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Research Article

Levels of Polycyclic Aromatic Hydrocarbons in Fried Tilapia Fish (*O. niloticus*) using GC-MS

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Abstract

This work was conducted to determine the safety levels of polycyclic aromatic hydrocarbons (PAHs) in deep oil fried tilapia fish obtained from two fish farms (A and B) located in Fayoum governorate, Egypt during June 2016. PAH compounds were determined by GC-MS. Results showed that the levels of total PAHs were 36.8 and 40.5 µg/kg in both fried samples from farm A and B. Levels of B[a]P Equivalent of PAHs in fried Tilapia fish were 1.7099 and 0.0405 in farm A and B samples. Based on our results, it could be concluded that benzo[a]pyrene compound was not detectable in all fried samples which are considered as a safe product for human consumption.

Keywords: Fish; Frying; PAHs; GC-MS

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of environmental contaminants that originate from the pyrolysis or incomplete combustion of organic matter [1]. They are universal contaminants of our environment and of the human food chain [2]. 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 [3-5]. The nature of PAHs makes them present as trace contaminants in air, water and soil [6]. They are ubiquitous environmental contaminants that are formed during the incomplete combustion of carbonaceous materials [7]. Although air and drinking water may be responsible for some human exposure, the highest PAHs intake is typically associated with their occurrence in diet (food) [7]. The U.S. Environmental Protection Agency (EPA) selected 16 priority PAHs based on their occurrence and carcinogenicity. In recent years, studies have found that benzo[a]pyrene is not a suitable marker for PAHs occurrence in foods, since it is not a good indicator of the concentration of other carcinogenic PAHs. Thus, the use of the sum of eight genotoxic PAHs, benzo[a]pyrene equivalents (BaPeq), as well as the sum of four PAHs, including benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene was recommended [8-9]. The presence of PAHs in food is usually a consequence of the nature of these compounds in the environment at their formation during cooking processes or as a result of the manufacturing processes [10]. Electric-plate grilling or frying would generate PAHs in cooked meat and fish, especially throughout the first cooking method which causes a highly rise in the formation of carcinogenic PAHs due to the direct contact of foods with the heat source leading to generate PAHs at a higher extent than the second one, as the result of incomplete combustion and pyrolysis of organic matter including proteins and fats [11]. Janoszka et al. [12], reported that the health hazard level of the PAHs daily ingested in the diet was found to be 3.7 µg in Great Britain, 5.17 µg in Germany, 1.2 µg in New Zealand and 3 µg in Italy. Also, FAO/WHO [13], reported that total PAHs level of 14 µg/kg in cooked and processed foods considered to be carcinogenic and mutagenic.

In [8], EFSA published an opinion on PAHs and concluded that BaP alone was not a suitable general marker for PAHs in food, but identified a group of 4 PAHs (PAH4) (the sum of benzo[a]pyrene, chrysene, benzo[a]anthracene, and benzo[b]fluoranthene) and a group of 8 PAHs (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) as better indicators based on data related to occurrence and toxicity. Measuring PAH8 offered little additional benefit compared with PAH4. Based on the EFSA opinion, the European Commission extended the scope of the regulation to include other types of food and to add limits for PAH4 [14]. As oil breaks down, it produces compounds that cause off-flavours and darkening, some of which may be toxic at high concentrations. Some volatile compounds formed during deep-fat frying are known to be toxic (e.g. 1,4-dioxane, benzene, toluene and hexyl-benzene) or potentially carcinogenic, such as carbonyl compounds or monoepoxides and some aldehydes produced from linoleic acid (e.g. 4-hydroxy-2-transnonenal which has been proven to be cytotoxic) [15]. Therefore, this study was designed to determine the safety level of PAHs in deep-oil fried tilapia fish samples that were obtained during June 2016 from two fish farms localized at El-Fayoum governorate, Egypt.

2. Materials and Methods

2.1 Fish samples

Tilapia fish (*O. niloticus*) 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 June 2016 at Fayoum Governorate. They were transported within two hours using ice box to Fish Processing and Technology Lab, Shakshouk Station for Water Resource, National Institute of Oceanography and Fisheries (NIOF), Egypt. Average weight was 303 ± 31.5 gm and length 25.9 ± 0.22 cm for raw samples from Farm A also; average weight and length of raw mullet samples from Farm B are 327 ± 93.8 gm and 26 ± 2.8 cm, respectively.

2.2 Frying process

Frying process was carried out as shown in Figure 1. The prepared fish was rubbed with flour left for 3-4 min and fried in pre-heated deep fried oil at 170-180ºC for 10-15 min using Electrical Fryer pan (Moulinex brand). Fried fish samples were removed when a golden brown color was appeared on their surfaces. They were placed in the frying basket to drain out the excess amounts of cooking oil then cooled and kept for analysis.

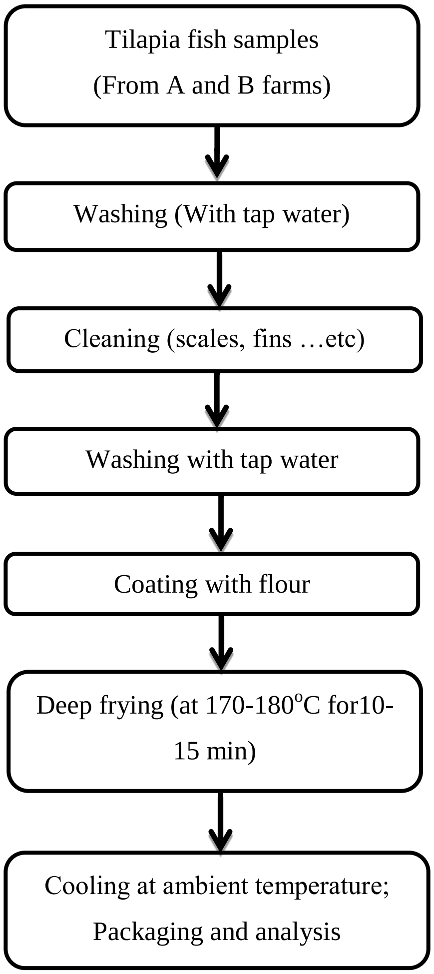

Figure 1: Flowchart of Tilapia fish frying.

2.3 Analytical methods

The edible of fried tilapia fish products was manually separated, homogenized, packed in polyethylene bags and then stored in a freezer at -20°C till analysis.

2.3.1 PAHs determination: 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 Forsberg et al. [16], Smoker et al. [17] and Khorshid et al. [18]. 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 1.0 μl 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.

B{a}P equivalent:

The B{a}Peq was calculated as the Σ B{a}Peqi value for individual PAHs. The B{a}Peqi calculated as the following equation:

$$\text{BaPeq} = \Sigma\,(\text{BaPeqi}) = \Sigma\,(\text{CPAHi} \times \text{TEFPAHi}) \qquad (1)$$

CPAHi: Concentration of each PAH in the sample; TEFPAHi: Toxic equivalency factor for each individual PAH.

2.3.2 Statistical analysis: The results obtained were analyzed statistically using the least significant difference test (LSD) at ($P \leq 0.05$) and were expressed as Mean, SD using SPSS 16 for windows.

3. Results and Discussion

3.1 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons compounds (PAHs) were determined in edible part of fried tilapia fish samples 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 obtained are given in Table (1). Benzo[a]anthracene, Phenanthrene and Chrysene were detected in farm A samples with amounts 16.9, 19.9 µg/kg and <LOQ (<2 µg/kg) respectively. The total amounts of PAHs were 36.8 µg/kg in farm A sample. While only Naphthalene was detected in farm B sample with amount 40.5 µg/kg. Our results agreed with Phillips [19] and Anderson et al. [11], who reported that electric-plate grilling or frying would generate PAHs in cooked meat and fish, and El-Badry et al. [20], who found that the amounts of PAHs increased after frying of tilapia fish.

3.2 B{a}P Equivalent of PAHs and toxic equivalent factors (TEFs)

Benzo[a]Pyrene (BaP) has been well characterized as the most potent carcinogenic PAH after ibenz[a,h]anthracene. Therefore, the total PAH concentration is expressed as Benzo[a]Pyrene Equivalents (BaPeq) to illustrate the toxic potency [21]. The BaPeqi was calculated as the sum of BaPeqi value for individual PAHs. The BaPeqi value was calculated for each PAH from its concentration in the sample (CPAHi) multiplied by its toxic equivalency factor (TEFPAHi) [22].

$$\text{BaPeqi} = \sum (\text{BaPeqi}) = \sum (\text{CPAHi} \times \text{TEFPAHi}) \qquad (2)$$

Table (2) shows the toxic equivalent factors (TEFs) and B[a]P Equivalent of PAHs in fried tilapia fish samples obtained from A and B farms. The B[a]P Equivalent of Phenanthrene and Benzo(a)anthracene were 0.0199 and 1.69 respectively for farm A and the sum of B[a]P Equivalents ∑ (BaPeqi) 1.7099. On other side the B[a]P Equivalent of Naphthalene was 0.0405 for farm B and the sum of B[a]P Equivalents ∑ (BaPeqi) 0.0405.

Compound	**Abbrev.**	**Mw**	**Rings**	**Concentration (µg/kg)**	
				Farm (A)	**Farm (B)**
Chrysene	CHR	228	4	<LOQ	ND
Anthracene	ANT	178	3	ND	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	ND	ND
Naphthalene	NA	128	2	ND	40.5
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	ND	ND
Pyrene	PYR	202	4	ND	ND
Benzo[a]anthracene	BaA	228	4	16.9	ND
Phenanthrene	PHE	178	3	19.9	ND
Σ 16PAHs				36.8	40.5

Farm (A): Al-Batts drain; Farm (B): El-Wadi drain; Mw: Molecular weight; LOQ: 2 µg / kg

Table 1: The concentrations of PAHs in fried tilapia samples.

Compound	**TEF**	**Farm (A)**		**Farm (B)**	
		Conc. (μg/kg)	**BaP_{eqi}**	**Conc. (μg/kg)**	**BaP_{eqi}**
Naphthalene	0.001	ND	ND	40.5	0.0405
Acenaphthylene	0.001	ND	ND	ND	ND
Acenaphthene	0.001	ND	ND	ND	ND
Fluorene	0.001	ND	ND	ND	ND
Phenanthrene	0.001	19.9	0.0199	ND	ND
Anthracene	0.01	ND	ND	ND	ND
Fluoranthene	0.001	ND	ND	ND	ND
Pyrene	0.001	ND	ND	ND	ND
Benzo[a]anthracene	0.1	16.9	1.69	ND	ND
Chrysene	0.01	<LOQ	ND	ND	ND
Benzo[b]fluoranthene	0.1	ND	ND	ND	ND
Benzo[k]fluoranthene	0.1	ND	ND	ND	ND
Benzo[a]pyrene	1	ND	ND	ND	ND
Indeno[1,2,3,c]pyrene	0.1	ND	ND	ND	ND
Dibenzo[a,h]anthracene	1	ND	ND	ND	ND
Benzo[g,h,i]perylene	0.01	ND	ND	ND	ND
$\sum$ (BaP_{eqi})			1.7099		0.0405

TEF: Toxic equivalent factor; BaP_{eqi}[a]: P equivalent; LOQ: 2 μg / kg; Farm (A): Al-Batts drain; Farm (B): El-Wadi drain

Table 2: Toxic Equivalent factors (TEFs) and B [a] P Equivalent of PAHs in fried Tilapia fish.

4. Conclusion

In conclusion, the safety of fried 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 levels of B[a]P Equivalent of PAHs in fried Tilapia fish were 1.7099 and 0.0405 in farm A and B samples. Based on our results, it could be concluded that Benzo[a]pyrene compound was not detectable in all fried samples which are considered as a safe product for human consumption.

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