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INCIDENCE OF HEAVY METALS RESIDUES IN SALTED AND SMOKED FISH PRODUCTS

(With 4 Tables)

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مدى تواجد بقايا المعادن الثقيلة فى منتجات الاسماك المملحة والمدخنة

ابراهيم على القويعى ، اميمة ابراهيم على ، اميمة عبد الفتاح صالح

أجريت هذه الدراسة لاستبيان مدى تواجد المعادن الثقيلة فى أسماك السردين المملحة وأسماك الرنجة المدخنة المعروضة للأستهلاك بأسواق السمك بمدينة دمنهور. وقد تم تجهيز العينات للقياس بواسطة جهاز الامتصاص الذرى الطيفى. وأوضحت النتائج تواجد عنصر الرصاص فى تلك المنتجات بنسبة ١٠٠٪ وبمتوسط ٢,٠٩٧ ±٠,٢٢٤ مجم/كجم وزن رطب فى أسماك السردين المملح و١,٩٢٩ ± ٠,٢١١مجم/كجم فى أسماك الرنجة المدخنة. بينما لم يتم اكتشاف الكادميوم فى جميع عينات أسماك السردين ما عدا عينة واحدة كانت قراءتها ٠,٠١٨ مجم/كجم وعلى العكس من ذلك وجد فى جميع أسماك الرنجة المدخنة بمتوسط٠,٢٠٣±٠,٠٤٤مجم/كجم. وكانت متوسطات عنصرى الحديد والزنك ٢٦,٥٨٣± ٣,٩٣١ و ١٥,٩٩٦± ١,٤٣٢ فى أسماك السردين المملحة و٢٧,٨١٠±٣,٦٨٦ و١١,٠٣٢± ٠,٦٥٨ مجم/كجم فى أسماك الرنجة المدخنةعلى التوالى. كما بينت الدراسة تواجد الرصاص فى جميع عينات المنتجات المفحوصة بنسب تتجاوز الحد المسموح به طبقا للمواصفة القياسية المصرية رقم ٢٣٦٠ لسنة ١٩٩٣ بينما جاوز٧٣,٣٪ من عينات أسماك الرنجة المدخنة فقط الحد المسموح به لعنصر الكادميوم. وكانت مستويات عنصرى الحديد والزنك فى الحدود المسموح بها عالميا ماعدا ١٣,٣٪ من كل هذه المنتجات جاوزت الحد المسموح به لعنصر الحديد. وقد تم مناقشة المخاطر الصحية أثر تناول منتجات غذائية تحتوى على تركيزات عالية من المعادن الثقيلة والتوصيات اللازمة للعمل على الإقلال من التلوث بها.

SUMMARY

A total of thirty samples of salted sardine and smoked herring fishes (15 of each) were randomely collected from fish markets in Damanhour city to estimate the concentrations of heavy metals using Flame Atomic Absorption Spectrophotometer. The obtained results showed that the mean values of lead in salted sardine and smoked herring were 2.097±0.224 and 1.929±0.211 mg/kg wet weight, respectively, with an incidence of 100% of each. While cadmium residue was detected only in one sample of salted sardine, with a reading of 0.018, but a mean value of 0.203±0.044 mg cadmium/kg was estimated in smoked herring with an incidence of 80%. Meanwhile, the means of iron and zinc elements were 26.583±3.931 and 15.996 ±1.432 mg/kg in salted sardine and were 27.81 ±3.686 and 11.032± 0.658mg/kg in smoked herring. The lead levels in all the examined samples of both fish products were above the permissible limit of Egyptian Organization of Standardization and Quality Control "EOSQC" (1993). Cadmium residues levels exceeded the permissible limit in 73.3% of smoked herring fish. The levels of both iron and zinc were within the permissible FAO/WHO guidelines except 13.3% of both fish products exceeded iron permissible limit. The health hazard of contaminated food products with heavy metals and the essential recommendations which should be taken were disccussed to safeguard the consumers.

Key words: *Fish, salted fish, smoked fish, heavy metals, lead, iron, zinc, cadmium.*

INTRODUCTION

Fish had long been regarded as a desirable and nutritional source of high quality protein and generous supply of minerals and vitamins. Smoked fish is widely consumed all over the world because of its high protein, low saturated fat, fat soluble vitamins, essential minerals and omega fatty acids known to support good health (Ikem and Egiebor, 2005) so, salted and smoked fishery products continues to occupy its important place as delicacy in the dishes of Egyptians.

Traditional fish products are usually produced by applying old preserving methods such as salting, fermenting, drying and smoking. These products are greatly varied amongst the countries as well as within the same country by using many different applications such as differences in additives, percentage of salt or vinger and maturing temperatures. Although such processing/preserving methods have been known as still old techniques for many decades, they still have wide acceptance around the world because of their specific taste and aroma.

Smoking process is considered as a sequal to curing, the result is a dry, golden brown surface which is imperative, besides, the wood smokes that are deposited on the fish products, inhibit the development of spoilage microorganisms and act as antioxidant (Fretheim *et al.*, 1980). The polluted salted and smoked fish constitute a consumer's health risk in Egypt especially in certain national occasions as feasts and Sham El-Neseem because a lot of these products are prepared by old traditional methods and consumed as ready-to-eat food. Salted sardine is produced by salting sardine fishes for a relatively long period with unrefined table salt (estimated as sodium chloride, 15-22%) fulfilled of impurities that included heavy metals to reduce the cost (Salah El-Dien *et al.*, 2005). Smoke was carried some heavy metals which may adsorped on smoked fish. Moreover salted and smoked fishes may be exposed to heavy metals pollutions during their sea-life before catching and processing (Küçüksezgin *et al.*, 2006). Heavy metals have been considered as dangerous substances causing a serious health hazard to human and animals, through progressive accumulation in their bodies as a result of a repeated consumption of small amount of these elements (Igado *et al.*, 2008). Among possible humans target organs of heavy metals, soft tissues such as the kidneys, liver and central nervous system appear to be especially sensitive (Apostoli, 2002). Studies of heavy elements in sardines (or herring) have been limited chiefly to fresh specimens from the Mediterranean (Canli and Atli, 2003; Falco *et al.*, 2006) and the northeast Atlantic (Food Standards Agency "FSA", 2005) and to canned products.

Therefore, it was important and necessary to conduct this study to determine the concentrations of some heavy metals in traditionally locally made salted sardine and smoked herring fishes and comparing the levels of pollution with the national and international permissible limits.

MATERIALS and METHODS

A. Collection of samples:

Thirty random fish products samples (15 each of salted sardine and smoked herring fishes) were collected at the consumer level from different fish markets of Damanhour city in El-Boheira governorate and each sample was wrapped in a light polyethylene bag and was placed in an ice box and was taken to the laboratory without delay.

B. Preparation of collected samples:

The collected fish products were washed with tape water several times and then with de-ionized water. The sample of each fish product was taken from the muscles below the dorsal fin and at the middle line, then was homogenized and was kept at fresh polyethylene bags at -20°C until analysis. Each weighted sample was transferred into a clean and acid washed screw-capped glass digestion tubes. All digestion tubes were identified for chemical analysis.

C. Digestion procedure:

Each prepared sample of fish products was digested according to Al-Ghrais (1995) by addition of 10 mls of nitric and perchloric acids mixture (4vol:1vol). Initial digestion was performed at room temperature for 4 hours followed by careful heating at 40-45°C for one hour in a water bath, then the temperature was raised to75°C with gentle shaking untill digestion was completed within 2-3 hours. The digest was allowed to cool at room temperature then diluted up to 25 mls with deionized water, then filtered through whatman's filter paper No.1. Blank solution was prepared to check the possible traces of heavy metals that may be present in the acids and deionized water which were used in the digestion and dilution of the samples. Lead, cadmium, iron and zinc levels were determined in all the examined samples by using Flame Atomic Absorpance Spectrophotometer (UNICAM 969 A.A. Spectrophotometer) in Toxicology Unit of the Animal Health Research Institute, Ministry of Agriculture, Dokki, Giza, Egypt.

Working condition for heavy metals analysis by atomic absorption spectrophotometer

Metal	Lamp wave Length (nm)	Slit width (nm)	Lamp Current (mA)	Fuel flow Rate (l/min.)	Burner Height (cm)	Detection limit
Lead (Pb)	217	0.7	12	30	8	0.02 ppm
Cadmium (Cd)	228.8	0.7	5	30	8	0.0006 ppm
Iron (Fe)	248.3	0.2	25	30	8	0.05ppm
Zinc (Zn)	213.9	0.7	10	30	8	0.002ppm

D. Quantitive determination of heavy metals in the examined samples:

The concentrations of lead (Pb), cadmium (Cd), iron (Fe) and zinc (Zn) in the examined samples were calculated according to the following equation: C=R x D/W
Where: C =Concentration of heavy metals (mg/kg) wet weight.
R=Reading of element concentration on digital scale of AAS.
D=Final volume of prepared sample in mls.
W=Weight of the wet sample.

The reading of absorbance values of Pb, Cd, iron and zinc concentrations were recorded and the results of analysed samples were calculated.

The obtained data in this study were statistically analysed according to the method of Petric and Waston (2006).

RESULTS

Table 1: Level of heavy metal concentrations (mg/kg, wet weight) in the examined samples of salted sardine fishes (n=15)

Element	Min.	Max.	Mean	S.E.
Lead (Pb)	0.633	3.823	2.097	0.224
Cadmium (Cd)	ND	0.018	0.001	0.001
Iron (Fe)	12.973	68.78	26.583	3.931
Zinc (Zn)	8.713	25.063	15.996	1.432

Min.:minmum Max.:maximum S.E.:standard error of mean
mg/kg=µg/g =ppm ND : not detected=14 samples"93.3%"

Table 2: Level of heavy metal concentrations (mg/kg,wet weight) in the examined samples of smoked herring fishes (n=15)

Element	Min	Max	Mean	S.E.
Lead (Pb)	0.488	3.57	1.929	0.211
Cadmium (Cd)	ND	0.653	0.203	0.044
Iron (Fe)	14.59	65.62	27.810	3.687
Zinc (Zn)	6.858	16.45	11.032	0.658

ND= 3 samples "20%"

Table 3: Comparison of the estimated heavy metals residues in the examined fish products with the recommended national and international permissible limits (n=15).

Element	Permissible Limit (PL) mg/kg	Salted Sardine				Smoked Herring			
		Within PL		Over PL		Within PL		Over PL	
		No	%	No	%	No	%	No	%
Lead (Pb)	0.3[a]	0	0	15	100	0	0	15	100
	0.2[b]	0	0	15	100	0	0	15	100
	0.1[c]	0	0	15	100	0	0	15	100
Cadmium (Cd)	0.1[c]	15	100	0	0	4	26.7	11	73.3
	0.05[b]	15	100	0	0	3	20	12	80
Iron(Fe)	40[d]	13	86.7	2	13.3	13	86.7	2	13.3
Zinc(Zn)	50[e]	15	100	0	0	15	100	0	0

[a]Codex (2008).
[b]Commission of the European Communities (2001).
[c]Egyptian Organization for Standardization and Quality Control (E.O.S.Q.C.), Egyptian Specification Standard"E.S.S." No.2360 (1993).
[d]Institute of Medicine (2003).
[e]FAO(1983) & MAFF (1995).

Table 4: Estimated daily and weekly intakes by adults consuming fishery products

Element	Provisional Permissible Tolerable Weekly Intakes (PTWI)[a] (µg/kg body weight)	Permissible Tolerable Intakes (PTI)/day "µg/day/60kg b.w."(PTDI,µg/kg b.w.)[b]	Salted Sardine		Smoked Herring	
			EWI(EDI)[c] (µg/day/60 kg b.w.)	EDI µg/kg, b.w.	EWI (EDI)[c] (µg/day/60kg b.w.)	EDI µg/kg, b.w.
Pb	25	214.3(3.57)	712.98 (101.85)	1.7	655.86 (93.69)	1.56
Cd	7	60(1.0)	0.34 (0.0457)	0.0008	69.02 (9.86)	0.164
Fe	5600	48000(800.0)	9038.22 (1291.14)	21.52	9455.4 (1350.77)	22.513
Zn	7000	60000(1000.0)	5438.64 (776.95)	12.95	3750.88 (535.84)	8.93

[a]Joint FAO/WHO Expert Committee on Food Additives(2004).
[b]Values in parenthesis represent permissible tolerable daily intakes(PTDI) measured by micrograms to every kilogram body weight.
[c]EWI:estimated weekly intakes from consumption of 340grams of fish every week by adult pearson of 60 kg body weight according to *US EPA(2004)*. Values in parenthesis represent estimated daily intakes (EDI) by adult person. - µg:microgram =0.001 milligram (mg). b.w.: body weight.

DISCUSSION

Lead (Pb): Short-term exposure to high levels of lead can cause brain damage, paralysis (lead palsy), anaemia and gastrointestinal symptoms, while longer-term exposure can damage the kidneys, reproductive, immune, nervous systems. The most critical effect of low-level of lead exposure is on intellectual development in young children, also, lead crosses the placental barrier and accumulates in the featus. Infants and young children are more vulnerable than adults to the toxic effects of lead where it absorped more readily. Consumption of food containing lead is the major source for the general population (Food Safety Autherity of Ireland, 2009).

The results obtained in Tables 1 and 2 indicated that lead concenteation levels in salted sardine and smoked fishes ranged from 0.633 to3.823 and 0.488 to 3.57 with means±standard error"S.E."values of 2.097±0.224 and 1.929±0.211mg/kg,wet weight, with 100% incidence of each, respectively.

Nearly similar results in salted fish in Egypt were reported by Nassar *et al.* (1996); Zaki (1998). Salah El-Dien *et al.* (2005) examined sixty salted fish (Feseakh) samples collected from different markets in Sharkia governorate, Egypt and recorded that the lead levels ranged from 1.45 to 48.59 with a mean value ±S.E. of 5.951±1.1051 ppm, wet weight. In addition, all the examined samples exceeded the Codex Alimentarius Commission limit (o.5 ppm) (FAO/WHO, 1992).

These results were higher than that previously reported by Sallam and El-Gazzar (1997). Daoud-Jehan and Abd EL-Aziz (2002) examined 60 ready-to-eat fish products samples, 30 from each of salted sardine and smoked herring of various sizes which were purchased from Alexandria governorate. Lead levels ranged from 0.041 to 0.723 and 0.03 to 0.371, with mean values of 0.275 ± 0.044 and 0.145 ± 0.017 ppm, wet weight and the percentage of samples exceeding the permissible limit (o.1 mg/kg according to EOSQC, Egyptian Standard Specification "E.S.S." No.2360, 1993) in salted sardine and smoked herring were 46.67% and 36.67%, respectively. In Finland, lower lead levels of 4.0 to 177.0 ppb were detected in salted fish (Tahvonen and Kumpulainen, 1996).

The results in Table 3 pointed that lead levels in all the examined samples were exceeding the permissible limit "0.1mg/kg" of EOSQC, No.2360 (1993). Also, these results were above the Codex (2008) guideline of 0.3mg/kg for lead element in fish and of 0.2 mg/kg set by the Commission of the European Communities (2001). These results were consistent with that of Salah El-Dien *et al.* (2005). Adekunle and Akinyemi (2004) reported elevated lead levels in street vended smoked fish in Nigerian markets, moreover, they stated that the food processing technique accounted for up to seven times increase in fish lead level. Since the United States Environmental Protection Agency (EPA, 1999) has never established a reference dose (RfD), a definitive level cannot be asserted for Pb in fish. The advisory limit would likely be 1 ppm or greater. Hodson *et al.* (1984) indicated that the Canadian Pb limit of 10 ppm was discontinued, but that the British limit remain at 2 ppm in fish, fish paste and canned fish (5 ppm in fried and salted fish). Abou-Arab *et al.* (1996) indicated that the FAO limit (1983) was 2.0 ppm.

Cadmium (Cd) is a cumulative toxicant that affects kidneys, bone metabolism, the reproductive tract and it is also carcinogenic and an endocrine disruptor. The kidney is the critical organ in humans and other mammals exposed for long periods to the relatively small amounts of Cd that might occur in foods (Satarug and Moore, 2004). Several epidemiological studies in human populations correlated the exposure to Cd with high blood pressure and cardiovascular disease (Telisman *et al.*, 2001).

The results illustrated in Tables 1 and 2 showed only one sample of salted sardine (6.7%) contained cadmium concentration of 0.018mg/kg and the remaining 93.3% were below the detectable limit of A.A.S. while in smoked herring samples it ranged from 0.10 to 0.653 with a mean value ± S.E. of 0.203±0.044 mg/kg, wet weight, with an incidence of 80%.

High cadmium levels detected in salted sardine and smoked fishes were recorded by Nassar *et al.* (1996); Sallam and El-Gassar (1997), Zaki (1998) and in salted fish "feseekh" by Salah El-Dien *et al.* (2005). Daoud-Jehan and Abd El-Aziz (2002) pointed out that Cd concentration in the examined salted sardine and smoked herring samples ranged from 0.04 to 0.129 and from 0.02 to 0.098 with mean values ± S.E.of 0.081±0.003 and 0.07±0.003 ppm wet weight, respectively. However, lower Cd residues in salted fish were reported by Tahvonen and Kumpulainen (1996) in Finland (9 - 42 µg/kg).

The results achieved in Table 3 showed that the Cd concentrations in all the examined salted sardine samples were below the permissible limit (0.1ppm) while 73.3% of smoked herring fishes were above it according to EOSQC, No.2360 (1993).

El-Tahan *et al.* (1999) found that Cd and Pb levels in the examined samples of salted fish (feseekh, melouha and sardine) purchased from Cairo markets were higher than the permissible level. In Egypt, Abou-Arab *et al.* (1996) reported Pb and Cd residues in whole imported sardines were 11.1 and 0.086 and in mackerel were 12.6 and 0.077 ppm, respectively.

The studied salted sardine samples were compared with the fresh samples of Moracean true sardine "Sardina pilchardus" analysed by Falco *et al.* (2006), their mean Pb was higher (2.097 vs 0.04µg/g), while mean concentration of Cd was about fourty times lower (0.001 vs 0.04µg/g).

The dietary Cd absorption rate in humans has been estimated at 5% (WHO, 1989 and IPCS, 1992) of its total intake. Acute toxicity caused by Cd contaminated food is very unusal but chronic exposure may be frequent (Satarug *et al.*, 2004).

Data reported in Table 4 were on a daily basis and for a 60 kg pearson. The United States Environmental Protection Agency (US EPA, 2004) has recommended weekly consumption of 12 ounces (approx.340g). Children and pregnant mothers are mostly at risk from exposure to toxic metals, so they are advised to strictly follow the recommendation of the US EPA minimum serving of 6 ounces (170g) of fish/week (US EPA, 2004). For Pb EDI, values of 1.7 and 1.56 µg Pb/kg of body weight which were estimated from consumption of salted sardine and smoked herring, respectively, were found below the maximum permissible daily intake proposed by WHO (3.57 µg of Pb/kg b.w.). For Cd, the intakes were found to be 0.0008 and 0.164 µg Pb/kg of body weight,also far below the WHO provisional daily intake level of 1 µg of Cd/kg of b.w.

Iron (Fe): It was reported that iron deficiency is the most wide-spread nutritional disorder, and that fish has the potential to contribute to food-based strategies to reduce the risk of iron deficiency anaemia (Roos *et al.*, 2007). One hundred gram portion of fish may provide almost 20% of daily Fe requirement of human. Therefore, both fish may be considered a good supplement source of Fe which performs several vital functions in human body. For example, it serves as a carrier of oxygen from the lungs to the tissues by red blood cells. It also helps to prevent some major health problems (Camara *et al.*, 2005). Also, iron has been shown to be important in the brain as impaired acquisition leads to neurological problems (Connor *et al.*, 2003).

Tables 1 and 2 showed that the iron concentrations in salted sardine and smoked herring ranged from 12.973 to 68.78 and from 14.59 to 65.62, with mean values ±S.E. of 26.583± 3.931 and 27.81±3.687 mg/kg, respectively.

The upper tolerable intake level (UL) of iron in children (0 months-8 years) and males/females (14-70years) is 40 and 45 mg/day, respectively. The recommended dietary allowance (RDA) of iron for 7-12 months infants and males/females 51-70 years is 11 and 8mg/day, respectively (Institute of Mediciene, 2003). In another study, Fe supplied in the diet must be in the range of 15 mg/day in order to meet daily requirement (Belitz *et al.*, 2004).

Results obtained in Tables 3 and 4 pointed to that 86.6% of the examined salted sardine and smoked herring samples were compatible with UL of iron. The estimated daily intakes 21.52 and 22.513 µg/kg b.w. were also below the permissible tolerable intake (800 µg/kg b.w.) stipulated by Joint FAO/WHO Export Committee on Food Additives, 2004.

The metal transporter protein Nramp 2, known also as DMT1 seems to be involved in Cd absorption (Tallkvist *et al.,* 2001). Increased expression of intestinal DMT1 has been found in iron deficiency and hemochromatosis. Increased expression of DMT1, in general, would provide individuals with greater capacity to absorp Fe and possibly Cd. This explains a 3.4-fold increase in Cd body burden in women with low Fe stores (Satarug *et al.,* 2004) and in individuals with low body Fe stores (Olsson *et al.,* 2002). Therefore, special attention should be given to ensure adequate Fe intakes to reduce Cd absorption, because iron deficiency is the most common nutritional disease in the world affecting around 2 billion people (Satarug and Moore, 2004).

Zinc (Zn) is an essential element and micronutrient required for normal growth by both plants and animals and contributes to the development and maintenance of the thymus gland. Zinc plays its role as an integral part of a number of metalloenzymes and as a catalyst for regulating the activity of specific zinc dependant enzymes such as carbonic anhydrase,alkaline phosphatase and alcohol hydrogenase (Moore and Ramamoorthy, 1984) DNA and RNA polymerases (Hayashi *et al.,* 2001).

Tables 1 and 2 revealed that the concentrations of zinc in salted sardine and smoked herring fishes were ranged from 8.713 to 25.063 and from 6.858 to 16.45 with mean values ±S.E.of 15.996±1.432 and 11.032±0.658 mg/kg wet weight, respectively. Also, the incidence of detection of zinc in each fish product was 100%. These levels were low in comparison with those in previous studies on salted fish in Egypt (Nassar *et al.,* 1996 and Zaki, 1998). Higher results of zinc level in salted fish (Feseekh) obtained by Salah El-Dien *et al. (2005)* were ranged from 20.68 to 125.92 ppm, with a mean value ± S.E.of 35.845 ± 2.6007 ppm, wet weight.

The results in Table 3 recorded that the zinc levels in examined samples not exceeded the maximum permissible limit (50 mg/kg, wet weight) proposed by FAO (1983) and MAFF (1995). Also, the estimated weekly and daily intakes were below PTI stipulated by Joint FAO/WHO Export Committee on Food Additives (2004), as shown in Table 4. The too little consumed zinc can cause problems, but also too much is harmful to human health (Agency for Toxic Substances and Disease Registry, 2004). The upper tolerable intake levels of zinc for children (1-3 years old) and males/females (19-70 years old) were 0.2 and 1.0 mg/day, respectively (Institute of Medicine, 2003).

Ako and Salihu (2004) cleared that the smoking as a method of fish preservation may raise the mineral composition of the product to levels that are either beneficial or toxic to humans.

Therefore, analytical data obtained from this study shows that there is health risks from consumption of locally produced smoked herring which were contaminated with high figures of Pb and Cd whereas salted sardine were found contaminated with high Pb level Table 3. Both low-risk group (adolescents and adults) and high-risk group (pregnant mothers and children) should consume fish in moderation since large consumption pattern especially for fishery products may increased health risks.

The finding of this study call for the need to standardize procedures and methods to enhance the quality of this fishery products which must be assessed not only on the basis of physical properties as texture,odour and flavour, but also by the mineral compostion. The method of smoking and duration of exposure to the smoke have been identified as an important factors that affect product quality and acceptability (Indranesa *et al.*, 2000). The crude salt used for salting this products may be contained a fraction of minerals of seawater and must be refined. Globally, further reduction in the levels of environmental contaminants emanating from power plants and other industrial emissions and effluent discharges are highly recommended to reduce contaminant inputs into aquatic environment.

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