

Safety Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in Cold Smoked Fish (*Mugil Cephalus*) Using GC-MS

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Abstract

This work was planned to determine the safety level of polycyclic aromatic hydrocarbons (PAHs) in cold smoked mullet fish obtained from two fish farms (A and B) localized at El-Fayoum Governorate, Egypt during August 2015. PAHs components were determined by GC-MS. Results showed that total of PAHs components was 28.8 and 5 ppb in both smoked fish products (A and B), respectively. Also, low molecular weight (LMW) of PAHs was found especially in smoked fish (A), followed by medium molecular weight (MMW) and however high molecular weight (HMW) was not detectable. Levels of Benzo [a] Pyrene (B {a} P) equivalent were 0.051 and 0.005 in products (A and B), respectively. However, PAH4 (BaP+CHR+BaA+BbF) and PAH8 (Benzo (a) anthracene, Chrysene, Dibenzo (a, h) anthracene, Benzo (g, h, i) perylene, Benzo (b) Fluoranthene, Benzo (k) fluoranthene, Benzo (a) Pyrene, Indeno (1, 2, 3-c, d) Pyrene) were not detectable. Also, categories of PAHs concentration are considered a minimally contaminated (10 to 99 µg/kg) and not contaminated (<10 ppb) compared with the maximum recommended levels. Based on our results, it could be concluded that Benzo (a) pyrene compound was not detectable in all smoked samples which are considered as a safe product for human consumption.

Keywords: Fish; Smoking; PAHs; GC-MS

Introduction

Contamination of food by polycyclic aromatic hydrocarbons (PAHs) can be resulted from food processing at high temperature such as frying, smoking or roasting Codex [1-3]. There are many parameters limited the amounts of PAHs in food such as composition of the smoke, technology used in smoking, combustion temperature, type of wood, and exposure of the edible parts to the smoke [3-5]. The ranges of MW could be divided into three classes; 152 g/mol to 178 g/mol, 202 and 228 g/mol to 278 g/mol of low, medium and high MW, respectively [6,7]. Seven of the PAHs have been considered human carcinogens; benzo (a) anthracene, benzo (b) fluoranthene, benzo (k) fluoranthene, chrysene, benzo (a) pyrene, dibenzo (a, h) anthracene, and indeno (1, 2, 3-c, d) pyrene. Therefore, this study was designed to determine the safety level of PAHs in cold smoked mullet fish samples that were obtained during August 2015 from two fish farms localized at El-Fayoum governorate, Egypt (Figure 1).

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 industrial for A and agricultural discharge for B during August 2015 at El-Fayoum governorate. They were transported within two hours using ice box to Fish Processing and Technology Lab, Shakhshouk Station for Water Resource, National Institute of Oceanography and Fisheries (NIOF), Egypt. Average of weight 453.3 gm ± 51.7 gm and length 35 gm ± 3 cm for raw samples from Farm A (Industrial discharge) also, average weight and length of raw mullet samples from Farm B (Agricultural discharge) are 526.6 gm ± 18 gm and 38 cm ± 2 cm respectively.

Smoking process and source of PAHs

After that, chilled whole fish samples were washed carefully with tap water, soaked in 10% brined solution for 2 hrs, rinsed with tap water for 1 min, semi-dried at 250°C for two hrs, traditional cold smoked at 35°C to 45°C for 8 to 10 hrs, using sawdust and finally cooled under ambient temperature.

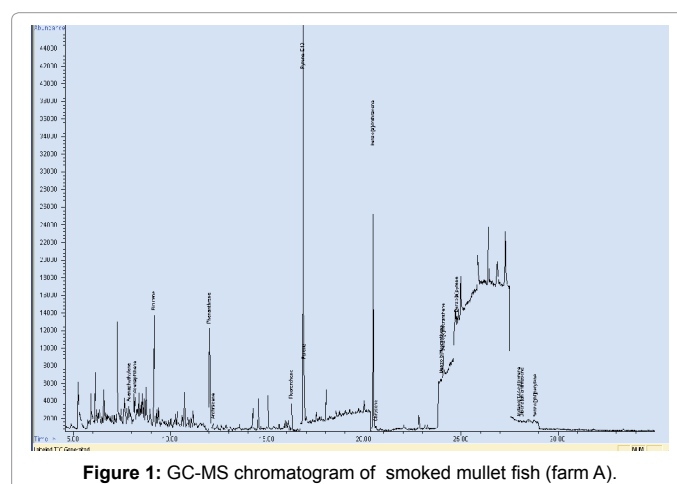


Figure 1: GC-MS chromatogram of smoked mullet fish (farm A).

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 determination: PAHs were determined at Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food

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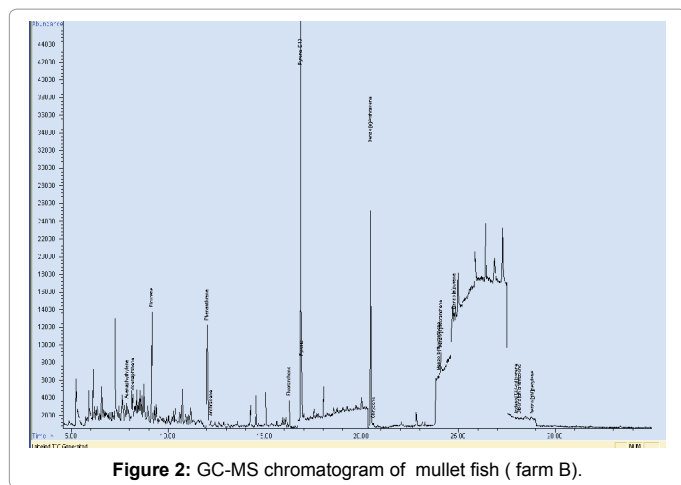


Figure 2: GC-MS chromatogram of mullet fish (farm B).

Compound	Abbrev.	Mw	Rings	Concentration (ppb)	
				Farm (A)	Farm (B)
Chrysene	CHR	228	4	ND	ND
Anthracene	ANT	178	3	2.8	ND
Acenaphthene	ACE	154	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	6.6	ND
Naphthalene	NA	128	3	ND	ND
Benzo(a)pyrene	BaP	252	5	ND	ND
Benzo(g,h,i)perylene	BghiP	276	6	ND	ND
Indeno(1,2,3,cd)pyrene	IcdP	276	6	ND	ND
Acenaphthylene	ACY	152	3	ND	ND
Fluoranthene	FLA	202	4	3.3	2.1
Pyrene	PYR	202	4	4.6	2.9
Benzo(a)anthracene	BaA	228	4	ND	ND
Phenanthrene	PHE	178	3	11.5	ND
Σ 16PAHs				28.8	5

Table 1: Molecular weight (MW), number of rings and concentration of PAHs in smoked mullet samples.

(QCAP), Agricultural Research Centre, Cairo, Egypt as described by Forsberg et al. [8], Smoker et al. [9] and Khorshid et al. [10].

B{a}P equivalent: The B{a}P_{eq} was calculated as the Σ B{a}P_{eqi} value for individual PAHs. The B {a}P_{eqi} calculated as the following equation [11]:

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

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 ≤ 0.05) and were expressed as Mean ± SD using SPSS 16 for windows.

Results and Discussion

As previous mentioned that raw mullet samples were obtained from different fish farms based on irrigation main resources; farm (A) was dependent on industrial discharge and localized at eastern Fayoum governorate farms whereas farm (B) was dependent on agriculture discharge and localized at Qarun lake farms (Figure 2).

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 products. Concentrations of ANT, FLA, PYR, FLU and PHE were 2.8, 3.3, 4.6, 6.6 and 11.5 ppb, respectively in smoked fish farm (A).

Farm (A): Industrial discharge. Farm (B): Agricultural discharge. Mw: Molecular weight.

In the other batch, FLA and PYR levels were 2.1 and 2.9 ppb, respectively in smoked fish farms (B). The PAHs have been grouped according to its molecular weights, we found that the high MW components of PAHs (228 g/mol to 278 g/mol), were Benzo (a) anthracene, Benzo (b) fluoranthene, Benzo (k) fluoranthene, Benzo (j) fluoranthene, Benzo (e) pyrene, Benzo (a) Pyrene, Benzo (ghi) perylene, dibenzo (h) anthracene, chrysene, cyclopenta (cd) pyrene, indo (1, 2, 3-c, d) Pyrene, and anthranthrene. The medium MW components (202 g/mol) were fluoranthene and pyrene. The low molecular weights (152 g/mol to 178 g/mol) included naphthalene, Acenaphthylene, acenaphthene, fluorene, anthracene and phenanthrene. Total of PAHs were 28.8 and 5 ppb in both smoked fish farms (A) and (B), respectively. In addition, rings number of PAHs in smoked fish farm (A) ranged from 3 to 4 rings and its MW ranged from 166 to 178 whereas in case of farm (B) only 4 rings and 202 as MW were found. Comparison of fish species smoked as fillets and as whole fish illustrated that the level of PAHs was higher for smoked fillets in comparison with the same fish species smoked as a whole fish.

Category of PAH concentration

Categories of concentration of PAH are considered a minimally contaminated (10 to 99 ppb) and not contaminated (<10 ppb) compared with recommended levels as set by Soares-Gomes et al. [12]. Concentrations of PAH were 28.8 and 5.0 µg/kg in smoked fish farms (A) and (B), respectively. Based on these results, Farm A (Industrial discharge) and B (Agricultural discharge) classified as minimally contaminated and Not contaminated respectively.

Molecular weight of PAHs in smoked fish

Also, Table 2 exhibits the molecular weight (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 high concentration of LWM in smoked fish farm (A) was 20.9 KDa. In other side, total concentration of medium molecular weights of PAHs was 5 KDa. Lipophilic nature of the PAHs and fish's skin may be make better protection from the HMW-PAHs than LMW as reported by Mohammadi et al. [13]. In addition, results showed that there is no found a HMW-PAHs in different smoked fish farms either (A) or (B). In all products, HMW-PAHs were below the limit of quantification or not detectable.

Toxic equivalent factors (TEFs) and B {a} P equivalent of PAHs

TEF is an estimate of the relative toxicity of individual PAH fraction

Fish farm	HMW	MMW	LMW
A	-	7.9	20.9
B	-	5	-
Farm (A): Industrial discharge. Farm (B): Agricultural discharge			

Table 2: Molecular weight (MW) of PAHs in cold smoked fish.

compared to benzo(a) pyrene. The toxic equivalent factors (TEFs) and B [a] P Equivalent of PAHs in smoked mullet fish are present in Table 3. Concentrations of phenanthrene, flourene, pyrene, fluoranthene and anthracene in smoked fish farm (A) were 11.5, 6.6, 4.6, 3.3 and 2.5 ppb, respectively and total B [a] P Equivalent was 0.051. On the other side, concentrations of pyrene and fluoranthene in smoked fish farm (B) were 2.9 and 2.1 ppb, respectively and total B [a] P Equivalent was 0.005.

Benzo [a] pyrene (BaP), PAH4 and PAH8

In this study the PAH4 (the sum of BaP, chrysene, benz [a] anthracene and benzo [b] fluoranthene) and PAH8 {Benzo (a) anthracene, Chrysene, Dibenzo (a, h) anthracene, Benzo (g, h, i) perylene, Benzo (b) Fluoranthene, Benzo (k) fluoranthene, Benzo (a) Pyrene, Indeno (1, 2, 3-c, d) Pyrene} were not detected in smoked fish samples too. So, in the current study, cold smoked products are safety for human consumption because BaP did not detect comparing to the maximum permissible limit 2 ppb as set by the European Commission Regulation (OJEU, 835/2011) [14]. These results are in accordance to the findings by El-Lahamy et al. [15] who reported that BaP not detected in cold and hot smoked catfish filets.

Sources and assessment of PAHs

Table 4 shows that source characterization and assessment of

Compound	TEF	Farm (A)		Farm (B)	
		PAHs (µg/kg)	BaP _{eqi}	PAHs (µg/kg)	BaP _{eqi}
Naphthalene	0.001	ND	-	ND	-
Acenaphthylene	0.001	ND	-	ND	-
Acenaphthene	0.001	ND	-	ND	-
Fluorene	0.001	6.6	0.0066	ND	-
Phenanthrene	0.001	11.5	0.0115	ND	-
Anthracene	0.01	2.5	0.025	ND	-
Fluoranthene	0.001	3.3	0.0033	2.1	0.0021
Pyrene	0.001	4.6	0.0046	2.9	0.0029
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	ND	-	ND	-
Indeno(1,2,3-c)pyrene	0.1	ND	-	ND	-
Dibenzo(a,h)anthracene	1	ND	-	ND	-
Benzo(g,h,i)perylene	0.01	ND	-	ND	-
Σ (BaP _{eqi})			0.051		0.005

TEF: Toxic equivalent factor; BaP_{eqi}: B[a] P equivalent; Farm (A): Industrial discharge; Farm (B): Agricultural discharge.

Table 3: Toxic Equivalent factors (TEFs) and B [a] P Equivalent of PAHs in smoked mullet fish.

Source of PAHs	PAH Ratios	
	[An/(An + Phen)]	[Fl/(Fl + Py)]
Wood combustion	178	202
Petroleum	>0.10	>0.5
Saw dust	Farm A	0.19
	Farm B	ND

[An/(An+Phen)]: anthracene plus phenanthrene. [Fl/(Fl+Py)]: fluoranthene to fluoranthene plus pyrene. Farm (A): Industrial discharge. Farm (B): Agricultural discharge.

Table 4: Sources and assessment of PAHs comparing with the previous reference.

PAHs comparing with the previous references. It is well known that the sawdust wood was used as source of PAHs in this work as mentioned above (material and methods part). Results showed that the ratio of anthracene to anthracene plus phenanthrene [An/(An+Phen)] was 0.19 in case of product obtained from farm A while it was not detected in farm B. This indicates that the wood combustion is the main source of PAHs compared with the mass 178. [An/(An+Phen)] ratio <0.10 usually is referred to petroleum while a ratio >0.10 indicates dominance of combustion [16-18]. The [Fl/(Fl+Py)] ratio also ranged from 0.41 to 0.42 in farm A and B products, respectively. The ratios ranged between 0.4 and 0.5 the refers to some amount of fossil fuel combustion sources (vehicular, fat and crude oil) of PAHs [19-22].

Conclusion

In conclusion, the safety of smoked fish has been controlled by measuring benzo (a) pyrene level, which is one of the most carcinogenic PAHs. European Commission has limited the maximum acceptable concentrations of benzo (a) pyrene at 2 ppb for smoked fish and smoked fishery products, excluding bivalve molluscs. In addition, the categories of concentration of PAH are considered a minimally contaminated and not contaminated compared with international recommended levels.

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