Hafez NE, et al., J Toxicol Cur Res 2019, 3: 008
DOI: 10.24966/TCR-3735/100008

HSOA Journal of Toxicology: Current Research

Research Article

Risk Assessment of Polycyclic Aromatic Hydrocarbons concentration in Cold Smoked Mullet Fish (Mugil Cephalus)

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Abstract

The purpose of this work was to determine the concentration and risk assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in cold-smoked mullet fish samples which were pre-frozen at -18°C for 6 months. Fish samples were obtained from two fish farms; A (Al-Batts drain) and B (El-Wadi drain), El-Fayoum governorate, Egypt. 16 components of PAHs concentration were determined by GC-MS. Results showed that the total concentration of PAHs recorded 16.2 and 7.4ppb sample of A and B-smoked mullet fish products, respectively. Also, levels of Benzo [a] Pyrene (B {a} P) equivalent were 0.0378 and 0.029ppb in A and B-products, respectively. Besides, content of Low Molecular Weight (LMW) components was higher in A-smoked mullet product than medium MW and vice versa in case B-smoked product however, high MW was not detected in products. In conclusion, PAHs concentration in smoked products processed from pre-frozen mullet samples for 6 months at -18°C are considered a minimally contaminated (16.2ppb) for A-smoked product and not contaminated (7.4ppb) for B-smoked product compared with recommended levels. In addition to the component of Benzo [a] Pyrene (B {a} P) was not detectable in all smoked fish products.

Keywords: Frozen mullet fish; GC-MS; PAHs; Smoking

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Citation: Hafez NE, Awad AM, Ibrahim SM, Mohamed HR, El-Lahamy AA (2019) Risk Assessment of Polycyclic Aromatic Hydrocarbons concentration in Cold Smoked Mullet Fish (Mugil Cephalus). J Toxicol Cur Res 3: 008.

Received: March 21, 2019; **Accepted:** April 21, 2019; **Published:** April 30, 2019

Introduction

Smoking is one of the oldest methods used to process and preserve fish. It is a process of treating fish by exposing it to smoke from smoldering wood or plant materials. This process is usually characterized by an integrated combination of salting, drying, heating and smoking operations in a smoking chamber. The preservation properties of smoking treatment are mainly due to the partial drying and the precipitation of aliphatic and aromatic vapors on fish surface [1-6]. Food cooking and processing methods at high temperatures such as smoking, drying, roasting, baking or frying are recognized as a major source of food contamination by PAHs [7-9].

PAHs compounds are containing 2 or more fused aromatic rings. Polycyclic aromatic hydrocarbons containing 4 rings such as chrysene and benzo [a] anthracene consider weakly carcinogenic compounds, while PAHs which have 5 or more rings are a potentially carcinogenic and mutagenic for human such as Benzo [a] Pyrene (BaP), benzo [g,h,i] perylene, benzo [b] fluoranthene, indeno [1,2,3-c,d] pyrene and benzo [k] fluoranthene [10-12].

Wood smoke contains a hundreds (at least 100) of PAHs and their derivatives which have carcinogenic compounds such as Benzo [a] Pyrene (BaP). BaP consider a marker for carcinogenic PAHs in smoked fish and the maximum level is 2µg/kg. After metabolic activation in mammalian cells to diol-epoxides, PAHs bind covalently to cellular macromolecules, including DNA, thereby causing errors in DNA replication and mutations that initiate the carcinogenic process. This mechanism of activation, with some modifications, occurs with all carcinogenic PAHs [13]. The classification of the International Agency of Research on Cancer for Benzo [a] Pyrene (BaP) was changed from group 2A (probably carcinogenic to humans) to group 1 (carcinogenic to humans), chrysene was changed from group 3 (not classifiable for humans) to group 2B (possibly carcinogenic to humans), and benzo [a] anthracene was re-grouped from 2A to 2B [14,15].

Therefore, the main purpose of this work was to determine the concentration and risk assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in cold-smoked mullet fish samples which were obtained from two fish farms, El-Fayoum governorate, Egypt and frozen storage at -18°C for 6 months.

Materials and Methods

Fish samples

Mullet fish (Mugil cephalus) samples were obtained after directly catch from two fish farms (A and B). The main resources of irrigation water were agricultural discharge for A (Al-Batts drain) and B (El Wadi drain) during August, 2015 at El-Fayoum governorate, Egypt. They were transported immediately to Fish Processing and Technology Lab, Shakshouk Station for Water Resource, National Institute of Oceanography and Fisheries (NIOF), Egypt. Average of weight 525±25gm and length 36±1cm for raw samples from Farm A (Al-Batts drain) while, the average weight of raw mullet samples

from Farm B (Agricultural discharge) was 545±5gm and length was 37.75±0.25cm, respectively. After that, fish samples were carefully washed with tap water, glazed, packed in polyethylene bags and stored at -18°C for 6 months.

Smoking process

After 6 months of raw mullet fish frozen storage, Fish samples (from A and B farm) were thawed at 4°C then soaked in 10% brined solution (Sodium chloride) for two hrs., rinsed with tap water for 1 min and semi-dried at 25°C for two hrs. The smokehouse had inside dimensions of 1.20×1.0×3.5m with pours-metal plates localized above the smoke source by 75cm. the Semi-dried fish samples were hooked at distance about 250cm in smoking house. Traditional cold smoking was carried out at 28-32°C for 8-10hrs. Using sawdust as smoke source. After smoking the fish samples were cooled under ambient temperature.

Analytical methods

The edible of smoked mullet fish products was manually separated, homogenized, packed in polyethylene bags and then stored in a freezer at -20°C till analysis. PAHs were determined at Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food (QCAP), Agricultural Research Centre. Cairo, Egypt as described by [16]. Chemicals and Reagents; acetone (Riedel-de Häen, purity 99.8%), acetonitrile (Sigma-Aldrich, purity>99.9%), toluene (Merck), dichlorom-ethane chromatography grade, and n-hexane (purity>99.0%) were used.

B {a} P equivalent

The BaP_{eqi} was calculated as the sum of BaP_{eqi} value for individual PAHs. The BaP_{eqi} value was calculated for each PAH from its concentration in the sample (C_{PAHi}) multiplied by its toxic equivalency factor (TEF_{PAHi}) [17].

$$BaP_{eqi}=\Sigma\ (BaP_{eqi})=\Sigma\ (C_{PAHi}\times TEF_{PAHi})$$

C_{PAHi}: Concentration of each PAH in the sample; TEF_{PAHi}: Toxic equivalency factor for each individual PAH.

Statistical analysis

The results obtained were analyzed statistically using the Least Significant Difference test (LSD) at ($P\leq0.05$) and were expressed as Mean±SD using SPSS 16 for windows

Results and Discussion

Polycyclic Aromatic Hydrocarbons (PAHs)

Table 1 shows the PAHs concentration of cold smoked mullet fish flesh. 16 components of PAHs were detected in edible part of investigated smoked products including; Naphthalene (NA), Acenaphthylene (ACL), Acenaphthene (ACE), Fluorine (FLU), Phenanthrene (PHE), Anthracene (ANT), Fluoranthene (FLA), Pyrene (PYR), Benzo [a] Anthracene (BaA), Chrysene (CHR), Benzo [b] Fluoranthene (BbF), Bonzo [k] Fluoranthene(BkF), Benzo [a] Pyrene (BaP), Dibenzo [a,h] Anthracene (DahA), Benzo [g,h,i] Pyrene (BghiP) and Indeno [1,2,3-cd] Pyrene (IcdP). The results showed that A-smoked samples contained 5 compounds; Phenanthrene (4.9ppb), Fluorene (3.8µg/kg), Fluoranthene (2.6ppb), Pyrene (2.5ppb) and Anthracene (2.5ppb). Σ16PAHs was 16.2ppb. While B-smoked samples contained 5 compounds; Fluoranthene (2.6ppb), Pyrene, Anthracene (2.4ppb), Fluorene and Phenanthrene (<LOQ) and they were lower than the limit of quantification of PAHs (<LOQ) (< 2ppb) and Σ16PAHs were 7.4ppb.

Compound	Abbrev.	Mw	Rings	Concentration (ppb)	
				Farm (A)	Farm (B)
Chrysene	CHR	228	4	ND	ND
Anthracene	ANT	178	3	2.4	2.4
Acenaphthene	ACE	153	3	ND	ND
Benzo(b)Fluoranthene	BbF	252	5	ND	ND
Benzo(k)fluoranthene	BkF	252	5	ND	ND
Dibenzo(a,h)Anthracene	DahA	278	5	ND	ND
Fluorene	FLU	166	3	3.8	<LOQ
Naphthalene	NA	128	2	ND	ND
Benzo(a)pyrene	BaP	252	5	ND	ND
Benzo(g,h,i)perylene	BghiP	276	6	ND	ND
Indeno(1,2,3-c,d)pyrene	IcdP	276	6	ND	ND
Acenaphthylene	ACY	152	3	ND	ND
Fluoranthene	FLA	202	4	2.6	2.6
Pyrene	PYR	202	4	2.5	2.4
Benzo(a)anthracene	BaA	228	4	ND	ND
Phenanthrene	PHE	178	3	4.9	<LOQ
Σ16PAHs				16.2	7.4

Table 1: Concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in cold-smoked mullet fish samples pre-frozen for 6 months at -18°C.

Note: Farm (A): Al-Batts Drain. Farm (B): El-Wadi Drain. Mw: Molecular weight. LOQ: <2µg/kg. ND: not detected.

Toxic Equivalent Factors (TEFs) and B {a} P equivalent of PAHs found in smoked mullet fish samples

Toxic Equivalency Factor (TEF) is an estimate of the relative toxicity of individual PAH fraction compared to benzo (a) pyrene. TEFs have been applied as a useful tool for the regulation of compounds with a common mechanism of actions (e.g. PAHs) [18]. Even if this presentation of PAH content is empirical because the effects of PAHs in a mixture are insufficiently understood, with this approach it is possible to express PAH contamination of food by a single value as reported by [19,20]. Benzo [a] Pyrene (BaP) has been well characterized as the most potent carcinogenic PAH after dibenz [a,h] anthracene. Therefore, the total PAH concentration is expressed as Benzo[a] Pyrene Equivalents (BaP_{eqi}) to illustrate the toxic potency [21].

The Toxic Equivalent Factors (TEFs) and B [a] P Equivalent of PAHs in smoked mullet fish are presented in table 2. The B [a] P Equivalent of Fluorene; Phenanthrene; Anthracene; Fluoranthene and Pyrene were 0.0038; 0.0049; 0.024; 0.0026 and 0.0025 respectively and the $\sum(BaP_{eqi})$ was 0.0378 for farm A smoked samples (Figure 1). In the other farm samples (B) the B [a] P Equivalent of Anthracene; Fluoranthene and Pyrene were 0.024; 0.0026 and 0.0024 respectively and the sum of B [a] P Equivalent $\sum(BaP_{eqi})$ were 0.029 (Figure 2).

Molecular weight of PAHs in smoked fish

Table 3 illustrates the Molecular Weights (MW) of PAHs in smoked mullet fish. The total concentration of the Low Molecular Weights (LWM) of PAHs was higher than the Medium Molecular

Weights (MMW) in smoked fish farm (A). The concentration of LWM in smoked fish farm (A) was 11.1ppb while MMW was 5.1ppb. On the other side, for samples from B farm the total concentration of medium molecular weights of PAHs 5µg/kg, and LMW was 2.4µg/kg. The high molecular weight compounds not detected in samples from both farms A and B, Most of the carcinogenic PAHs fall within the group of the HMW [22]. This can be suggested to have been influenced by low fat and pyrolysis resulted from melted dropping onto the heat source. This is due to the average temperature of the smoking processes does not favor the production of HMW PAHs. The temperature range of 500-900°C is known to favor the production of HMW PAHs from thermal breakdown of lignin in lignocelluloses during wood combustion and also from pyrolysis of fats in fish [23-27].

Compound	TEF	Farm (A)		Farm (B)	
		Conc.(ppb)	BaP_{eqi}	Conc. (ppb)	BaP_{eqi}
Naphthalene	0.001	ND	-	ND	-
Acenaphthylene	0.001	ND	-	ND	-
Acenaphthene	0.001	ND	-	ND	-
Fluorene	0.001	3.8	0.0038	<LOQ	-
Phenanthrene	0.001	4.9	0.0049	<LOQ	
Anthracene	0.01	2.4	0.024	2.4	0.024
Fluoranthene	0.001	2.6	0.0026	2.6	0.0026
Pyrene	0.001	2.5	0.0025	2.4	0.0024
Benzo(a)anthracene	0.1	ND	-	ND	-
Chrysene	0.01	ND	-	ND	-
Benzo(b)fluoranthene	0.1	ND	-	ND	-
Benzo(k)fluoranthene	0.1	ND	-	ND	-
Benzo(a)pyrene	1.0	ND	-	ND	-
Indeno(1,2,3,c)pyrene	0.1	ND	-	ND	-
Dibenzo(a,h)anthracene	1.0	ND	-	ND	-
Benzo(g,h,i)perylene	0.01	ND	-	ND	-
$\sum(BaP_{eqi})$			0.0378		0.029

Table 4: Toxic Equivalent Factors (TEFs) and B [a] P Equivalent of PAHs found in pre-frozen cold smoked mullet fish for6 months at -18°C.

Note: TEF: Toxic Equivalent Factor; BaP_{eqi}[a]: P equivalent.Farm (A): Al-Batts Drain; Farm (B): El-Wadi Drain.

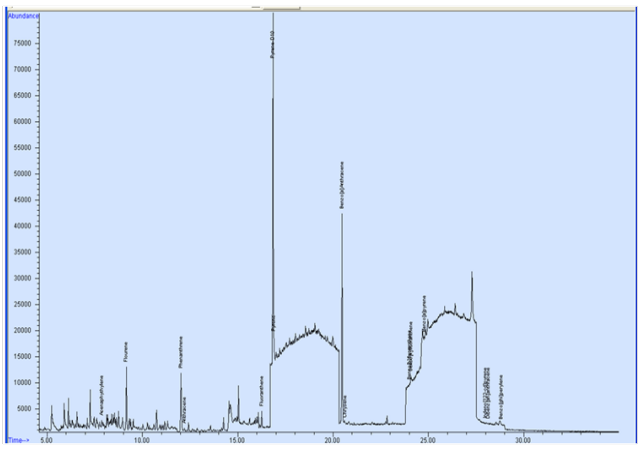

Figure 1: GC-MS chromatogram of smoked mullet fish (farm A).

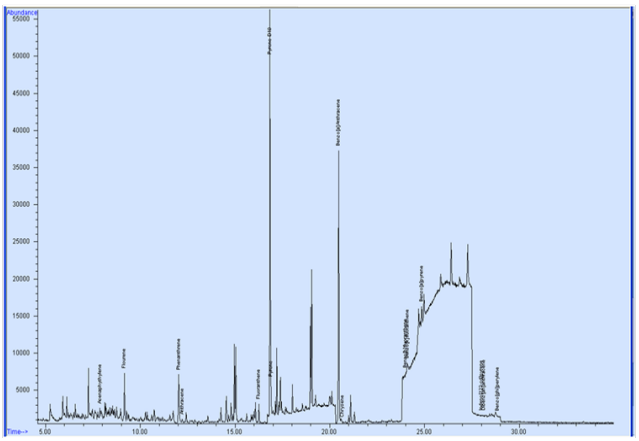

Figure 2: GC-MS chromatogram of smoked mullet fish (farm B).

Concentrations (ppb) of the PAHs for Farm A Samples			Concentrations (ppb) of the PAHs for Farm B Samples		
HMW	MMW	LMW	HMW	MMW	LMW
-	5.1	11.1	-	5	2.4

Table 3: Total mean concentration (ppb) of PAHs in cold smoked fish, according to their molecular weights.

Note: HMW: High Molecular Weight; MMW: Medium Molecular Weight; LMW: Low Molecular Weight.

Farm (A): Al-Batts Drain; Farm (B): El-Wadi Drain: was not detectable.

Category of PAH concentration

The categories of PAHs concentration as not contaminated (<10ppb); minimally contaminated (10-99ppb); moderately contaminated (100-1000 ppb) and highly contaminated(>1000ppb) [29].

Category of PAH concentration (ppb) in the studied smoked samples is shown in table 4. Concentrations of PAHs were 16.2 and 7.4ppb in smoked fish from farms (A) and (B), respectively. Based on these results, categories of concentration of PAH are considered a minimally contaminated (10-99ppb) for A-smoked product, may be due to pollutants presented in Al-Batsdrain and not contaminated (<10ppb) for B-smoked product compared with recommended levels as set by [28].

A-Smoked Mullet Product		B-Smoked Mullet Product	
Category	ΣPAHs (ppb)	Category	ΣPAHs (ppb)
Minimally contaminated	16.2	Not contaminated	7.4

Table 3: Category of PAH concentration (ppb) in the studied cold smoked mullet samples.

Note: Farm (A): Al-Batts Drain. Farm (B): El-Wadi Drain.

Conclusion

It could be concluded PAHs concentration in smoked mullet products processed from pre-frozen mullet samples for 6 months at -18°C are considered a minimally contaminated (16.2ppb) for A-smoked mullet product and not contaminated (7.4ppb) for B-smoked product compared with recommended levels. In addition to the component

of Benzo [a] Pyrene (B {a} P) was not detectable in all smoked fish products.

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