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Assessment of Ochratoxin A and Aflatoxin B1 Levels in the Smoked Fish with Special Reference to the Moisture and Sodium Chloride Content

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ABSTRACT

A total of 60 samples of smoked fish (*Clupea harengus*) were purchased randomly during summer of 2010 from Port-Said markets. The samples were examined for the levels of ochratoxin A and aflatoxin B1 and the contents of moisture and sodium chloride. The obtained results revealed that the mean values of ochratoxin A and aflatoxin B1 levels in the examined smoked fish were 0.73 ± 0.22 and 4.66 ± 0.94 ppb, respectively. While the contents of moisture and sodium chloride (on dry basis) in the examined samples were 66.07 ± 0.27 and $7.36 \pm 0.08\%$, respectively. On the assessment of the toxin levels, ochratoxin A and aflatoxin B1 were not detected in 80% (48) and 68.33% (41) of the examined smoked fish samples, respectively. On the other hand the contents of moisture and sodium chloride (on dry basis) in the examined samples were ranged from 61.83 to 70.98% and from 6.14 to 8.34%, respectively. From the sum of the examined smoked fish samples, 20% (12) and 31.67% (19) were exceeded the permissible limits for ochratoxin A and aflatoxin B1, respectively. The relationship between the levels of ochratoxin A and aflatoxin B1 and the contents of moisture and sodium chloride as well as the public health significance of these toxins were discussed.

Key words: Ochratoxin A, aflatoxin B1, moisture, sodium chloride, smoked fish

INTRODUCTION

Fish is an important and good quality source of protein, supplies a good balance of protein, vitamins, minerals and polyunsaturated fatty acids. Their flesh is tender and firmly texture and easily digested than beef or other type of protein. Most fish are consumed in the fresh form but some undergo processing particularly salting, drying and smoking (Haugen and Undeland, 2003; Gonzalez-Fandos *et al.*, 2005; Adebayo-Tayo *et al.*, 2008). Smoking is one of the most ancient processing technologies used for preservation and keeping fish from spoiling by using a combination of salt and smoke to reduce moisture content; consequently inhibition the microbial and enzymatic spoilage (Omojowo *et al.*, 2010). This process still used and remains popular for the flavor it gives to such fish. Oily fish (high in fat) such as herring of genus *Clupea harengus* are recommended for smoking because they absorb smoke faster and have better texture than lean fish. From the processing centers to the markets centers, smoked fish are often contaminated with microorganisms

including molds. Some species of these molds are major cause of spoilage and others are able to produce specific mycotoxins in these smoked fish (Essien *et al.*, 2005).

Mycotoxins are natural poisonous metabolites excreted extracellularly by toxigenic molds in/on foods and feeds. Ochratoxin A and aflatoxin B1 are the most important secondary metabolites/toxins produced in specific ecological niches by some strains of toxigenic molds. Ochratoxin A is produced by fungi belonging to the genus *Aspergillus* such as *Aspergillus aluaceus* var. *aluaceus* (Formerly *A. ochraceus*), *A. niger*, *A. carbonarius*, or *A. flavus*, genus *Penicillium* (*P. verrucosum*) or the genera *Petromyces* and *Neopetromyces* (Bayman *et al.*, 2002; Guillaumont *et al.*, 2005; Anli and Alkis, 2010), while aflatoxin B1 is produced by other strains belonging to the group of *Aspergillus flavus*, *Aspergillus parasiticus* and the rare species *Aspergillus nomius* (Milita *et al.*, 2010). The entry of ochratoxin A and aflatoxin B1 to humans takes place through direct and/or indirect contamination of food. Direct contamination can take place when the ochratoxin A and aflatoxin B1 producing molds grow on the smoked fish and food under favorable conditions such as high humidity, temperature and moisture levels that enhanced mold growth and toxin production. Indirect contamination can take place when the fish ingested feed containing ochratoxin A and aflatoxin B1 (Halasi *et al.*, 2005; Toscani *et al.*, 2007; Alkhalaf *et al.*, 2010; Sørensen *et al.*, 2010). Feed containing agricultural commodities such as cereals is considered the major source for transmission of molds and mycotoxins to fish and animals that further transmitted to humans (Guillaumont *et al.*, 2005). From the point of food technology, ochratoxin A and aflatoxin B1 pose a serious problem; whereas they fall into the group of heat stable compounds resistant to degradation and have a long toxic effect during food production process (Moss, 2000; Karan *et al.*, 2005). Also, human exposure to ochratoxin A and aflatoxin B1 is principally through ingestion of contaminated foods. Thus they are considered the most serious to public health in humans. (Herzallah, 2009).

The health hazards of ochratoxin A (OTA) in humans include hepatotoxic, carcinogenic, teratogenic, genotoxic, nephrotoxic and immunosuppressive. In addition, it may be implicated in human disease Balkan Endemic Nephropathy (BEN) and in the development of urinary tract tumors (Toscani *et al.*, 2007; Anli and Alkis, 2010). OTA was classified as a possible human carcinogen within Group 2 B (IARC, 1993). The toxic effect of aflatoxin B1 (AFB1) in fish varies depending on the species, age, sex and nutrition. The acute aflatoxicosis form includes pallid gills, impaired blood clotting, anemia, poor growth rates and lack of weight gain. Prolonged exposure to low concentrations of AFB1 may induce a chronic form that includes liver tumors, as yellow nodules which metastasize to the kidney and increase in mortality rate. Also, it depresses the immune system making fish more susceptible to bacterial, viral or parasitic opportunistic infections (Almeida *et al.*, 2011). In humans, aflatoxin B1 is considered the most serious to public health and associated with both acute and chronic toxicity. The acute toxicity in humans has been encountered only rarely, which includes fever, vomiting, jaundice, abdominal pain, pulmonary edema, convulsions, coma and death with cerebral edema and fatty involvement of liver, kidney and heart. While in chronic form aflatoxin B1 poses hepatocarcinogenic, teratogenic, carcinogenic, estrogenic and mutagenic effects in humans and animals. (Herzallah, 2009). Also, AFB1 incites reproductive and neuro-disorders as well as immunosuppression (Horn and Dömer, 2002). AFB1 was classified as a possible human carcinogen within Group 1 A (IARC, 1993).

The objectives of this study were to assess the ochratoxin A and aflatoxin B1 toxins in smoked fish and to evaluate the effect of moisture and sodium chloride content on the levels of ochratoxin A and aflatoxin B1 in the examined samples.

MATERIALS AND METHODS

Samples collection: A total of 60 samples of smoked fish (*Clupea harengus*) were purchased randomly during summer of 2010 from Port-Said markets. Individual fish, approximately ranged from 250-300 g, was placed separately into sealed plastic bags and thoroughly identified. The smoked fish samples were carried out immediately after delivered to the laboratory to assess the levels of ochratoxin A, aflatoxin B1, moisture and sodium chloride.

Preparation of the samples: The muscle of each smoked fish sample was separately passed rapidly three times through food chopper with plate opening equal to 1/8th inch (3 mm), mixed thoroughly after each grinding to obtain a uniform mass and finally began all determinations promptly. The homogenate sample was transferred to a wide mouth glass stoppered. The analysis was carried out as soon as possible, if any delay, the sample was chilled to inhibit the decomposition (AOAC, 2000).

Assessment of ochratoxin A and aflatoxin B1 levels: By high performance liquid chromatography (Agilent 1200 Series-Germany), the prepared samples were conducted individually for determination of ochratoxin A levels by a validated methods recorded by Toscani *et al.* (2007) while aflatoxin B1 levels were determined according to AOAC (2000).

Reagents: All used chemicals were HPLC grade. Ochratoxin A and aflatoxin B1 standards, diatomaceous earth, sodium chloride, anhydrous sodium sulphate, acetic acid and tetrahydrofuran obtained from Sigma-Aldrich (Sigma, St. Louis, MO, USA), while acetone, hexane, methanol, chloroform, toluene, ethyl acetate, formic acid, orthophosphoric acid, Tris-Hydrochloric acid, acetonitrile, glacial acetic acid and HPLC water obtained from Merck Inc. (Merck, Darmstadt, Germany).

Ochratoxin A

Sample extraction: An aliquot of 10 g of the prepared sample and 100 mL solution of (chloroform: 85% orthophosphoric acid 100:4, v/v") were mixed and homogenized in a blender at high speed for 2 min. After filtration through a fast filtering Whatman No. 3 filter paper, 60 mL of the filtrate was transformed into a separating funnel and extracted twice with 5 mL of (buffer 0.2 M Tris-Hydrochloric acid : Acetonitrile 90:10, v/v). The upper aqueous layer was carefully collected and thoroughly mixed.

Clean-up procedure: A 50 mL of the aliquot was passed through the Agilent ZORBAX C18 (3 µm, 2.1×250 mm) column for cleanup. After the solution had passed, the column was washed by water and dried by air. The ochratoxin A was eluted with 2 mL methanol with a vacuum manifold. The methanol was dried under gentle nitrogen stream and the residue was redissolved in mobile phase (water: acetonitrile: glacial acetic acid 49.5: 49.5: 1.0) before the injection in HPLC.

Preparation of ochratoxin A working standards: Analytical grade standard for ochratoxin A (OTA) was purchased from Sigma (St. Louis, MO, USA). Extreme handling precautions and safety measures should be applied during the preparation of ochratoxin A standard due to its carcinogenicity. Ochratoxin A standard solution was obtained by dissolving the solid standard, in toluene-acetic acid (99:1, v/v) and stored at -20°C. The concentration and purity of standard

solutions were evaluated according to AOAC (2000) official methods -970.44B. The stock solution was made in 4 mL of toluene-acetic acid mixture (99:1, v/v) at 250 $\mu\text{g mL}^{-1}$. An intermediate solution was prepared at 10 $\mu\text{g mL}^{-1}$ by diluting 1 mL of stock solution with 25 mL of toluene-acetic acid mixture (99:1, v/v). The working solution was prepared in toluene-acetic acid mixture (99:1, v/v) at 1000 ng mL^{-1} . For calibration curve, working standards solutions were prepared by diluting the solution to the following concentration: 2.5, 5.0, 10.0, 15.0, 20.0 and 25.0 ng mL^{-1} . These solutions were prepared daily from the stock solution. All solutions were maintained in amber flasks to protect them from light and stored in refrigerator and equilibrate to room temperature before use.

HPLC determination: The assessment of ochratoxin A was carried out with HPLC (Agilent 1200 Series, Germany) with fluorescent detector (Model FL 2475) at wavelength 380 and 440 nm for excitation and emission, respectively. The column used was the Agilent ZORBAX C18 (3 μm , 2.1 \times 250 mm) and the mobile phase was composed of water: acetonitrile: glacial acetic acid 49.5: 49.5: 1.0; which pumped with constant flow at 1 mL min^{-1} . Twenty microliter of the reconstituted sample were injected in the HPLC at 24°C to achieve the optimum resolution of ochratoxin A. Several blanks (methanol only) and ochratoxin A standard solution were injected also. The examined samples were done in triplicates. Calculations to get the level of ochratoxin A in the examined samples were carried out automatically by Agilent ChemStation Software System.

Aflatoxin B1

Sample extraction: In a blender, 50 g of the prepared homogenized sample were mixed with 100 mL of acetone and 100 mL of water for 10 min, 10 g of diatomaceous earth were added and stirred gently for 5 min then filtered through fast filtering Whatman No. 1 filter paper. One hundred milliliter of the filtrate were transferred to 500 mL wide mouth glass stoppered Erlenmeyer volumetric flask and mixed with 50 mL of 5% NaCl and 50 mL of hexane then shaken gently on a mechanical shaker (IKA, GmbH, Germany) for 5 min at 2400 rpm. The hexane layer was discarded. Next, 50 mL of 5% NaCl and 150 mL of chloroform (3 \times 50 mL) were added to the aqueous layer and shaken gently for 5 min each time. The chloroform layer was collected from the three extractions, dried over anhydrous sodium sulphate and evaporated using rotary evaporator. The residues were redissolved in 1 mL chloroform.

Clean-up procedure: The clean-up of the extract was carried out with Agilent ZORBAX C18 (5 μm , 4.6 \times 250 mm) column. The column was conditioned with 2 mL of 0.5% aqueous acetic acid, then 1 mL of the filtered extract followed by 4 mL of 0.5% acetic acid were loaded over the conditioned C18 column. The column then washed with 0.5 mL of 20% Tetrahydrofuran (THF) in 0.5% aqueous acetic acid. This was followed by passing 2 mL of hexane through the column tube and then dried under nitrogen. The column tube was washed with 3 mL of 25% THF in hexane and dried for 1 min under nitrogen. The retained aflatoxins B1 were eluted with 2 \times 2 mL from 1% THF in methylene chloride then dried over a stream of nitrogen. The dried aflatoxins B1 reconstituted in 0.5 mL of toluene before the injection in HPLC.

Preparation of aflatoxin B1 working standards: Analytical grade standards for Aflatoxin B1 (AFB1) were purchased from Sigma (St. Louis, MO, USA). Extreme handling precautions and safety measures should be applied during the preparation of aflatoxin B1 standard due to its

carcinogenicity. Aflatoxin B1 standard solution was obtained by dissolving the solid standard, in methanol: water 1:1, v/v and stored at -20°C. The concentration and purity of standard solutions were evaluated according to AOAC (2000) official methods -970.44B. The working standard solutions were prepared using concentration: 0.05, 1.0, 3.0, 6.0, 12.0 and 24.0 ng mL⁻¹ in eluent (methanol: water 1:1, v/v) to make the standard calibration curves. These solutions were prepared daily from the stock solution. All solutions were maintained in amber flasks to protect them from light and stored in refrigerator and equilibrate to room temperature before use.

HPLC determination: The determination of aflatoxin B1 was carried out with HPLC (Agilent 1200 Series, Germany) with fluorescent detector (Model FL 2475) at wavelength 365 and 440 nm for excitation and emission, respectively. The column used was C18 (5 µm, 4.6×250 mm) and the mobile phase was composed of toluene, ethyl acetate, formic acid and methanol (90:5:2.5:2.5, v/v) which pumped with constant flow at 1.0 mL min⁻¹. Twenty microliter of the reconstituted sample were injected in the HPLC at 24°C to achieve the optimum resolution of aflatoxins. Several blanks (methanol only) and aflatoxin B1 standard solution were injected also. The assessment of the given samples were done in triplicates and the sample regarded as positive for aflatoxin B1, if its retention time and peak corresponded to that of the standard. Calculations to get the level of aflatoxin B1 in the examined samples were carried out automatically by Agilent ChemStation Software System.

Assessment of moisture content: Empty dish (≥ 50 mm diameter and ≥ 40 mm deep) with its lid was dried in hot air ovens (INCUCCELL-MMM Group, LSIS-B2V/VC111) at 105°C for 3 h and cooled in the desiccator (at room temperature). The lid was removed and 5 g of the prepared sample was spread over the base of the dish then covered by its lid and Weighed (W1). The dish, partially covered by lid, was placed in the oven at 105°C for 3 h then tightened cover, cooled in the desiccator and weighed quickly. The dish with its cover was returned to the oven and dried for another 1/2 h then cooled and weighed. This was repeated until 2 successive constant Weights (W2) were obtained. Losses in weight was recorded as moisture and calculated according to the following equation (AOAC, 2000).

$$\text{Moisture content (\%)} = \frac{(W1 - W2)}{W1} \times 100$$

W1 = Weight (g) of sample before drying.

W2 = Weight (g) of sample after drying.

Assessment of sodium chloride level

Reagents: All reagents used were of analytical grade. Silver nitrate, ferric ammonium sulphate and standard ammonium thiocyanate were purchased from, Sigma-Aldrich while nitric acid was obtained from Merck Inc (Merck, Darmstadt, Germany).

Determination: In a 250 mL beaker, 2 g of the dried sample was taken with known volume of 0.1 N standard silver nitrate solution (16.99 g AgNO₃ in halogen free water and diluted to 1000 mL), more than enough to precipitate all Chloride (Cl) as AgCl and 20 mL of dilute nitric acid (1 part H₂NO₃ acid to 4 parts halogen free water v/v). The homogenate was boiled gently on hot plate until all solids except AgCl dissolved (usually 15 min) then cooled to room temperature. Fifty

microliter of distilled water free from halogen and 1 mL of ferric alum indicator (saturated solution of ferric ammonium sulphate $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12 \text{H}_2\text{O}$ in halogen free water) were added to the aliquot and titrated with 0.1 N standard ammonium thiocyanate solution (7.612 g of NH_4SCN in halogen free water and diluted to 1000 mL) until the appearance of permanent light brown color. The level of sodium chloride was calculated according to the following equation (AOAC, 2000).

$$\text{Sodium chloride (on dry basis) m / m} = \frac{5.85 \times (V1N1 - V2N2)}{M}$$

- V1 = Vol. of standard solution of silver nitrate.
- N1 = Normality of standard silver nitrate solution.
- V2 = Vol. of standard pot. thiocyanate solution.
- N2 = Normality of standard pot. thiocyanate solution.
- M = Mass of dried material taken for test

Statistical analysis: One-Way ANOVA test was performed on the treatment means for each parameter studied using Statistical Package for Social Scientists (SPSS) for windows 16.0 (SPSS Inc., Chicago, IL and USA) to examine whether there were significant differences in parameters analyzed. Correlations between the ochratoxin A, aflatoxin B, moisture and sodium chloride levels were also done (SPSS, 2007).

RESULTS

Assessment of ochratoxin A and aflatoxin B1 levels: A total of 60 samples of smoked fish were estimated by high performance liquid chromatography (Agilent 1200 Series-Germany), with fluorescent detector (Model FL 2475) at wavelength 380 and 440 nm for excitation and emission of ochratoxin A levels respectively but at wavelength 365 and 440 nm for excitation and emission of aflatoxin B1 levels respectively. The quantitative assessment of ochratoxin A and aflatoxin B1 levels were not detected in 80% (48) and 68.33% (41) but detected in 20% (12) and 31.67% (19) of the examined smoked fish samples, respectively as shown in Table 1 and Fig. 1. This indicates defect in sanitation of the aquatic ecosystem and during catching, improper smoking process, bad condition of storage and loss of preservative effect of wood smoking during storage.

Assessment of moisture and sodium chloride contents: The contents of moisture and sodium chloride (on dry basis) were measured in the examined samples and showed high moisture contents as apparent in Table 1. This regards to the improper brining time and methods, salt gradients used and the frozen state of fish which consequently affecting the salts uptake.

Frequency distribution of the examined parameters: The frequency distribution of the levels of ochratoxin A, aflatoxin B1, moisture and sodium chloride in the examined smoked fish showed in Table 2 an increase in the moisture content and lower of sodium chloride in most samples. This initiates the mold growth and enzymatic activity consequently production of ochratoxin A and aflatoxin B1 in the samples.

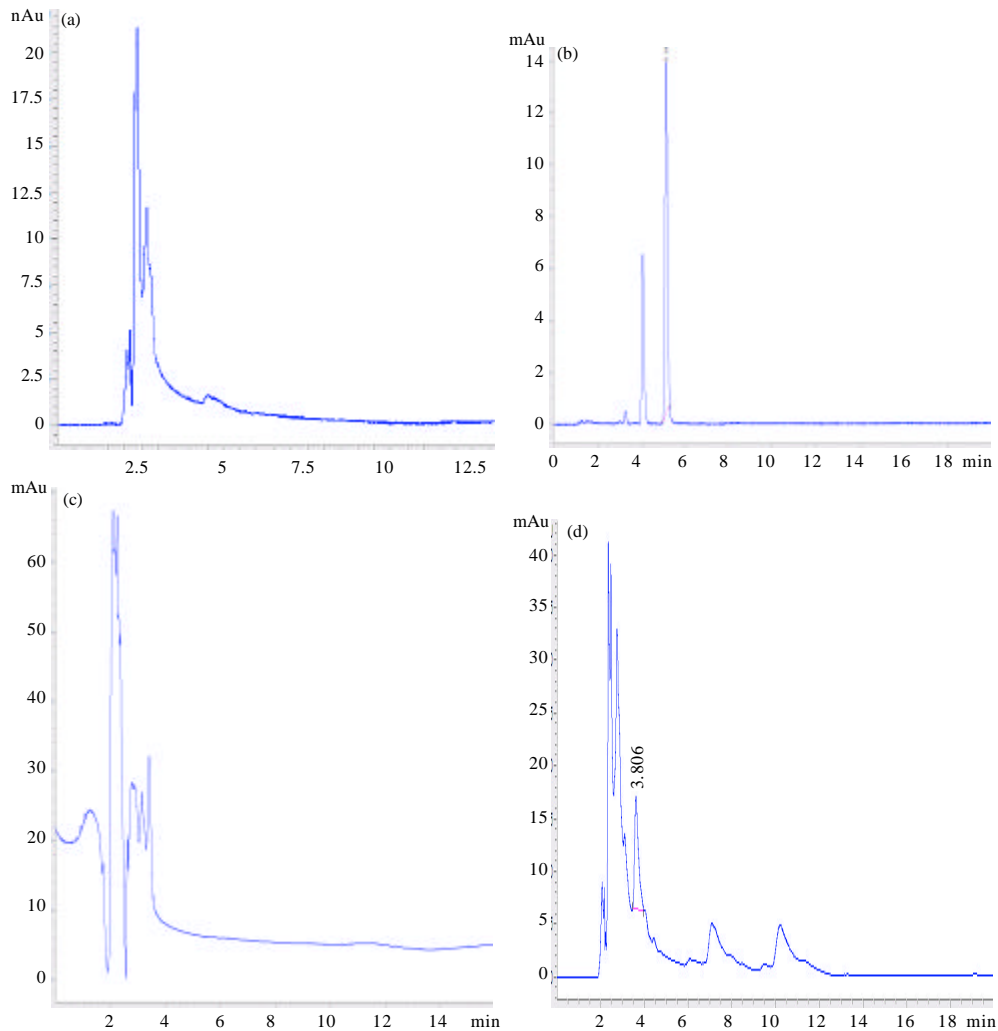


Fig. 1(a-d): Ochratoxin A and aflatoxin B1 chromatograms for analysed smoked fish samples and blank using fluorescent detector (a): Ochratoxin A blank. (b): Extracted smoked fish sample containing ochratoxin A. (c): Aflatoxin B1 blank. (d): Extracted smoked fish sample containing aflatoxin B1

The relationship between the examined parameters: By using one-way ANOVA test showed a highly significant relationship between the mean values of ochratoxin A, aflatoxin B1, moisture and sodium chloride (Table 3). This indicates that the high concentration of sodium chloride showed greatest reduction of toxins and water contents.

Effects of ochratoxin A and aflatoxin B1 on public health: Comparison of the levels of ochratoxin A and aflatoxin B1 in the examined smoked fish with their permissible limits established by EOSQC (2005) as shown in Table (4). Although ochratoxin A and aflatoxin B1 not detected in most of examined smoked fish samples, some samples exceeded the permissible limits recommended by EOSQC (2005) and consider a public health hazardous for the consumers and enhanced the ability of ochratoxin A to increase the mutagenic ability of aflatoxin B1.

Table 1: Statistical analytical results of ochratoxin A, aflatoxin B1, moisture, sodium chloride (on the basis of dry weight) levels recovered from smoked fish samples (n = 60)

		Ochratoxin A (ppb)	Aflatoxin B1 (ppb)	Moisture (%)	Sodium Chloride ^(A) (%)
Samples	Total	No. 60	60	60	60
		% 100	100	100	100
(ND)	No.	48	41	-----	-----
	%	80	68.33	-----	-----
(D)	No.	12	19	60	60
	%	20	31.67	100	100
Statistic for the examined parameters	Min.	0.00	0.00	61.83	6.14
	Max.	6.41	24.35	70.98	8.34
	Mean	0.73	4.66	66.07	7.36
	S.E.	0.22	0.94	0.27	0.08

^(A): On the basis of dry weight. ppb: Part per billion. ND: Non detectable level (<1 ppb). D: Detectable level. Min.: Minimum. Max.: Maximum. SE: Standard error

Table 2: Frequency distribution of ochratoxin A, aflatoxin B1, moisture, sodium chloride (on the basis of dry weight) levels in smoked fish samples (n = 60)

Ochratoxin A			Aflatoxin B1			Moisture			Sodium chloride ^(A)		
Levels range (ppb)	No.	(%)	Levels range (ppb)	No.	(%)	Percentage range (%)	No.	(%)	Levels range (%)	No.	(%)
0 (ND)	48	80.00	0 (ND)	41	68.33	>61-<63	2	3.33	>6-<6.5	7	11.67
>1-<2	2	3.33	>10-<13	11	18.33	>63-<65	23	38.33	>6.5-<7	6	10.00
>2-<3	3	5.00	>13-<16	3	5.00	>65-<67	13	21.67	>7-<7.5	17	28.33
>3-<4	1	1.67	>16-<19	1	1.67	>67-<69	16	26.67	>7.5-<8	19	31.67
>4-<5	3	5.00	>19-<22	2	3.33	>69-<70	4	6.67	>8-<8.5	11	18.33
>5-<6	1	1.67	>22-<25	2	3.33	>70-<71	2	3.33	-----	-----	-----
>6-<7	2	3.33	-----	-----	-----	-----	-----	-----	-----	-----	-----
Total	60	100	Total	60	100	Total	60	100	Total	60	100

^(A): On the basis of dry weight. ppb: Part per billion. ND: Non detectable level (<1 ppb)

Table 3: Correlation between the different mean values of ochratoxin A, aflatoxin B1, moisture and sodium chloride (on the basis of dry weight) levels in smoked fish samples

	Ochratoxin A	Aflatoxin B1	Moisture	Sodium chloride ^(A)
Ochratoxin A	-----	0.000 (**)	0.000 (**)	0.000 (**)
Aflatoxin B1	0.000 (**)	-----	0.000 (**)	0.003 (**)
Moisture	0.000 (**)	0.000 (**)	-----	0.000 (**)
Sodium chloride ^(A)	0.000 (**)	0.003 (**)	0.000 (**)	-----

^(A): On the basis of dry weight. (**): Highly significant correlation at <0.01 by using one way ANOVA test

DISCUSSION

The quantitative assessment of ochratoxin A and aflatoxin B1 levels in the examined smoked fish samples were showed in Table 1 and Fig. 1. The mean values of ochratoxin A and aflatoxin B1 levels were 0.73±0.22 and 4.66±0.94 ppb, respectively. These results were agree with the result recorded by Adebayo-Tayo *et al.* (2008) who recorded that the mean values of aflatoxin B1 recovered from smoked stock fish (*Gadus morhua*) was 4.750 ppb but disagree with the other results recorded by the same author for the different other types of smoked fish.

Adebayo-Tayo *et al.* (2008) recorded that the mean values of aflatoxin B1 for other types of smoked fish were 1.505, 2.100, 2.205, 2.515, 2.805, 3.0005, 3.005, 3.55, 7.52 and 8.105 ppb. Also these results were lower than the results recorded by Hassan *et al.* (2011) who recorded that the mean values of aflatoxins in smoked fish was 65.1 ± 0.05 ppb. Table 1 showed an incidence of 20% (12) and 31.67% (19) of the examined smoked fish samples for ochratoxin A and aflatoxin B1, respectively. This incidence was lowered than that recorded by Adebayo-Tayo *et al.* (2008) who found that 100% (55) of the examined smoked fish samples contain aflatoxin B1 but more than that of the results (26.6% 8) recorded by Hassan *et al.* (2011). The differences between the obtained results and results of other studies may be attributed to the variation in the sanitation of the aquatic ecosystem and during catching. Improper smoking process i.e. contamination of fish during salting and smoking process, handling manufactured, unventilated storage, transportation and marketing of smoked fish initiate the mold attaches. Also the condition of storage (temperature and humidity) and a gradual loss of the preservative effect of wood-smoking over long storage time have essential role in elevation of toxigenic mold counts and increase the levels of their metabolites (i.e., ochratoxin A and aflatoxin B1) (Basti *et al.*, 2003; Hassan, 2003; Hassan *et al.*, 2007; Adebayo-Tayo *et al.*, 2008).

The moisture and sodium chloride (on dry basis) contents in the examined smoked fish samples were 66.07 ± 0.27 and $7.36 \pm 0.08\%$, respectively (Table 1). This results were disagree with the results recorded by Lin *et al.* (2003) who found that the mean values of sodium chloride and moisture content in hot smoked king were 3.51 and 64.16%, respectively. Also the moisture contents were higher than the results (mean values of moisture in different fish samples were ranged from 22.7 to 27.6% recorded by Adebayo-Tayo *et al.* (2008). The variation between the obtained results and results of other studies may be attributed to the variation in the type and condition of fish, fish thickness, brining time and the brining methods and salt gradients used (Lin *et al.*, 2003). Additional factors such as post mortem condition of the fish, fish species and the whether a sample had been previously frozen were affecting the salts uptake rate and the final equilibrium salt concentration (Wang *et al.*, 1998).

Regarding the frequency distribution of ochratoxin A, aflatoxin B1, the moisture and sodium chloride levels in the examined smoked fish in Table 2, showed that a high contents of moisture (ranged from >63 to <69 with a percentage of 86.67% 52) which initiates the mold growth and enzymatic activity i.e., production of extracellular metabolites such as ochratoxin A and aflatoxin B1 which ranged from >1 to <5 ppb and from >10 to <16 ppb in a percentage of 14% (9) and 23.33% (14), respectively. The sodium chloride contents in the examined smoked fish were ranged from >7 to <8.5 with a percentage of 78.33% (47). The assessed parameters showed high levels of sodium chloride in the examined smoked fish and this may be attributed to the variation between the brining and smoking level during processing. Also the disturbance between the moisture content/ water activity besides the ratio between the hot/traditional smoking and the antimicrobial agents present in wood smoke has ability to increase the moisture content; consequently enhanced mold growth (Eyabi *et al.*, 2001; Omojowo *et al.*, 2010).

Also by using one-way ANOVA test to compare the means values of the studied parameter ochratoxin in the examined smoked fish samples (Table 3), showed a highly significant relationship between the mean values of ochratoxin A, aflatoxin B1, moisture and sodium chloride. These parameters relationship indicates that the high concentration of sodium chloride showed greatest reduction of toxins and water contents and vice versa (Omojowo *et al.*, 2010).

Table 4: A Comparison between the mean values of ochratoxin A, aflatoxin B1 and sodium chloride (on the basis of dry weight) with the permissible limits recommended by E.O.S.Q.C

Ochratoxin A					Aflatoxin B1					Sodium chloride ^(A)					
Results					Results					Results					
Allowed	Not allowed	Permissible			Allowed	Not allowed	Permissible			Allowed	Not allowed	Permissible			
No.	(%)	No.	(%)	limits (ppb)	No.	(%)	No.	(%)	limits (ppb)	No.	(%)	No.	(%)	limits	
E.O.S.Q.C. ^(B)	48	80	12	20	0	41	68.33	19	31.67	0	60	100	0	0	4.5-15%

ppb: Part per billion. ^(A): On the basis of dry weight. ^(B): = EOSQC (2005)

Table 5: A Comparison between the mean values of ochratoxin A and aflatoxin B1 with the action level recommended by different organization

Organization	Ochratoxin A				Action level ^(A)	Aflatoxin B1				Action level ^(A)
	No.	(%)	No.	(%)		No.	(%)	No.	(%)	
FDA ^(B)	----	----	----	----	None ^(C)	56	93.33	4	6.67	= 20 ^(D)
WHO ^(E)	60 ^(F)	100	----	----	= 0.5 ^(G)	56	93.33	4	6.67	= 20

^(A): The maximum limits allowed to be found in human food. ^(B): FDA (2000). ^(C): Non set by FDA. ^(D): ppb (Part per billion). ^(E): WHO (1996) ^(F): at 70 kg body weight. ^(G): $\mu\text{g kg}^{-1}$ (ppb) body weight day⁻¹

In comparison with the permissible limits of the ochratoxin A and aflatoxin B1 and sodium chloride with the permissible limits established by EOSQC (2005) for smoked fish showed in Table 4, it is evident that ochratoxin A and aflatoxin B1 were not detected in 80% (48) and 68.33% (41) and this percentages were agree with the limits recommended by EOSQC (2005) for ochratoxin A and aflatoxin B1 in smoked fish respectively. Also 100% (60) of the examined samples were agreed the limits recommended by EOSQC (2005) for sodium chloride contents. On the other hand, 20% (12) and 31.67% (19) of the examined smoked fish samples were exceeded the permissible limits of ochratoxin A and aflatoxin B1 established by EOSQC (2005) for smoked fish respectively. The samples which exceed the permissible limits of ochratoxin A and aflatoxin B1 may be regarded to an error in the processing include the sanitation condition pre and post smoking process and the condition and time of the storage (Hassan *et al.*, 2011). From the public health significant the samples which exceed the permissible limits of ochratoxin A and aflatoxin B1 according to EOSQC (2005), consider a public health hazardous for consumers besides the ability of ochratoxin A to increase the mutagenic ability of aflatoxin B1 (Sedmikova *et al.*, 2001).

Table 5 showed a comparison between the action level of different organization for ochratoxin A and aflatoxin with the obtained results in the examined smoked fish samples. It showed that 100% (6) and 93.33% (56) of the examined sample were agreed the action levels recommended by FDA (2000) and WHO (1996). The percentage of acceptable samples according to the permissible limits recommended by EOSQC (2005) and that of the action levels recommended by FDA (2000) and WHO (1996) were differed. This indicates that there are several regulations for ochratoxin A

and aflatoxin B1, a specific regulation should be cited for these toxins especially in food of animal origin and harmonized in all countries for all commodities.

CONCLUSION

In conclusion, screening of smoked fish for the presence and the levels of ochratoxin A and aflatoxin B1 are a relevant strategy to assure the safety condition of smoked fish and guarantees to smoking process. While the sodium chloride and moisture contents may relevant to enhance safety guarantees and regulatory scrutiny to smoking fish production. Application of good manufacture practices and Hazards Analysis Critical Control System (HACCP) from the processing centers to the markets centers till reach to the consumers must be done for improving the safety condition of smoked fish. Such control include prevention the synthesis of molds producing ochratoxin A and aflatoxin B1 and their toxins with the retaining of the nutritive quality and palatability of smoked fish. Maintaining humidity, temperature and moisture levels within safe limits is on of the most important hurdles contributing to the stability and safety of smoked fish products. The levels of Water Phase Salt (WPS) should be within the safe limits to inhibit the growth of molds and production of toxin during brining regime, curing process and dehydration steps during the smoking process. Using modern technology and technological improvements should be continued to prevent any contamination, cross contamination and poor condition during manufactured process and storage. Finally, a new modern safely, easily and economic biologically method must introduced to prevent or reduce the ochratoxin A and aflatoxin B1 levels with retaining of the nutritive quality and palatability of the smoked fish. The authority and organization should established a new easily and rapidly quantitative methods for the detection and assessment of ochratoxin A, aflatoxin B1 and anther mycotoxins in all food specially of animal origin. Also the maximum permissible limits of ochratoxin A, aflatoxin B1 and anther mycotoxins should be reduced to the lowest levels and become the same as limits in all country worldwide.

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