Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

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Original Paper

Chemical residues in ready to eat fish products

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ARTICLE INFO

Keywords

Aldrin

Cadmium

Lead

Malathion

Mercury

Received 05/10/2020

Accepted 17/10/2020

Available On-Line 20/01/2021

ABSTRACT

A total of one hundred random samples of ready to eat (RTE) fish products represented by fried Oreochromis niloticus, grilled Oreochromis niloticus, smoked Herring and salted Sardine (25 of each) were collected from different fish markets and restaurants in Menofia governorate, Egypt. The aim of this study was to estimate the concentration levels of chemical residues such as heavy metals residues (mercury, lead and cadmium) and pesticides residues (aldrin and malathion) through subjection the examined samples to atomic absorption spectrophotometer for detecting the residues of mercury, lead and cadmium and gas chromatography for the residues of aldrin and malathion. The mean values of mercury (mg/Kg) in fried O. niloticus, grilled O. niloticus, smoked herring and salted sardine were 0.43±0.01, 0.59±0.01, 0.70±0.01 and 0.94±0.02, respectively. The mean values of lead (mg/Kg) in fried O. niloticus, grilled O. niloticus, smoked herring and salted sardine were 0.27±0.01, 0.33±0.01, 0.45±0.01 and 0.61±0.01, respectively. The mean values of cadmium (mg/Kg) in fried O. niloticus, grilled O. niloticus, smoked herring and salted sardine were 0.11±0.01, 0.14±0.01, 0.20±0.01 and 0.29±0.01, respectively. The mean values of aldrin (ppb) only detected in fried O. niloticus, grilled O. niloticus and salted sardine were 82.95±4.19, 151.26±7.40 and 193.02±8.81, respectively. The mean values of malathion (ppb) only detected in grilled O. niloticus and salted sardine were 96.57 and 248.19±10.32, respectively. As conclusion, chemical examination of RTE fish products is a sensitive indicator verifying the quality and good hygienic status of RTE fish products.

1. INTRODUCTION

Ready to eat fish products have been recognized as nutritional source due to their high-quality proteins and unsaturated fatty acids especially, omega-3 which are regarded as preventive compounds from many diseases as coronary heart diseases, fatty liver and cancer (Payap, 2011). Unfortunately, during the last few decades the aquatic environment had exposed to chemical pollution by heavy metals and this became as a one of the most serious problems in the world. Untreated municipal, industrial and agricultural wastes, coal and oil combustion and phosphate fertilizers are the primary sources of heavy metal pollution of fish, especially in areas close to industrial and agricultural activities (Jarup, 2003). Fish are more sensitive to heavy metals because of their ability to accumulate them in their tissues by absorption along the gill surface and skin to higher levels several hundred times more than the concentration of metals in their surrounding water medium. Lead, mercury and cadmium are among the most dangerous heavy metals as they can be very harmful even at low concentrations when ingested over a long time periodor when their intake is excessive (Celik and Oehlenschager, 2007). Lead is highly toxic substance which accumulates in body due to its low rate of elimination. When lead levels in fish rise over the permissible limits, chronic lead toxicity occurs resulting in encephalopathy, anemia and renal tubular dysfunction (Andreji et al. 2005). Mercury is highly toxic compound, as it is fat soluble and easily absorbed and accumulates in erythrocytes and CNS leading to mercury poisoning symptoms including incoordination, ataxia, memory loss and progressive intellectual dullness (Castoldi et al. 2003). Cadmium is highly carcinogenic substances as, chronic exposure to cadmium leads to heart diseases, anemia, chronic renal failure and liver diseases (Alonso et al. 2002). On the other side, the widespread use of pesticides has created potential toxic hazards for aquatic life. These chemicals can enter and contaminate water through direct application, runoff and atmospheric deposition leading to poisoning of fish and threaten the human health due to their cumulative nature resulting in cancer, renal failure and neuropathy of nervous system. Organophosphorus pesticides (malathion) are less persistent than organochlorine type (aldrin) and these compounds have short persistence and could change into metabolites which are mostly more toxic than the original compounds (Bedi et al. 2007). The toxicity of aldrin may be acute or chronic; the chronic effect leads to some critical diseases such as cancer, neurological damage, endocrine disruption and birth defects. Exposure to high amounts of malathion in the contaminated RTE fish products may cause difficulty in breathing, chest tightness, cramps, watery eyes, blurred vision, loss of

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consciousness and death (ATSDR, 2003). For these reasons, chemical hazards such as heavy metals residues (mercury, lead and cadmium) and pesticides residues (aldrin and malathion) in fish has become an important worldwide concern because of the health risks associated with RTE fish products consumption by consumers. Consequently, all necessary sanitary measures and recommendations should be taken to control the contamination of RTE fish products with such harmful chemical hazards (Begum et al., 2013).

2. MATERIAL AND METHODS

2.1. Collection of samples:

A grand total of one hundred random samples of ready to eat fish products represented by fried *O. niloticus*, grilled *O. niloticus*, smoked Herring and salted Sardine (25 of each) were collected from different fish markets and restaurants in Menofia governorate, Egypt. The collected samples were transferred in an ice box to the laboratory under aseptic conditions without undue delay. All collected samples were analyzed by Atomic Absorption Spectrophotometer for estimation of heavy metals concentrations (mercury, lead and cadmium) and by Gas chromatography for estimation of pesticides concentrations (aldrin and malathion).

2.2. Determination of heavy metals:

2.2.1. Washing procedures (AOAC, 2006)

2.2.2. Digestion technique (Voegborlo and Akagi, 2007)

2.2.3. Preparation of blank and standard solutions (Shibamoto and Bjeldanes, 2000).

2.2.4. Analysis: By Atomic Absorption Spectrophotometer (AAS) (VARIAN, Australia, model AA240 FS).

2.2.5. Quantitative determination of heavy metal residues:

Mercury absorbency was recorded directly from the digital scale of AAS and its concentration was calculated according to then following equation:

$C_1 = (A_1 / A_2) \times C \times (D/W)$ mg/kg

Where, C_1=concentration of mercury (mg/kg) wet weight. A_1=Absorbency reading of sample solution. A_2= Absorbency reading of standard solution. C=Concentration of mercury on the standard solution. D=Dilution factor of sample and W= Weight of each sample.

While the concentration of lead and cadmium was estimated according to the following equation: $C = R \times (D/W)$

Where, C=concentration of lead (mg/kg) wet weight. R=reading of digital scale of AAS. D= Dilution of prepared sample and W= Weight of the sample.

2.3. Determination of pesticides:

2.3.1. Determination of aldrin as organochlorine pesticide:

The procedures for estimation of aldrin in the examined samples were applied using Gas chromatography (GC) according to Heck et al. (2007) as follow:

2.3.1.1. Extraction

2.3.1.2. Clean up with florisil method (Darko and Acquaah, 2008)

Finally, the concentrations of the aldrin residues (ppb) were calculated as follows:

Aldrin concentration= S/ W (ppb)

Where, S= Concentration of standard solution (ppb) in corresponding to the spot of reference pesticide standard. W= Weight of the tested sample.

2.3.2. Determination of malathion as organophosphorus pesticide:

2.3.2.1. Extraction and purification (Bonsir et al. 2007)

2.3.2.2. Partitioning (AOAC, 2006)

2.3.2.3. Clean-up (Serrano et al. 2008)

2.3.2.4. Preparation of stock standards

2.3.2.5. Preparation of chromatographic working standards

2.3.2.6. Preparation of the extracted sample

2.3.2.7. Chromatography: By High Performance Liquid Chromatography (HPLC).

Finally, Quantitative determinations of malathion were carried out in accordance the following formula:

$$Log W = \text{Log ws} \frac{A - As}{Ao - A} Log D$$

Where W= Amount of the analyzed material, Ws= Amount of standard applied, As= Spot area of the standard, Ao= Spot area of the diluted analyzed material, and D= Dilution factor

2.4. Statistical Analysis:

The evaluation and interpretation of obtained results were statistically carried out according to Feldman et al. (2003).

3. RESULTS

Mercury

The results in table (1) revealed that, the incidence of mercury (mg/kg) in the examined samples were 55 (55%) samples represented as: 11(44%) fried *O. niloticus* with average of 0.43±0.01mg/kg; 12(48%) grilled *O. niloticus* with average of 0.59±0.01mg/kg; 14 (56%) smoked herring with average of 0.70±0.01mg/kg and 18(72%) salted sardine with average of 0.94±0.02mg/kg. The acceptability of examined samples was shown in table(1) as 67accepted samples represented as 20(80%), 18(72%), 17(68%) and 12(48%) from fried *O. niloticus*, grilled *O. niloticus*, smoked herring and salted sardine, respectively. The acceptability was according to the permissible limits of mercury (0.5mg/kg) recommended by Egyptian Standards "E. S" (2010).

Table 1 Statistical analytical results of mercury levels (mg/Kg) in the examined samples of ready to eat fish products and their acceptability (n=25).

Fish products	Mercury incidence		Mean ±S.E	Acceptability * Accepted samples	
	No. of +ve samples	%. of +ve samples		No.	%
Fried *O. niloticus*	11	44	0.43 ± 0.01	20	80
Grilled *O. niloticus*	12	48	0.59 ± 0.01	18	72
Smoked Herring	14	56	0.70 ± 0.01	17	68
Salted Sardine	18	72	0.94 ± 0.02	12	48
Total	55	55		67	67

*Egyptian Standards "E.S" (2010). Maximum Permissible Limit of Mercury according to "E.S" (2010) is 0.5mg/Kg.

Lead

The results in table (2) revealed that, the prevalence of lead (mg/kg) in the examined samples were 47(47%) samples represented as: 10(40%) fried *O. niloticus* with average of 0.27±0.01mg/kg; 10(40%) grilled *O. niloticus* with average of 0.33±0.01mg/kg; 12(48%) smoked herring with average of 0.45±0.01mg/kg and 15(60%) salted sardine with average of 0.61±0.01mg/kg. The acceptability of the examined samples was shown in table(2) as 74accepted samples represented as 21(84%), 20(80%), 18(72%) and 15(60%) from fried *O. niloticus*, grilled *O. niloticus*, smoked herring and salted sardine, respectively. The acceptability was according to the permissible limits of lead (0.1mg/kg) recommended by Egyptian Standards "E. S" (2010).

Table 2 Statistical analytical results of lead levels (mg/Kg) in the examined samples of ready to eat fish products and their acceptability (n=25).

Fish products	Lead incidence		Mean ±S.E	Acceptability * Accepted samples	
	No. of +ve samples	%. Of +ve samples		No.	%
Fried *O. niloticus*	10	40	0.27 ± 0.01	21	84
Grilled *O. niloticus*	10	40	0.33 ± 0.01	20	80
Smoked Herring	12	48	0.45 ± 0.01	18	72
Salted Sardine	15	60	0.61 ± 0.01	15	60
Total	47	47		74	74

*Egyptian Standards "E.S" (2010). Maximum Permissible Limit of Lead according to "E.S" (2010) is 0.1mg/Kg

Cadmium

The results in table (3) revealed that, the prevalence of cadmium (mg/kg) the examined samples were 36 (36%) samples represented as: 7(28%) fried *O. niloticus* with average of 0.11±0.01mg/kg; 8(32%) grilled *O. niloticus* with average of 0.14±0.01mg/kg; 10(40%) smoked herring with average of 0.20±0.01mg/kg and 11(44%) salted sardine with average of 0.29±0.01mg/kg. The acceptability of examined samples was shown in table (3) as 77accepted samples were represented as 21(84%), 21(84%), 19(76%) and 16(64%) from fried *O. niloticus*, grilled *O. niloticus*, smoked herring and salted sardine, respectively. The acceptability was according to the permissible limits of cadmium (0.05mg/kg) recommended by Egyptian Standards "E. S" (2010).

Table 3 Statistical analytical results of cadmium levels (mg/Kg) in the examined samples of ready to eat fish products and their acceptability (n=25).

Fish products	Cadmium incidence		Mean ±S.E	Acceptability * Accepted samples	
	No. of +ve samples	%. of +ve samples		No.	%
Fried *O. niloticus*	7	28	0.11 ± 0.01	21	84
Grilled *O. niloticus*	8	32	0.14 ± 0.01	21	84
Smoked Herring	10	40	0.20 ± 0.01	19	76
Salted Sardine	11	44	0.29 ± 0.01	16	64
Total	36	36		77	77

*Egyptian Standards "E.S" (2010). Maximum Permissible Limit of Cadmium according to "E.S" (2010) is 0.05mg/Kg

Aldrin as organochlorine pesticide

The results in table (4) revealed that, the prevalence of aldrin (ppb) in the examined samples were 7(7%) samples represented as: 2(8%) fried *O. niloticus* with average of 82.95±4.19ppb; 2(8%) grilled *O. niloticus* with average of 151.26±7.40ppb and 3(12%) salted sardine with average of 193.02±8.81ppb. While all the examined samples of smoked Herring were free from aldrin. The acceptability of examined samples was shown in table (4) as 98accepted samples represented as 25(100%), 24(96%), 25(100%) and 24(96%) from fried *O. niloticus*, grilled *O. niloticus*, smoked herring and salted sardine, respectively. The acceptability was according to the permissible limits of aldrin (200ppb) recommended by Egyptian Standards "E. S" (2010).

Malathion as organophosphorus pesticide

The results in table (5) revealed that, the prevalence of malathion (ppb) in the examined samples were 3 (3%) samples represented as: 1(4%) grilled *O. niloticus* with average of 96.57ppb and 2(8%) salted sardine with average of 248.19±10.32ppb. While all the examined samples of fried *O. niloticus* and smoked Herring were free from malathion. The acceptability of examined samples was shown in table (5) as 99 accepted samples represented as 25(100%), 25(100%), 25(100%) and 24(96%) from fried *O. niloticus*, grilled *O. niloticus*, smoked herring and salted sardine, respectively. The acceptability was according to the permissible limits of malathion (200ppb) recommended by Egyptian Standards "E. S" (2010).

Table 4 Statistical analytical results of aldrin levels (ppb) as organochlorine pesticide in the examined samples of ready to eat fish products and their acceptability (n=25).

Fish products	Aldrin incidence		Mean ±S.E	Acceptability * Accepted samples	
	No. of +ve samples	%. of +ve samples		No.	%
Fried *O. niloticus*	2	8	82.95 ± 4.19	25	100
Grilled *O. niloticus*	2	8	151.26 ±7.40	24	96
Smoked Herring	Free	Free	Free	25	100
Salted Sardine	3	12	193.02 ±8.81	24	96
Total	7	7		98	98

*Egyptian Standards "E.S" (2010). Maximum Permissible Limit of Aldrin according to "E.S" (2010) is 200 ppb

Table 5 Statistical analytical results of malathion levels (ppb) as organophosphorus pesticide in the examined samples of ready to eat fish products and their acceptability (n=25).

Fish products	Malathion incidence		Mean ±S.E	Acceptability * Accepted samples	
	No. of +ve samples	%. of +ve samples		No.	%
Fried *O. niloticus*	Free	Free	Free	25	100
Grilled *O. niloticus*	1	4	96.57^{b}	25	100
Smoked Herring	Free	Free	Free	25	100
Salted Sardine	2	8	248.19 ± 10.32^{a}	24	96
Total	3	3		99	99

* Means with different superscripts were significantly different (P<0.05). Egyptian Standards "E.S" (2010). Maximum Permissible Limit of Malathion according to "E.S" (2010) is 200 ppb

4. DISUCSSION

Contamination of fish with different chemical hazards is a matter of great concern worldwide because of its bad deleterious role in human health and nutrition. The rate of urbanization and industrialization is usually increased day by day leading to increase of chemical pollution of aquatic life world over (Turkmen et al. 2009) .

Mercury was released from electric generating stations and various industries operation. Mercury is recognized as a highly toxic metal and there are two forms of mercury inorganic and methyl mercury (organic form). Methyl mercury is more toxic to man than inorganic one because it cannot be excreted from the body and can cross the blood brain barrier to nervous system resulting in progressive and irreversible brain damage (Alonso et al. 2002). The incidence of mercury residues in the examined samples and their acceptability was showed in table(1), the obtained results recorded that the prevalence of mercury in the examined samples were 55(55%) samples represented as: 11(44%) fried O. niloticus with average of 0.43±0.01mg/kg; 12(48%) grilled O. niloticus with average of 0.59±0.01mg/kg; 14(56%) smoked herring with average of 0.70±0.01mg/kg and 18(72%) salted sardine with average of 0.94±0.02mg/kg. The acceptability of the examined samples was 67accepted samples represented as 20(80%), 18(72%), 17(68%) and 12(48%) from fried O. niloticus, grilled O.

niloticus, smoked herring and salted sardine, respectively. The acceptance was according to the permissible limits of mercury (0.5mg/kg) recommended by Egyptian Standards "E. S" (2010). The obtained results are being higher than those obtained by Amin (2011) who recorded the mean concentration of mercury in RTE samples of salted sardine was 0.51±0.04mg/kg. The current results are being lower than those recorded by Abd El-Aty (2010) detected the mean value of mercury in RTE samples of smoked herring was 0.85±0.04mg/kg.

Lead reaches the aquatic system because of superficial soil erosion and atmospheric deposition. Lead is considered as toxic substance as, accumulates in the body leading to lethargy, mental dullness and anorexia (ATSDR, 2005). The incidence of lead residues in the examined samples and their acceptability was showed in table(2), the obtained results recorded that the prevalence of lead in the examined samples were 47 (47%)samples represented as: 10(40%) fried O. niloticus with average of 0.27±0.01mg/kg; 10(40%) grilled O. niloticus with average of 0.33±0.01mg/kg; 12(48%) smoked herring with average of 0.45±0.01mg/kg and 15(60%) salted sardine with average of 0.61±0.01mg/kg. The acceptability of the examined samples was 74accepted samples represented as 21(84%), 20(80%), 18(72%) and 15(60%) from fried O. niloticus, grilled O. niloticus, smoked herring and salted sardine, respectively. The acceptability was according to the permissible limits of lead (0.1mg/kg) recommended by Egyptian Standards "E. S" (2010). The obtained results were higher than those obtained by Mohamed (2009) (0.09±0.01mg/kg in Tilapia niloticus), while lower than those recorded by AbdElgwad (2003) (2.419±0.282mg/kg in salted sardine).

Cadmium is classified as a probable human carcinogen because of its long-term storage in the body. Cadmium reaches to fish via sulphur phosphate fertilizer and sewage sludge (ATSDR, 2003). The incidence of cadmium residues in the examined samples and their acceptability was showed in table(3), the obtained results recorded that the prevalence of cadmium in the examined samples were 36 (36%) samples represented as: 7(28%) fried O. niloticus with average of 0.11±0.01mg/kg; 8(32%) grilled O. niloticus with average of 0.14±0.01mg/kg; 10(40%) smoked herring with average of 0.20±0.01mg/kg and 11(44%) salted sardine with average of 0.29±0.01mg/kg. The acceptability of the examined samples was 77accepted samples represented as 21(84%), 21(84%), 19(76%) and 16(64%) from fried O. niloticus, grilled O. niloticus, smoked herring and salted sardine, respectively. The acceptability was according to the permissible limits of cadmium (0.05mg/kg) recommended by Egyptian Standards "E. S" (2010). The obtained results were higher than those obtained by Tuzen and Soylak (2007) (0.19±0.01mg/kg in salted sardine). The effect of cooking process such as frying and grilling have only a very limited value as a means of reducing lead, mercury and cadmium concentrations and sometimes, heat treatment of fish by frying and grilling for long period of time cannot destroy the heavy metals (Dhatrak and Nandi, 2009) .

Aldrin as organochlorine pesticide can enter water system directly through area of treatments to damage the algae or indirectly through the inflow of sewage and industry wastewater. Exposures to moderate levels of aldrin for a long-time cause irritability, uncontrollable muscle movements and destroy blood cells in the body of some sensitive people (ATSDR, 2003). The incidence of aldrin residues in the examined samples and their acceptability was showed in table (4), the obtained results recorded that the frequency distribution of aldrin in examined samples were 7 (7%) samples represented as: 2(8%) fried O. niloticus with average of 82.95±4.19ppb; 2(8%) grilled O. niloticus with average of 151.26±7.40ppb and 3(12%) salted sardine with average of 193.02±8.81ppb. While all the examined samples of smoked Herring were free from aldrin. The acceptability of the examined samples was 98accepted samples represented as 25(100%), 24(96%), 25(100%) and 24(96%) from fried O. niloticus, grilled O. niloticus, smoked herring and salted sardine, respectively. The acceptability was according to the permissible limits of aldrin (200ppb) recommended by Egyptian Standards "E. S" (2010). The obtained results are being higher than those obtained by El-Sayed (2010) (70.05±3.19ppb in Tilapia niloticus). Cooking process such as deep frying seems to significantly reduce the levels of persistent aldrin in fish, as this method of frying accelerates drying of the fillets and evaporation of water and persistent aldrin due to the high temperature of the cooking oil and by transfer of aldrin to the cooking oil which itself could be acting as an extraction solvent (Witczak, 2009) .

Malathion as organophosphorus pesticide in RTE fish products could lead to some critical sings of toxicity as cramps, watery eyes, neurological damage, endocrine disruption and birth defects (Ahmed et al. 2010).The incidence of malathion in the examined samples and their acceptability was showed in table(5), the obtained results recorded that the prevalence of malathion in the examined samples were 3 (3%) samples represented as: 1(4%) grilled O. niloticus with average of 96.57ppb and 2(8%) salted sardine with average of 248.19±10.32ppb. While all the examined samples of fried O. niloticus and smoked Herring were free from malathion. The acceptability of examined samples were 99accepted samples represented as 25(100%), 25(100%), 25(100%) and 24(96%) from fried O. niloticus, grilled O. niloticus, smoked herring and salted sardine, respectively. The acceptance was according to the permissible limits of malathion (200ppb) recommended by Egyptian Standards "E. S" (2010). The obtained results were higher than those obtained by Soumis et al. (2003) (80.37ppb in grilled Tilapia niloticus), while lower than those recorded by El-Sayed (2010) (270.19±10.12 ppb in salted sardine). Grilling is a good and highly recommended method to reduce malathion residues. Application of grilling which release or remove fat from the fish products revealed a great reduction in malathion concentrations by ratio of 11% to 100% (Domingo, 2016) .

Fish is highly susceptible to deterioration immediately after harvest. So, if fish is not sold fresh, preservation or processing methods such as smoking and chemical preservation (salting) are usually applied to extend its shelf-life (Okonta and Ekelemu, 2005). During, hot smoking high heat results in direct destruction and decrease in the concentration of heavy metals and pesticide residues, due to the reduced moisture content (Abolagba and Igbinevbo, 2010). Salting is one of the oldest techniques for fish preservation and is essentially intended to increase the shelf life of the product. Unfortunately, the crude salt, bad quality spices and other additives used for curing may be increase the level of heavy metals and pesticide as, they consider a good source of some of the heavy metals and pesticide residues and the chemical residues may come from some of the unapproved insecticides commonly used for curing (Berhimpon et al. 2011).

To control the entrance of these chemical, a good quality raw fish should be used in the preparation of RTE fish products. RTE fish products should be purchased from reputable sources and markets with a history of providing safe food to customers. Healthy, high quality and hygienically prepared salts, spices and other additives free from any contaminants should be used. HACCP system should be applied to ensure the quality of RTE fish products and safety of consumers (Cuadrado et al. 2000).

5. CONCULOSION

In conclusion, the examined samples of fried *O. niloticus*, grilled *O. niloticus*, smoked Herring and salted Sardine proved to be contaminated with various chemical hazards such as heavy metals (mercury, lead and cadmium) and pesticides (aldrin and malathion).Therefore, RTE fish products constitute, at times, a public health hazard. Consequently, all monitoring systems should be put in place to control the residues of chemical hazards in RTE fish products and to ensure the safety of consumers

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