people at Arabian Gulf Countries to avoid health hazards of these contaminants [3, 14]. However, little effort has been made, as well as few reports have been published in this concern.

The target of this research was to asses the heavy metals pollution of marine hammour fish (*Epinephelus areolatus*), as well as to determine the remainder concentration from these contaminants in cooked and frozen fish tissues.

MATERIALS AND METHODS

Materials: Marine Hammour *Epinephelus areolatus* fishes (belong to the family Serranidae, order Perciformes and class Actinopterygii) were obtained from local market

in Jeddah city, Saudi Arabia during the season of 2008-2009. The purchased fishes were carefully washed with tap water, packaged in iceboxes and transported to the Research Laboratory of Nutrition and Food Science Department, Faculty of Education for Home Economics and Art Education, King Abd-Elaziz University, Jeddah, Saudi Arabia.

The refined sunflower oil, tomato sauce and sodium chloride were obtained from the local markets in Jeddah, Saudi Arabia.

Standard heavy metals including; iron (Fe), lead (Pb), chromium (Cr), copper (Cu), manganese (Mn), arsenic (As), cadmium (Cd), cobalt (Co), zinc (Zn) and mercury (Hg) were obtained from Merck Co., Darmstadt, Germany.

Methods

Preparation of Whole Fish Samples for Freezing

Process: Each whole fresh hammour fish was cleaned carefully with tap water and then the glazing process was carried out by submerging the fish in cold water at $4\pm 1^{\circ}\text{C}$ for about 1-2 minutes. The glazed fishes were packaged in polyethylene bags and stored at $-18\pm 2^{\circ}\text{C}$.

Preparation of Fish Samples for Cooking:

Fresh marine hammour fishes were dressed carefully by removing scales, fins, head, tail and viscera. The fish body was washed with tap water to remove any traces of blood and viscera. After that, the fish flesh was divided into desired consistent size fillets and then the fillets were trimmed, washed carefully by tap water, drained for a few minutes and then immersed in 10 % NaCl solution for 10 minutes. Whereas, fish fillets were washed with tap water and treated with 3 % spices' mixture (composed of black pepper powder, cumin powder and fresh garlic paste at ratio of 1:1:2 respectively). Then, the prepared fillets were used for analysis and cooking. The other internal fish organs (brain, gills and viscera) were separated from fresh fishes and collected carefully for analysis.

Cooking of Fish Flesh Fillets:

Prepared fresh hammour fish fillets were cooked according to the procedures of [33]. The prepared fish fillets divided into 3 batches, the first was cooked by roasting method in shallow pans containing adequate quantity of tomato sauce and refined sunflower oil using gas oven at $160\pm 5^{\circ}\text{C}$ for 20 min. or to an internal temperature of about 80°C . The second batch was thinly coated with wheat flour (72 % extraction) and then deep-fat fried in a household electric stainless steel pans (1 liter-capacity, 20 cm-diameter and 6 cm-high)

containing the heated frying oil medium; refined sunflower oil, to a temperature of $170\pm 5^{\circ}\text{C}$ and then deep-fat frying process was carried out for 6-8 min. at the previous temperature. The third batch of prepared hammour fish fillets was thinly coated with wheat bran and broiled using laboratory electric-plate broil (Model Suteskv, Russia, size of the flat was 30×30 cm) at $160\pm 5^{\circ}\text{C}$ for 12 min of the first side and then turned over to cook the other side for 8 min. After roasting, deep-fat frying or broiling was performed, all cooked fish samples were allowed to drain until they had cooled to ambient temperature ($\approx 35^{\circ}\text{C}$), and then were ground and homogenized by electric grinder (Oster Heavy Duty Food Grinder, USA). Whereas, the ground cooked fish samples were packaged in polyethylene bags and stored at about $-18\pm 2^{\circ}\text{C}$ until they analyzed for heavy metals determination.

Freezing process: Fresh prepared whole hammour fish samples were frozen at $-18\pm 2^{\circ}\text{C}$ and stored under the same former frozen temperature. The heavy metals level was determined in frozen fish samples after both 24 hr. (initial zero time) and 6 months of frozen storage.

Determination of Heavy Metals: The concentrations of the tested heavy metals were determined in fresh hammour fish organs (brain, gills, viscera and muscles), and in fresh, cooked and frozen fish flesh according to the procedure of [33]. Adequate weight of 2g for fish tissues was digested by using a mixture of nitric acid and perchloric acid (4:1 v/v). The digest was quantitatively transferred to a 25 ml. volumetric flask with twice-distilled water, adjusted to pH 2 with nitric acid (1 N) and the volume made up to the mark. The heavy metals were measured using atomic absorption spectrophotometer (Perkin Elmer Model 2380) at specific wave length for each metal.

Statistical Analysis: The obtained data were subjected to analysis of variance using SAS program for the multiple comparison between fresh and treated hammour fish samples; the procedure of ANOVA and Duncan's Multiple Range Test were used [35].

RESULTS

Proximate Composition of Tested Fresh, Cooked and Frozen Hammour Fish Samples: As illustrated in Table 1, the raw whole hammour fish contained moisture, crude

protein, crude lipid and ash at level of 76.16, 18.62, 3.26 and 1.84 %, on wet weight basis respectively. In addition that the moisture content of fillets was ranged between 61.91 and 64.73 % depending upon the kind of cooking method. On the other hand, the other chemical components in cooked fish fillets were ranged from 70.60 to 78.94 % for crude protein, 8.45 to 19.53 % for crude lipid and 8.14 to 10.60 % for ash content.

The obtained results showed that freezing process and frozen storage temperature ($-18\pm 2^{\circ}\text{C}$) caused, somewhat, slight effect on gross chemical components. Whereas, the frozen whole fish had crude protein, crude fat and ash contents at level of 78.87, 12.93 and 8.11 %, on dry weight basis (18.46, 3.02 and 1.90 %; on wet weight basis) respectively. The moisture content of fillets ranged between 61.91 and 64.73 % depending upon the kind of cooking method. On the other hand, the other chemical components in cooked fish fillets ranged from 70.60 to 78.94 % for crude protein, 8.45 to 19.53 % for crude lipid and 8.14 to 10.60 % for ash content.

Distribution of Heavy Metals in the Different Organs of Hammour Fish: The differences among the heavy metals' concentration in different organs of marine hammour fish were determined and then the obtained data were recorded as in Table 2.

As shown in Table 2, all tested heavy metals were detected in the different tested fresh organs and tissues of hammour fish at different concentrations depending upon the type of fish organ and the heavy metal itself. The present results (Table 2) evident that the predominant heavy metals in tested fish organs were Zn, Fe and Pb which were found at greatly higher concentrations than the other tested heavy metals in the tested fish organs. Furthermore, the highest concentrations (ppm) of Mn (2.762), Co (3.529) and Zn (49.08) were observed in hammour fish brain. While, the highest accumulation level (ppm) of Fe (16.165), Cr (3.059), Cu (10.713), As (5.391) and Cd (1.160) was found in the fish gills. In addition, hammour fish viscera contained the highest concentration of Pb (12.489) and Hg (1.308). On the contrary, the lowest accumulation rate of all tested heavy metals was observed in the fish muscles, with the exception of Pb and Mn which were found at the lowest concentrations in fish gills and viscera respectively.

Influence of the Popular Cooking Methods on Heavy Metals' Level in Hammour Fish Flesh: As shown in Table 3, roasting caused a significant ($p < 5$) reduction in Pb, Cr, Mn, As and Zn of hammour fish flesh by 16.2, 9.3,

Table 1: Proximate composition (%) of fresh, cooked and frozen marine hammour fish samples

Chemical Component (%)									
Variables	Moisture	Crude Protein		Crude lipid		Ash		Carbohydrates	
		W.w	D.w	W.w	D.w	W.w	D.w	W.w	D.w
Raw Whole Fish	76.16	18.62	78.1	3.26	13.68	1.84	7.72	0.12	0.50
Prepared Fresh fillets	76.02	18.55	77.36	3.19	13.3	2.10	8.76	0.14	0.58
Cooked Fish Fillets									
Roasted	63.86	28.53	78.94	4.37	12.09	2.94	8.14	0.30	0.83
Fried	61.91	26.89	70.60	7.44	19.53	3.27	8.58	0.49	1.29
Broiled	64.73	27.70	78.54	2.98	8.45	3.74	10.6	0.85	2.41
Frozen Whole Fish*	76.60	18.46	78.87	3.02	12.93	1.90	8.11	0.02	0.10

W.w: Chemical component percentage on wet weight basis D.w: Chemical component percentage on dry weight basis. Frozen whole fish*: after 6 months of frozen storage (at -18±2°C)

Table 2: Distribution of heavy metals (ppm; on wet weight basis) in the different organs of marine hammour fish

Heavy Metal Level (M±SE)*					
Heavy Metal	Flesh	Brain	Gills	Viscera	MPL**
Fe	3.618±0.23 ^a	7.903±0.57 ^b	16.165±1.09 ^d	12.018±0.74 ^c	--
Pb	2.105±0.29 ^b	9.830±0.57 ^c	1.207±0.31 ^a	12.489±0.79 ^d	2
Cr	0.172±0.05 ^a	1.146±0.29 ^c	3.059±0.46 ^d	0.466±0.08 ^b	0.20
Cu	0.498±0.12 ^b	0.295±0.07 ^a	10.713±0.79 ^d	7.109±0.63 ^c	20
Mn	1.751±0.23 ^b	2.762±0.29 ^c	1.027±0.16 ^a	0.957±0.11 ^a	---
As	0.218±0.09 ^a	0.937±0.16 ^b	5.391±0.43 ^d	1.184±0.17 ^c	0.20
Cd	0.029±0.003 ^a	0.063±0.002 ^b	1.160±0.29 ^d	0.482±0.09 ^c	0.05
Co	0.160±0.07 ^a	3.529±0.43 ^d	2.032±0.31 ^c	0.246±0.005 ^b	0.20
Zn	19.814±1.09 ^c	49.077±3.16 ^d	41.539±2.84 ^c	36.071±2.63 ^b	30
Hg	0.261±0.04 ^a	0.440±0.09 ^b	0.692±0.06 ^c	1.308±0.31 ^d	0.50

M±SE*: Mean±Standard error of heavy metal level; Means (in the same row) having different superscripts are significantly varied. MPL**: Maximum Permissible limit of heavy metal for human consumption of fish.

Table 3: The influence of common cooking methods on heavy metal level (ppm; on wet weight basis) in marine hammour fish

Heavy Metals	Fresh	Roasted	Change (%)	Fried	Change (%)	Broiled	Change (%)	MPL**
	M±SE*	M±SE*		M±SE*		M±SE*		
Fe	3.618±0.53 ^a	9.275±0.67 ^b	+ 7.6	9.747±0.59 ^c	+ 13.1	9.401±0.56 ^{bc}	+ 9.1	---
Pb	2.105±0.18 ^d	1.763±0.21 ^b	-16.2	1.670±0.16 ^a	-20.7	1.823±0.27 ^c	-13.2	2
Cr	0.172±0.05 ^c	0.156±0.03 ^b	-9.3	0.143±0.05 ^a	-16.4	0.16±0.07 ^b	-7.1	0.20
Cu	0.498±0.12 ^b	0.498±0.07 ^b	0	0.447±0.11 ^a	-10.2	0.526±0.13 ^c	+ 5.6	20
Mn	1.751±0.31 ^d	1.385±0.23 ^a	-20.9	1.528±0.26 ^b	-12.8	1.608±0.23 ^c	-8.2	---
As	0.218±0.09 ^c	0.193±0.11 ^b	-11.4	0.176±0.07 ^a	-19.3	0.181±0.09 ^a	-16.9	0.20
Cd	0.029±0.003 ^c	0.029±0.005 ^c	0	ND±0.0 ^a	-100	0.023±0.002 ^b	-20.7	0.05
Co	0.166±0.19 ^a	0.169±0.16 ^a	+ 2.1	0.166±0.12 ^a	0	0.166±0.14 ^a	0	0.20
Zn	19.87±1.24 ^c	23.04±1.27 ^c	+ 15.9	22.31±1.29 ^b	+ 12.3	23.1±1.32 ^c	+ 16.3	30
Hg	0.261±0.09 ^b	0.261±0.06 ^b	0	0.240±0.10 ^a	-7.9	0.274±0.09 ^c	+ 5.1	0.50

M±SE*: Mean±Standard error of heavy metal level in cooked fish; Means (in the same row) having different superscripts are significantly varied. MPL**: Maximum Permissible limit of heavy metal for human consumption of fish.

Table 4: Effect of freezing process and frozen storage (-18±2°C) on heavy metals level (ppm; on wet weight basis) of whole hammour fish

Heavy Metal	Frozen Fish Flesh			Change (%)	MPL**
	Fresh Prepared	Initial Zero time	After 6 months		
	M±SE*	M±SE*	M±SE*		
Fe	4.146 ±0.23 ^c	3.920±0.31 ^b	3.617±0.22 ^a	-12.75	--
Pb	2.732±0.29 ^a	2.630±0.27 ^a	2.517±0.16 ^a	-7.80	2
Cr	0.284±0.07 ^a	0.284±0.09 ^a	0.284±0.05 ^a	0	0.20
Cu	0.673±0.10 ^b	0.641±0.13 ^{a,b}	0.606±0.09 ^a	-10.29	20
Mn	2.520±0.27 ^c	2.348±0.30 ^b	2.120±0.22 ^a	-15.87	--
As	0.436±0.09 ^a	0.436±0.09 ^a	0.409±0.11 ^a	-6.14	0.20
Cd	0.039±0.002 ^b	0.039±0.003 ^b	ND±0.00 ^a	-100	0.05
Co	0.195±0.06 ^b	0.203±0.08 ^{a,b}	0.214±0.06 ^a	+ 9.74	0.5
Zn	21.82±1.17 ^c	20.65±1.22 ^b	19.08±1.13 ^a	-12.58	30
Hg	0.339±0.21 ^a	0.339±0.13 ^a	0.339±0.17 ^a	0	0.20

M±SE*: Mean±Standard error of heavy metal level; Means (in the same row) having different Superscripts are significantly varied. MPL**: Maximum Permissible limit of heavy metal for human consumption of fish.

20.9, 11.4 and 7.05 % respectively. While, the roasting process led to a significant (p<5) increase in Fe and Zn levels of fish fillets respectively

The obtained data (Table 3) also indicated that deep-fat frying method caused a significant (p<5) loss in Pb, Cr, Cu, Mn, As, Cd and Hg of hammour fish flesh by about 20.7, 16.4, 10.2, 12.8, 19.3, 100 and 7.9 %, while it caused a significant (p<5) increment in Fe and Zn by 13.1 and 12.3 % respectively. In addition that broiling cooking caused a significant (p<5) decrease in Pb, Cr, Mn and Cd by about 13.2, 8.2, 16.9 and 20.7 % of fish flesh respectively. On the contrary, it caused a significant (p<5) increase in Fe, Cu, Zn and Hg of fish flesh by about 9.1, 5.6, 16.3 and 5.1 % of fish fillets respectively. The predominant heavy metals in all cooked hammour fish samples were Zn (19.87-23.10 ppm) and Fe (9.275-9.747 ppm).

Effect of Freezing Process and Frozen Storage Temperature (-18±2°C) on Heavy Metals Level in Whole Hammour Fish: The Effect of freezing process on heavy metals level in whole hammour fish and the frozen storage (-18±2°C for 6 monthes) stability of these pollutants were illustrated as shown in Table 4,

As given in Table 4, the levels of most determined heavy metals in whole hammour fish did not significantly (p<5) influence by freezing process, with the exception of Fe (-5.45%), Mn (-6.83) and Zn (-8.80%) concentrations which were, somewhat, significantly (p<5) decreased. Furthermore, the most tested heavy metals in fish exhibited a highly stability during frozen storage for

6 months. On the other hand, the predominant metals in frozen fish were Zn (19.08 ppm), Fe (3.617 ppm), Pb (2.517 ppm) and Mn (2.120 ppm) at the end of tested frozen storage period

DISCUSSION

Knowledge of the proximate chemical composition of tested marine hammour fish samples is particularly important for determination of their nutritive value as well as for investigating the effects of the other treatments, cooking methods and processing for extents of pollution with variable contaminants. The present proximate analysis results indicated that all tested hammour fish samples had high protein contents and considerable levels of fat. This aspect is of dietary significance to the consumer since it is a known fact that protein is essential for maintaining and building muscles [3, 36]. The high fat content in tested hammour fish products is of nutritional value as a great amount of evidence from epidemiological studies and clinical trials in Spain supports a protective effect against coronary heart disease for fish consumption and intake of marine omega-3-fatty acids which reduce serum lipids and lipoproteins, impair platelet aggregation, increase cell membrane fluidity and lower blood pressure in humans [3,4]. The present results also have evidence that all tested cooking methods; i.e. roasting, pan-fat frying and broiling, caused a high loss in the moisture content of tested hammour fish samples as the result of evaporation by thermal treatments used in cooking processes. The other gross chemical components of fish

samples may be diluted or concentrated throughout cooking as a reflection of one or more of possible alterations including; the moisture loss, absorption of some gross components from cooking medium, the destruction and leaching out of some fish components throughout cooking process [22,32]. On the other hand, the gross chemical components in whole hammour fish were slightly effected by freezing process and frozen storage conditions (-18±2 C for 6 monthes), as previously reported [3, 37]

The present results also illustrated that all determined heavy metals were detected in the different hammour fish organs which were greatly varied in their ability for accumulation of the tested heavy metals depending upon fish organ and upon the heavy metal itself as reported in literature [21,26,38,39,40]. The present results also showed that hammour fish flesh contained the all determined heavy metals at lower concentration than the maximum permissible limits (MPLs) of these contaminants reported by codex alimentarius [41, 42] with the exception of Pb and As which were found at level of 2.105 and 0.218 ppm, on wet weight basis, higher than their MPLs being 2 and 0.2 ppm, respectively.

With regards to the effect of cooking processes on the tested pollutants, the cooking operations (i.e. roasting, deep-fat frying and broiling) exhibited vary effect on the tested heavy metals' level in marine hammour fish flesh depending upon the cooking method and the heavy metal itself. The reduction in heavy metals' content in the tested fish which was taken place throughout cooking process may be related to release of these metals with the loss of drip as free salts, possibly in association with soluble amino acids and uncoagulated proteins bounded with metals [43-45]. On the other hand, the increase in some heavy metals in cooked fish could be attributed to the loss of moisture throughout cooking that caused the concentration of some metals in fish muscles [32, 44, 45]. It is fortune to mention that all cooked hammour fish products, under investigation, were safe for human consumption as they contained all determined heavy metals within the MPLs reported by previously mentioned International Legislations.

Concerning the effect of freezing process and frozen storage conditions on heavy metals concentration in hammour fish, it could be observed that, freezing process did not significantly affect the concentrations of most determined heavy metals in whole marine hammour fish. Furthermore, the most tested heavy metals in fish exhibited a highly stability during frozen storage for 6 months. On the other hand, the predominant metals in

frozen fish were Zn (19.08 ppm), Fe (3.617 ppm), Pb (2.517 ppm) and Mn (2.120 ppm) at the end of tested frozen storage period. These results are in quite accordance with those previously reported [22, 44].

It could be concluded that regardless of the highly pollution of marine hammour fish with heavy metals, the cooked fish flesh products were safe for human consumption as they contained these pollutants within the maximum permissible limits (MPLs). It is recommended that we should do our best to overcome the problem of pollution the Arabian Gulf and the Red Sea water with these contaminants by continuous assessing and controlling on the pollution sources by specialized organization having all required instruments. Moreover, it is a devisable to remove inedible parts including viscera, brain and gills from the fish prior to cooking and processing and not to eat them to avoid potential risks to human health arising from the high toxicity and persistence of heavy metals.

REFERENCES

1. Damsgaard, C.T., L. Schack-Nielsen, K.F. Michaelsen, M.B. Fruekilde, O. Hels and L. Lauritzen, 2006. Fish oil affects blood pressure and the plasma lipid profile in healthy Danish infants. *J. Nutr.*, 136: 94-99.
2. Mayer, K., M.B. Schaefer and W. Seeger, 2006. Fish oil in the critically ill: From experimental to clinical data. *Curr. Opin. Clin. Nutr. Metab. Care*, 9: 140-148.
3. Musaiger, A.O. and R. D'Souza, 2008. Chemical composition of raw fish consumed in Bahrain. *Pakistan J. Biol. Sci.*, 11(1): 55-61.
4. Alonso, A., M.A. Martinez-Gonzalez. and M. Serrano-Martinez, 2003. Fish omega-3 fatty acids and risk of coronary heart disease. *Med. Clin.*, 121: 28-35.
5. Dahl, L., T. Bjorkkjaer, I.E. Graff, M.K. Malde and B. Klementsén, 2006. Fish-more than just omega 3. *Tidsskr. Nor. Laegeforen.*, 126: 309-311.
6. Mozaffarian, D., J.S. Gottdiener. and D.S. Siscovick, 2006. Intake of tuna or other broiled or baked fish versus fried fish and cardiac structure, function and hemodynamics. *Am. J. Cardiol.*, 97: 216-222.
7. Al-Jedah, J.H. and R.K. Robinson, 2001. Aspects of the safety of the fish caught off the Coast of Qatar. *Food Control*, 12: 549-552.
8. Burger, J., K.F. Gaines, C.S. Boring, J. Snodgrass, W.L. Jr. Stephens and M. Gochfeld, 2004. Effects of cooking on radio cesium in fish from the Savannah River: Exposure differences for the Public. *Arch. Environ. Contam. Toxicol.*, 46: 231-235.

9. De-Mora, S., S.W. Fowler, E. Wyse and S. Azemard, 2004. Distribution of heavy metals in marine bivalves, fish and coastal sediments in the Gulf and Gulf of Oman. *Marine Pollution Bulletin*, 49: 410-424.
10. Sadiq, M., T. Saeed and S.W. Flower, 2002. Seafood contamination, In: Khan, N.Y., Munawar, M., Price, A.R.G. (Eds.), *The Gulf Ecosystem: Health and Sustainability*. Bakhuy Publishers, Leiden, pp: 327-351.
11. Dua, A. and N. Gupta, 2005. Mercury toxicology as assessed through fish scales. *Bull. Environ. Contam. Toxicol.*, 74: 1105-1110.
12. Jarup, L., 2003. Hazards of heavy metal contamination. *Br. Med. Bull.*, 68: 167-182.
13. Boguszewska, A. and K. Pasternak, 2004. Mercury-influence on biochemical processes of the human organism. *Ann. Univ. Mariae Curie Sklodowska*, 59: 524-527.
14. Al-Saleh, I. and N. Shinwari, 2002. Preliminary report on the levels of elements in four fish species from the Arabian Gulf of Saudi Arabia. *Chemosphere*, 48: 749-755.
15. Al-Yousuf, M.H., M.S. El-Shahawi and S.M. Al-Ghais, 2000. Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex. *Sci. Total Environ.*, 256: 87-94.
16. UNEP, 1993. Guidelines for monitoring chemical contaminants in the sea using marine organisms (United Nations Environmental Programme). Reference methods for marine pollution studies, Report 6, Athens.
17. Edwards, J.W., K.S. Edyvane, V.A. Boxalls, M. Hamann and K.L. Soole, 2001. Metal levels in Seston and marine fish flesh near industrial and metropolitan centers in South Australia. *Marine Pollution Bulletin*, 42(5): 389-396.
18. Sivaperumal, P., T.V. Sankar and P.G. Viswanth-Nair, 2007. Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-à-vis international standards. *Food Chemistry*, 102: 612-620.
19. McCrea, R.C. and J.D. Fischer, 1986. Heavy metals and organ-chlorine contaminants in the five major Ontario Rivers of the Hudson Bay Lowland. *Poll. Res. J. Can.*, 21: 225-234.
20. El-Gamal, I.M., 1993. An environmental evaluation of industrial waste water as a source of irrigation. Proc. 3rd International Conf. on Environ. Protect. Is A Must, April 13-15. Alexandria, Egypt, pp: 207-219.
21. Qiao-qiao, H.I., Z.H.U. Guang-Wei and A. Langdon, 2007. Bioaccumulation of heavy metals in fishes from Taihu Lake, China. *J. Environ. Sci.*, 19: 1500-1504.
22. Rowayshed, G.H., A.M. Sharaf. and S.M. El-Sayed, 2002. Heavy metals pollution in boliti (*Oreochromis niloticus*) fish as affected by the common cooking and processing methods. *Annals of Agric. Sci.*, Moshtohor, 40(4): 2211-2222.
23. Watanabe, I. and S. Tanabe, 2003. Trace element accumulation in 20 species of fishes from Lake Baikal, Caspian Sea and Japanese coast water. *J. Environ. Chem.*, 13: 31-40.
24. Sankar, T.V., A.A. Zynudheen, R. Anandan and P.G. Viswanathan-Nair, 2006. Distribution of organochlorine pesticide and heavy metal residues in fish and shellfish from Colicut region, Kerala, India. *Chemosphere*, 65: 583-590.
25. Turkmen, A., M. Turkmen, Y. Tepe and I. Akyurt, 2005. Heavy metals in three commercially valuable fish species from Iskenderun Bay, North East Mediterranean Sea, Turkey. *Food Chemistry*, 91: 167-172.
26. Dural, M., M.Z.L. Goksu and A.A. Ozak, 2007. Investigation of heavy metal levels in economically important fish species captured from the Tuzal lagoon. *Food Chemistry*, 102: 415-421.
27. Baji, A.H., Z.B. Awang and M.S. Embong, 1986. Monitoring heavy metals contents of coastal water fishes in Malaysia Proc. Intl. Conf. Dev. Managt. Trop. Living Aquat. Resources, Serdang, Malaysia, 2-5 Aug, 1983, pp: 219-224.
28. Marzec, M.A. and R. Bulinski, 1996. Studies on some trace elements in Polish food products. XX11. Lead, cadmium, zinc and copper contents in canned fish. *Brometologiai-Chemia-TokyKologicza*, 29(4): 367-373.
29. FDA, 2001. Fish and Fisheries Products Hazards and Controls Guidance, third ed. Center for Food Safety and Applied Nutrition, US Food and Drug Administration.
30. Ellias, L.D., 1996. Chemical and toxicological studies on hazardous waste sites in Harris country. M.Sc. Thesis, Faculty of Science, Texas Solution Univ., USA.
31. Friberg, L., G.F. Norberg and V.B. Voak, 1986. Book on the toxixology of metals. E/sevier north Holand Biomedical Press, Amsterdam, pp: 320.
32. EPA, 2002. Environmental protection Agency. Risk Assessment: Technical Background Information. RBG table. Available from <http://www.epa.gov/reg3hwmd/risk/rbc1003.pdf>.

33. Mustafa, F.A. and D.M. Medeiros, 1985. Proximate composition, mineral content and fatty acids of catfish (*Ictalurus punctatus, Rofinesque*) for different seasons and cooking methods. J. Food Sci., 50: 585-588.
34. AOAC, 1995. Association of Official Analytical Chemists, Official Methods of Analysis. Metals and other elements at trace levels in foods. Published by AOAC International suite 400 2200 Wilson Boulevard Arlington, Virginia 22201-3301 USA.
35. Helwing, J.T., 1983. SAS introductory guide, Revised Edition SAS Institute INC. Cary, North Carolina, USA, 27500: 55-61.
36. Bonjour, J.P., 2005. Dietary protein. An essential nutrient for bone health. J. Am. Coll. Nutr., 24: 526S-536S.
37. Beklevik, G., A. Polat and F. Ozogul, 2005. Nutritional value of sea bass (*Dicentrarchus labrax*) fillets during frozen (-18°C) storage. Turk. J. Vet. Anim. Sci., 29: 891-895.
38. Ashraf, W., 2005. Accumulation of heavy metals in kidney and heart tissues of Epinephelus microdon fish from the Arabian Gulf. Environ. Monit. Assess, 101: 311-316.
39. Ploetz, D.M., B.E. Fitts and T.M. Rice, 2007. Different accumulation of heavy metals in muscle and liver of a marine fish, (*King Mackerel, Scomberomorus cavalla Cuvier*) from the northern Gulf of Mexico, USA, Bull. Environ. Contam. Toxicol., 78: 124-127.
40. Uysal, K., E. Emre and E. Kose, 2008. The determination of heavy metals accumulation ratios in muscle, skin and gills of some migratory fish species by inductively coupled plasma-optical emission spectrometry (ICP-OES) in Beymelek Lagoon (Antalya/ Turkey), Microchem. J., 90: 67-70.
41. IPCS/WHO, 1987. Environment health criteria for food. Report. UNEP.ILO and WHO. International Programme on chemical safety. WHO, Geneva, Switzerland, pp: 131.
42. FAO/WHO, 1989. Toxicological evaluation of certain food additives and committee thirty-third meeting of the joint FAO/WHO expert Cambridge University Press. Cambridge.
43. Atta, M.B., L.A. El-Sebaie, M.A. Noaman and H.E. Kassab, 1997. The effect of cooking on the content of heavy metals in fish (*Tilapia nilotica*). J. Food Chemistry, 58(1-2): 1-4.
44. Gokoglu, N., P. Yerlikaya and E. Cengiz, 2004. Effects of cooking methods on the proximate composition and mineral concentrations of rainbow trout (*Oncorhynchus mykiss*). Food Chemistry, 84: 19-22.
45. Ersoy, B., Y. Yanar, A. Kucukgulmez and M. Celik, 2005. Effects of four cooking methods on the heavy metal concentrations of sea bass fillets (*Dicentrarchus labrax Linne, 1785*). Food Chemistry, 99: 748-751.