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Detection of Polycyclic Aromatic Hydrocarbons (Pahs) in Cold Smoked Mullet Fish Samples after 60 Days of Frozen Storage at-18°C

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ABSTRACT

The current investigation aimed to study the effect frozen storage followed by cold smoking process on polycyclic aromatic hydrocarbons (PAHs) in Mullet fish. PAHs in smoked samples after 60 days of raw frozen storage, the total PAHs content were 23.6 and 11.9 µg/kg for samples from farms A and B, respectively. Benzo(a)Pyrene, PAH4 and PAH8 not detected in all smoked samples. Toxic Equivalent Factor (TEFs) values were 0.0497 and 0.0344 in smoked samples from farms A and B, respectively.

Keywords: *Mullet fish, cold smoking, PAHs, frozen storage.*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants that are formed during the incomplete combustion of carbonaceous materials (Suchanová *et al.*, 2008). Polycyclic aromatic hydrocarbons (PAHs) comprise the largest class of chemical compounds, containing two or more fused aromatic rings made up of carbon and hydrogen atoms, known to be genotoxic agents (SCF 2002). However, PAHs also are usually penetrating into smoked products such as fish, where they are protected from light and oxygen, so after some time; the polycyclic aromatic hydrocarbon concentration in the fish stabilizes at a certain constant level (Simko and Knezo, 1992).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) (Isioma *et al.*, 2017). 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 AFSSA (2003) and Vincent *et al.*, (2007). 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_{eq}) to illustrate the toxic potency (Perugini *et al.*, 2007).The temperature range of 500–900°C is known to favor the production of high molecular weights HMW PAHs from thermal breakdown of lignin in lignocelluloses during wood combustion and also from pyrolysis of fats in fish (Essumang *et al.*, 2013 and Chukwujindu *et al.*, (2016). Seyedeh et *al.*,(2013) reported that the categories of PAHs concentration as not contaminated (<10 µg/kg); minimally contaminated (10-99 µg/kg); moderately contaminated (100-1000 µg/kg) and highly contaminated (> 1000 µg/kg). Therefore the current study aimed to know the effect of cold smoking process on polycyclic aromatic hydrocarbons levels in per frozen Mullet fish.

MATERIAL AND METHODS

Mullet Fish (M. Cephalus)

Fresh mullet fish samples (Mugil*cephalus)* about 20 kg were purchased from two fish farms (A and B). The main resources of irrigation water were El batts drain for (A) and El-Wadi drain for (B) at Fayoum Governorate during August, 2015. An average of weight was 453.3±89.6g and length was 35.00±3cm of raw

samples from Farm A. And 526.6± 25.1g and 38±1cm, respectively for raw samples from Farm B.

Sodium Chloride

Fine refined table salt of Sodium chloride (BONO) produced by Egyptian Salts and Minerals Company (EMISAL) was used. It composed of 98.5% sodium Chloride, 30-70 ppm, potassium iodate, and 0.3% humidity.

Sawdust as Smoke Source

Sawdust was purchased from local market located in Fayoum Governorate.

Preparation

Fish samples were immediately transported in ice box from farms to Fish Processing and Technology Lab., Shakshouk Research Station for Aquatic Resources, National Institute of Oceanography and Fisheries (NIOF), Egypt. After that, fish samples were carefully washed with tap water, glazed, packed in polyethylene bags and stored at -18ºC for 2 months. Raw and frozen fish samples were cold smoked.

Smoking Process

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

Polycyclic Aromatic Hydrocarbons (Pahs)

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 **Khorshid *et al.*, (2015)** as follows:.

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. Agilent QuEChERs salts and buffers were pre-packaged in anhydrous packages for EN 15662 containing 4 g magnesium sulfate ($MgSO_4$), 1 g sodium chloride (NaCl), 1 g sodium citrate, and 0.5 g disodium. PFTE or polyethylene 50 mL tubes with screw cap and 15mL tubes contain1g magnesium sulfate were obtained for sample extraction. Centrifuge up to 4000 rpm (Heraeus Labofuge 400), Vortex, Automatic Pipettes (Hirschmann Laborgerate) suitable for handling volumes of 10μl to100μl and100μl to 1000μl, 10 ml solvent dispenser (Hirschmann Laborgerate) for Acetonitrile. The glassware were washed with detergent and water then rinsed with acetone and dried at 90°C before use. Agilent 6890N series gas chromatography instrument equipped with 5975 series mass selective detector and Agilent GC Column of model J&W HP-5ms Ultra Inert with the specifications (30 m length, 0.25 mm internal diameter, 0.25μm film thickness) were used for both qualitative and quantitative determination of PAHs.

Helium gas was used as the carrier gas; the column was maintained at a constant flow rate of 1.3 ml/min. The back injector line was maintained at 260°C. Injection volumes were 1.0μl in the split less mode. The column temperature was initially held at 90°C for 2 min, ramping to180°C at a rate of 15°C/min, held at 180°C for 15 min, ramping to 250°C at a rate of 10°C/min, held for 2 min, ramping to 290°C at a rate of 10°C/min, and held for 10 min.

The mass spectrometer was operated in the ionization mode and spectra were acquired using a mass range of 45–450 m/z. Quality control and assurance of each batch were passed by monitoring the performance of the GC-MS and the mass selective detector daily by tuning the mass detector and monitoring the sensitivity and linearity of the calibration curve, respectively, and also analyzing blank sample to confirm that there in contamination effect on the results during analysis.

Results and Discussion

Table (1) shows the PAHs concentration of cold smoked Mullet fish flesh which has previously frozen for 60 days. 16 components of PAHs were detected in edible part of investigated products. The total concentration of PAHs was 23.6 µg / kg. Phenanthrene have the highest concentration (9 µg / kg), then Fluorene (4.8 µg / kg), Pyrene (3.7 µg / kg), Fluoranthene (3.2 µg / kg) and Anthracene (2.9 µg / kg). While the total concentration of PAHs residues in farm B samples was 11.9 µg / kg. The highest concentration was for Pyrene (3.9 µg / kg), then Fluoranthene (3.2 µg / kg), Anthracene (2.5 µg / kg) and Phenanthrene (2.3 µg / kg). 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. Our results are in agreement with those findings reported by Duedahl-Olesen *et al.* (2006); and Wretling *et al.*, *(*2010), who found that the variation in PAHs is due to the source of smoke, combustion temperature, flame intensity, surface and size of product, skin on and skin off, and the structure of the product.

Toxic Equivalent Factors (Tefs) and B {A} P Equivalent of Pahsfound in Smoked Mullet Fish Samples

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}) (**Nisbet and LaGoy, 1992).**

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

After 60 days of frozen storage of raw fish samples (2), the samples were smoked. the TEFs factors of Fluorene; Phenanthrene; Anthracene; Fluoranthene and Pyrene were 0.001; 0.001 ; 0.01; 0.001 and 0.001 and their B [a] P Equivalent were 0.0048; 0.009; 0.029; 0.0032 and 0.0037 for samples from farm A, respectively. On the other, hand the TEFs factors of Fluorene, Phenanthrene; Anthracene; Fluoranthene and Pyrene were 0.001, 0.001; 0.01; 0.001 and 0.001 and their B [a] P Equivalent were 0.0048, 0.009; 0.029; 0.0032 and 0.0037 for farm A smoked samples respectively. While farm B were 0.0023, 0.025, 0.0032 and 0.0039 for Phenanthrene; Anthracene; Fluoranthene and Pyrene, respectively. The sum of B [a] P Equivalent (∑ (BaPeqi) of PAHs compounds were 0.0497 and 0.0344 for farm A and B respectively.

Table1. *The concentration of polycyclic aromatic hydrocarbons (PAHs) in cold smoked mullet fish samples after 60 days of frozen storage at -18°C*

Compound	Abbrev.	Mw	Rings	Concentration (µg/kg)	
				Farm (A)	Farm (B)
Chrysene	CHR	228	4	ND	ND
Anthracene	NT	178	3	**2.9**	**2.5**
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	**4.8**	ND
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,cd)pyrene	IcdP	276	6	ND	ND
Acenaphthylene	ACY	152	3	ND	ND
Fluoranthene	FLA	202	4	**3.2**	**3.2**
Pyrene	PYR	202	4	**3.7**	**3.9**
Benzo(a)anthracene	BaA	228	4	ND	ND
Phenanthrene	PHE	178	3	**9**	**2.3**
Σ 16PAHs				**23.6**	**11.9**

Farm (A): *Al-Batts Drain,* ***Farm (B):*** *El-Wadi Drain,* ***Mw:*** *Molecular,* ***ND:*** *not detected*

Table2. *Toxic Equivalent Factors (TEFs) and B [a] P Equivalent of PAHs found in pre-frozen cold smoked mullet fish after 60 days of frozen storage at -18°C*

Compound	TEF	Farm (A)		Farm (B)	
		Conc. (µg/kg)	BaP_{eqi}	Conc. (µg/kg)	BaP_{eqi}
Naphthalene	0.001	ND	-	ND	-
Acenaphthylene	0.001	ND	-	ND	-
Acenaphthene	0.001	ND	-	ND	-
Fluorene	0.001	**4.8**	**0.0048**	ND	-
Phenanthrene	0.001	**9**	**0.009**	**2.3**	**0.0023**
Anthracene	0.01	**2.9**	**0.029**	**2.5**	**0.025**
Fluoranthene	0.001	**3.2**	**0.0032**	**3.2**	**0.0032**
Pyrene	0.001	**3.7**	**0.0037**	**3.9**	**0.0039**
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	-
∑ (BaP_{eqi})			**0.0497**		**0.0344**

TEF: *Toxic equivalent factor,* ***BaP_{eqi}[a]:*** *P equivalent,* ***Farm (A):*** *Al-Batts Drain,* ***Farm (B):*** *El-Wadi Drain*

Molecular Weight of Pahs in Smoked Fish

Table (3) exhibits 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) throughout storage periods.

In the other side, for samples from B farm the total concentration of medium and low molecular weights of PAHs were 7.1 and 4.8 µg / kg after 60 days of frozen. This may be due to the lipophilic nature of the PAHs and it may be that the fish's skin protected them from the HMW-PAHs than LMW as reported by Mohammadi *et al.*, (2013). Most of the carcinogenic PAHs fall within the group of the HMW (EFSA, 2002). In addition, results showed that the HMW-PAHs did not detected in different smoked fish farms either (A) or (B). In all products, HMW-PAHs were below the limit of quantification or not detectable. The increase in the concentration of low molecular weight hydrocarbons over the smoking 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 (Essumang *et al*., 2013 and Chukwujindu *et al*., (2016).

Category of PAH Concentration

Category of PAH concentration (µg/kg) in the studied smoked samples is shown in Table (4). Concentrations of PAHs were 23.6 and 11.9µg/kg in smoked fish from farms (A) and (B), respectively after 60 days. Based on these results, categories of concentration of PAH are considered a minimally contaminated (10-99 µg/kg) for farm A and B respectively compared with recommended levels as set by Seyedeh et *al.*, (2013).

Table3. *Total mean concentration (µg / kg) of PAHs in cold smoked fish, according to their molecular weights*

Storage period (days)	**Concentrations (µg /kg) of the PAHs for Farm A samples**			**Concentrations (µg/kg) of the PAHs for Farm B samples**		
	HMW	**MMW**	**LMW**	**HMW**	**MMW**	**LMW**
60	-	**6.9**	**16.7**	-	**7.1**	**4.8**

HMW: *high molecular weight,* ***MMW:*** *medium molecular weight,* ***LMW:*** *low molecular weight,* ***Farm (A):*** *Al-Batts Drain,* ***Farm (B):*** *El-Wadi Drain*

Table4. *Category of PAH concentration (µg/kg) in the studied cold smoked samples*

Storage Period (days)	**Farm A**		**Farm B**	
	Category	**ΣPAHs**	**Category**	**ΣPAHs**
60	Minimally contaminated	**23.6**	Minimally contaminated	**11.9**

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

Sources and Assessment of Pahs

Table (5) shows that Source characterization and assessment of 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 0.24 and 0.52 for farm A and B respectively after 60 days of frozen storage. 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 an indication of petroleum while a ratio > 0.10 indicates dominance of combustion(Zhang et al., 2006; Pies et al., 2008 and Placha´ et al., 2009).The [Fl/(Fl + Py)] ratio(Fluoranthene to Fluoranthene plusPyrene) also ranged from 0.46 and 0.45 for farm A and B respectively . The levels from smoked fish

samples with ratios between 0.4 and 0.5 imply some amount of fossil fuel combustion sources (fat, vehicular and crude oil) of PAHs. The (especially pyrolysis of fat) pyrogenic source can be attributed to the high levels of fat content (Phillips, 1999 and Kazerouniet al., 2001). [BaA/(BaA + Chry)], [BaP/(BghiP)] and [Ind/(Ind + BghiP)] did not detected in all the tested cold smoked products. While in the other studies, it could be noticed that the mass 228, [BaA/(BaA + Chry)] ratio was < 0.20 implies petroleum, 0.20–0.35 indicates either petroleum or combustion (mixed), and >0.35 implies combustion(Maher and Aislabie, 1992; Gilbert et al., 2006; Zhang et al., 2006; Pies et al., 2008 and Placha´ et al,. (2009).

Table5. *Sources and assessment of PAHs for pre-frozen cold smoked samples*

Source of PAHs			PAH Ratios				
			[An/ (An + Phen)] 178	[Fl/ (Fl + Py)] 202	[BaA/ (BaA + Chry)] 228	[BaP/ (BghiP)]	[Ind/ (Ind + BghiP)]
Wood combustion			>0.10	>0.5	1.2–5.0	1.2–5.0	>0.5
Petroleum			<0.10	0.40	<0.20	>0.6	<0.5
Sawdust	**60 days**	**Farm A**	**0.24**	**0.46**	ND	ND	ND
		Farm B	**0.52**	**0.45**	ND	ND	ND

(An): *anthracene* ***(Phen):*** *phenanthrene,* ***(Fl):*** *Fluoranthene* ***(Py):*** *pyrene,* ***(BaA):*** *benzo [a] anthracene* ***(Chry):*** *chrysene,* ***(BaP):****benzo [a] pyrene* ***(BghiP):*** *benzo [g,h,i] perylene,* ***(Ind)****: indeno[1,2,3- cd]pyrene* ***(BghiP) :*** *benzo[g,h,i]perylene,* ***Farm (A):*** *Al-Batts Drain,* ***Farm (B):*** *El-Wadi Drain.*

REFERENCES

[1] AFSSA (2003).AFSSA opinion on a request for an opinion on the risk assessment of benzo [a] pyrene B [a] P and other polycyclic aromatic hydrocarbons (PAHs), present in various commodities or in certain vegetable oils, as well as PAH concentration levels in commodities beyond which health problems may arise. French Food Safety Agency.Referral no. 2000-SA-0005.

[2] Chukwujindu M. A., Francisca, I. B., Iwekumo, A., Eferhire, A., and Grace, O.B.I., 2016. Concentrations and risks of polycyclic aromatic hydrocarbons in smoke-cured fish products in Nigeria. International J. of Environmental Studies, 2016.

[3] Duedahl-Olesen, L., White, S. and Binderup, M.L., (2006). Polycyclic aromatic hydrocarbons (PAHs) in Danish smoked fish and meat products. Polycyclic Aromatic Compounds 26 (3), 163–184.

[4] EFSA (2002) European Food Safety Authority.Scientific Committee on Food. Opinion on the risks to human health of polycyclic aromatic hydrocarbons in food, Italy

[5] Essumang, D.K., Dodoo, D.K. and Adjei, J.K., 2013, Effect of smoke generation sources and smoke curing duration on the levels of polycyclic aromatic hydrocarbon (PAH) in different suites of fish. Food and Chemical Toxicology, 58, 86–94.

[6] Gilbert, E., Dodoo, D.K., Okai-Sum, F., Quagraine, E.K., (2006). Characterization and source assessment of heavy metal and Polycyclic Aromatic Hydrocarbons (PAHs) in sediments of the Fosulegoon, Ghana. J. of Environmental Science and Health, Part A 41, 2747–2775.

[7] Isioma T., Ozekeke O. and Lawrence E. (2017). Human health risk assessment of polycyclic aromatic hydrocarbons (PAHs) in smoked fish species from markets in Southern Nigeria. Toxicology Reports 4 (2017) 55–61.

[8] Kazerouni, N., Sinha, R., Hsu, C.H., Greenberg, A. and Rothman, N. (2001). Analysis of 200 food items for benzo (a)pyrene and estimation of its intake in an epidemiologic study. Food and Chemical Toxicology 39, 423–436.

[9] Khorshid, M., Souaya, E. R., Hamzawy, A. H. and Mohammed, M. N. (2015). QuEChERS Method Followed by Solid Phase Extraction Method for Gas Chromatographic Mass Spectrometric Determination of Polycyclic Aromatic Hydrocarbons in Fish. Int. J. of Analytical Chem. Article ID 352610.

[10] Maher, W.A. and Aislabie, J., 1992. Polycyclic aromatic hydrocarbons in near shore marine sediments of Australia. Science of the Total Environment 112, 143–164.

[11] Mohammadi A., Ghasemzadeh-Mohammadi, V., Haratian, P., Khaksar, R. and Chaichi, M. (2013). Determination of polycyclic aromatic hydrocarbons in smoked fish samples by a new microextraction technique and method optimisation using response surface methodology. J Food Chem 141: 2459-2465

[12] Nisbet, I. C. T. and LaGoy, P. K., (1992). Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs)," Regulatory Toxicology and Pharmacology, vol. 16, pp. 290-300.

[13] Perugini, M., Visciano, P., Giammarino, A., Manera, M., Di Nardo, W. and Amorena, M. (2007).Polycyclic aromatic hydrocarbons in marine organisms from the Adriatic Sea, Italy.Chemosphere, 66, 1904e1910.

[14] Phillips, D.H.(1999). Polycyclic aromatic hydrocarbons in the diet, Mutat. Res./Genet. Toxicol. Environ. Mutagenesis 443 (1) 139–147

[15] Pies, C., Hoffmann, B., Petrowsky, J., Yang, Y., Ternes, T.A. and Hofmann, T. (2008). Characterization and source identification of polycyclic aromatic hydrocarbons (PAHs) in river bank soils. Chemosphere 72, 1594–1601.

[16] Placha´, D. ,Raclavska´ , H. , Maty´ sek, D. and Ru¨ mmeli, M.H.,(2009).The polycyclic aromatichydrocarbon concentrations in soils in the Region of ValasskeMezirici, theCzech Republic.Geochemical Transactions (10), 12, http://dx.doi.org/10.1186/1467-4866-10-12.

[17] Scientific Committee on Foods of EC, SCF, (2002).Opinion of the Scientific Committee on Food in the Risk to Human Health of PAHs in Food.Scientific Committee on Foods of EC, SCF, Brussels.Seyedeh, L. M. N., Wan, R. I., and Mohamad, P. Z. (2013). Residual Concentration of PAHs in Seafood from Hormozgan Province, Iran: Human Health Risk Assessment for Urban Population. International J. of Environmental Science and Development, Vol. 4, No. 4, August 2013.

[18] Simko, P., and J. Knezo, (1992). Influence of cooking on benzo(a)pyrene content in frankfurters. J. Mol. Nutr. Food Res., 36(2): 208-209.

[19] Suchanová, M., Haj, L. J., Tomaniová, M., Kocourek, V. and Babika, L. (2008).Polycyclic aromatic hydrocarbons in smoked cheese. J. Sci. food Agric., 88(8): 1307-1317.

[20] Vincent, V., Thierry, S., Fabrice, M., Bruno, L., and Carole, P. (2007). Determination of PAH profiles by GC–MS/MS in salmon processed by four cold-smoking techniques. J. Food Additives and Contaminants, July 2007, 24(7): 744–757.

[21] Wretling, S., Eriksson, A., Eskhult, G.A. and Larsson, B., (2010). Polycyclic aromatic hydrocarbons (PAHs) in Swedish smoked meat and fish. J. of Food Composition and Analysis 22, 264–272.

[22] Zhang, H.B., Luo, Y.M., Wong, M.H., Zhao, Q.G. and Zhang, G.L. (2006).Distributions and Concentrations of PAHs in Hong Kong Soils. Environmental Pollution 141, 107–114.

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