



Polycyclic Aromatic Hydrocarbons as Food Toxicant in Smoked Fishes

Emel ÖZ 

Atatürk University, Faculty of Agriculture, Department of Food Engineering, Erzurum, Turkey
e-mail: emel.oz@atauni.edu.tr
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ABSTRACT: Fish plays important role in human nutrition and health due to its nutritional value. On the other hand, fresh fish is perishable material because of its high moisture content. Therefore, various methods are used to maintain and extend the shelf life of fish. Smoking is one of the most common food preservation methods in fish processing. However, wood smoke used in the smoking process contains hazardous chemical compounds like polycyclic aromatic hydrocarbons. Polycyclic aromatic hydrocarbons (PAHs) are organic compounds consisting of two or more fused aromatic rings. Epidemiological studies have shown that a number of PAHs are mutagenic and/or carcinogenic. Therefore, it is needed more information about PAHs found and/or formed in smoked fishes. In the present review, the structure and toxicity of PAHs, the factors that affect PAH concentration and the studies conducted in this context, and legal limits about PAHs in smoked fishes were reviewed.

Keywords: Food toxicant, Smoking, Fish, Carcinogenicity, Mutagenicity

Tütsülenmiş Balıklarda Gıda Toksikantı Olarak Polisiklik Aromatik Hidrokarbonlar

ÖZ: Balık, sahip olduğu besin değeri nedeniyle insan beslenmesinde ve sağlığında önemli rol oynamaktadır. Diğer taraftan taze balık, yüksek su içeriği nedeniyle kolay bozulabilir bir materyaldir. Bu nedenle, balığın raf ömrünü korumak ve uzatmak amacıyla çeşitli metotlar kullanılmaktadır. Tütsüleme, balık işlemede en yaygın kullanılan gıda muhafaza yöntemlerinden biridir. Ancak tütsüleme prosesinde kullanılan odun dumanı, polisiklik aromatik hidrokarbonlar (PAH) gibi bazı zararlı bileşikler içermektedir. PAH'lar iki veya daha fazla kaynaşmış aromatik halkadan oluşan organik bileşiklerdir. Epidemiyolojik çalışmalar, bazı PAH'ların mutajenik ve/veya karsinojenik olduğunu göstermiştir. Bu nedenle, tütsülenmiş balıkta bulunan ve/veya oluşan PAH'lar hakkında daha fazla bilgiye ihtiyaç vardır. Bu derlemede PAH'ların yapısı ve toksisitesi, tütsülenmiş balıklarda PAH'ların oluşumunu etkileyen faktörler, bu bağlamda yapılan çalışmalar ve tütsülenmiş balıklardaki PAH'ların yasal sınırları derlenmiştir.

Anahtar kelimeler: Gıda toksikantı, Tütsüleme, Balık, Karsinojenlik, Mutajenlik

INTRODUCTION

Functional compounds found in foods include anticholesterolemic, antioxidant, antivirals, antimutagens and anticarcinogens. On the other hand, many chemical compounds that are mutagenic and carcinogenic have been found in foods. Therefore, foods are responsible for the prevention and treatment of certain diseases, as well as the emergence of certain diseases (Sun et al., 2019).

Epidemiological and toxicological studies have shown that nutrition is clearly associated with health problems such as coronary heart disease, stroke, certain types of cancer, diabetes and atherosclerosis. Therefore, nutrition is very important for human health. The nutritional benefits of fish relate to the content of protein (high-quality protein and essential amino acids), fat content (fatty acid composition, especially omega-3 fatty acids), as well as minerals and vitamins (Domingo, 2016). However, fish spoils easily due to its high moisture content, nutritional

value, low connective tissue content and neutral pH (Gokoglu, 2019). Therefore, it is considered as highly perishable material, when left unprocessed (Turhan et al., 2013; Slamova et al., 2017). Smoking is one of the oldest methods widely used in the preservation of fish. In fish processing technology, smoking process is used not only for its taste and aroma, but also for inactivating effect of smoke on enzymes and microorganisms (Simko, 2002; Slamova et al., 2017). On the other hand, smoking of fishes may cause the formation of some mutagenic and/or carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs) (Stolyhwo and Sikorski, 2005).

PAHs or polyarenes are organic compounds consisting of two or more fused aromatic rings without any heteroatom or substituent (Plaza-Bolanos et al., 2010). To date, 660 different PAHs have been identified (Stolyhwo and Sikorski, 2005). More than

160 PAHs were found in nature, but United States - Environmental Protection Agency (EPA) selected only 16 of them as primary contaminants (Chen and Chen, 2001). Thus, it is needed more about information about these food toxicants.

The objective of the present review is to give information about PAHs contamination and/or formation in smoked fishes. Therefore, in the present study, the structure and toxicity of PAHs, the factors affecting the formation of PAHs in smoked fish, the studies conducted in this context, and the legal limits about PAHs in smoked fishes were presented.

Structure, mutagenicity and carcinogenicity of PAHs

PAHs can be classified into two groups according to their relative molecular weight and the number of benzene rings: light and heavy PAHs. The compounds containing 2-4 aromatic rings are called as “light” PAHs, while the compounds containing

more than four rings are known as “heavy” PAHs (Sun et al., 2019). All PAHs are high lipophilic, non-polar compounds and solid at room temperature and their general characteristics are high melting and boiling point, and very little water solubility (Chen and Chen, 2005; Plaza-Bolanos et al., 2010). On the other hand, heavy PAHs are more toxic and stable compounds than the light PAHs (Purcaro et al., 2013).

The carcinogenicity of PAHs depends on their structure. The chemical structure of the 16 PAHs that selected as priority pollutants by the EPA were given in Figure 1.

The light PAHs with molecular mass of below 216 Da are regarded as not carcinogenic. On the other hand, benzo[a]pyrene (BaP) with molecular mass of 252 Da are very mutagenic and carcinogenic. Therefore, BaP in smoked products is selected as a marker of carcinogenic PAHs (Scientific Committee on Food, 2002).

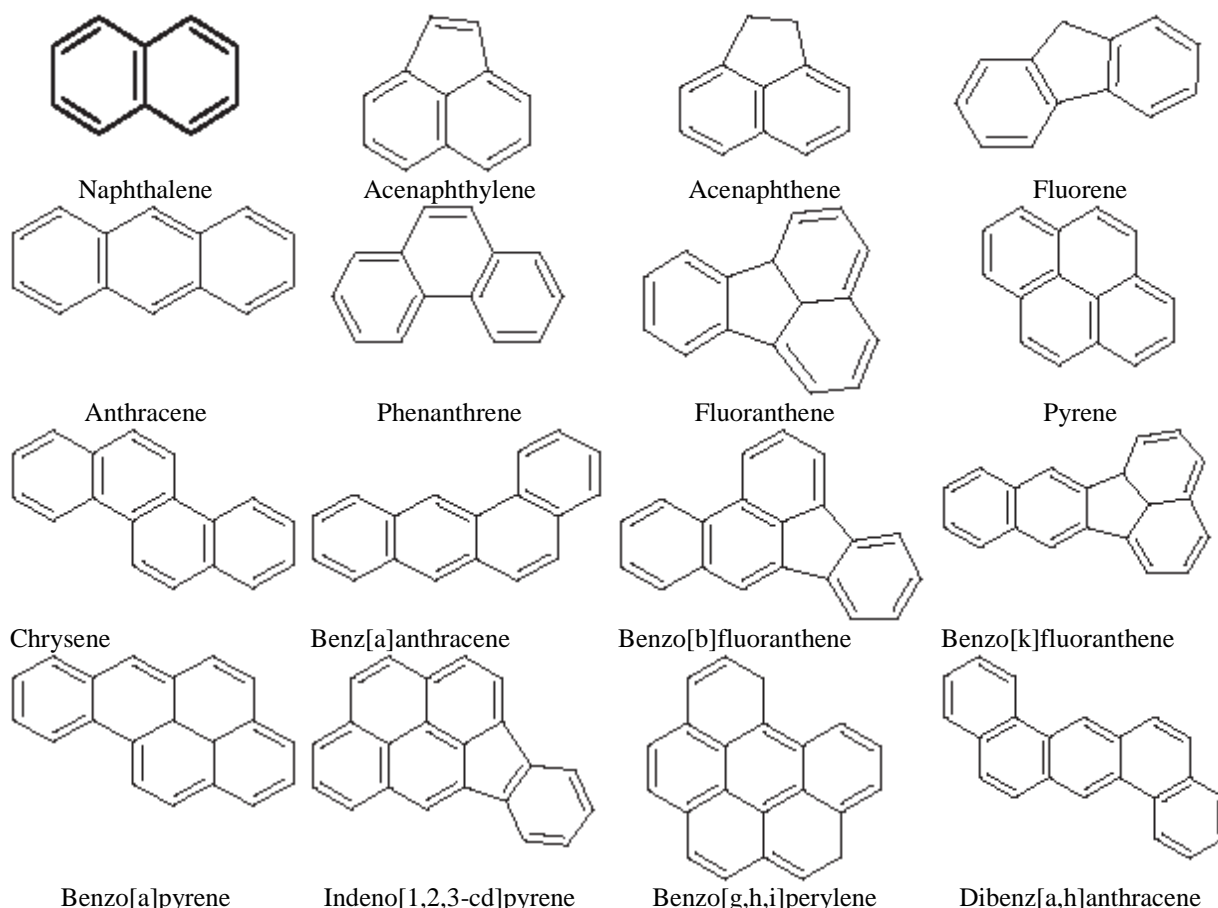


Figure 1. Chemical structure of the 16 PAHs listed by the US Environmental Protection Agency as priority pollutants (Mottier et al., 2000).

Mutagenicity and carcinogenicity of PAHs have been reported by the food and nutrition authorities such as the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the International Agency for Research on Cancer (IARC), the European Scientific

Committee on Food (SCF), the European Food Safety Authority (EFSA) and the US Environmental Protection Agency (EPA) (Ledesma et al., 2016). The mutagenic and carcinogenic properties of PAHs were given in Table 1.

Table 1. Carcinogenic and mutagenic properties of PAHs

| Compound names | Genotoxicity | IARC classification* |
|-----------------------------|--------------|----------------------|
| Acenaphthene | Questionable | Not yet evaluated |
| Acenaphthylene | Questionable | Not yet evaluated |
| Anthracene | Negative | 3 |
| Benz(a)anthracene | Positive | 2B |
| Benzo(b)fluoranthene | Positive | 2B |
| Benzo(k)fluoranthene | Positive | 2B |
| Benzo(g,h,i)perylene | Positive | 3 |
| Benzo(a)pyrene | Positive | 1 |
| Chrysene | Positive | 2B |
| Dibenz(a,h)anthracene | Positive | 2A |
| Fluoranthene | Positive | 3 |
| Flourene | Negative | 3 |
| Indeno(1,2,3-cd)pyrene (IP) | Positive | 2B |
| Phenanthrene | Questionable | 3 |
| Pyrene | Questionable | 3 |
| Naphthalene | Positive | 2B |

* 1: Carcinogenic, 2A: Probably carcinogenic, 2B: Possibly carcinogenic, 3: Not classifiable

While BaP was classified as a Group 1 carcinogen (known humans carcinogen), 16 others of PAHs were also classified as either Group 2A (probably human carcinogen) or Group 2B (possible human carcinogen) carcinogens (IARC, 2010).

Some of PAHs are considered as genotoxic carcinogens, while some of PAHs are not defined as carcinogens but may act as synergists (Plaza-Bolanos et al., 2010). People are exposed to PAHs through air, water and cigarette smoke. On the other hand, the most common source of the major routes of human exposure to PAHs in non-smoking people is food such as meat, fish, fruits and vegetables (Falco et al., 2003; Paris et al., 2018). Epidemiologists have estimated that approximately 30-40% of the colon, breast, lung, prostate, thyroid, skin, ovary, esophagus, endometrium and stomach cancers are related to foods (Ferguson, 2002a; 2002b). PAHs are the largest chemical compounds known to cause cancer (Chen and Chen, 2005; Öz and Yüzer, 2016). It is reported that benz[a]anthracene (BaA), BaP, benzo[b]fluoranthene (BbF), benzo[j]fluoranthene (BjF), benzo[k]fluoranthene (BkF), chrysene (Chry), dibenzo[a,h]anthracene (DahA), and indeno[1,2,3-c,d]pyrene (IncdP) caused tumors in laboratory animals through inhalation and oral exposure, as well as through long periods of skin contact (Falco et al., 2003).

The analysis of PAHs

PAHs are usually found in foods at the level of ppb (Simko, 2002). The main problems with the determination of PAHs in foods are low detector levels, various potential interfere compounds present in the sample and complexity of foods. Therefore, chromatographic methods have been frequently used in recent years.

There are two main approaches to determining PAHs in foods. The first is to determine the 15-20 common PAHs. These PAHs contain both carcinogenic compounds such as BaP, DahA, BbF and IncdP, and non-carcinogenic compounds such as phenanthrene (Phe), anthracene (AnT), pyrene (Pyr) and benzo[ghi]perylene (Bghip). The other approach is the determination of BaP as an indicator for all PAHs. Of course, the first approach gives a more accurate result, because each PAH has a different concentration (Phillips, 1999).

It is necessary to perform preconcentration prior to the chromatographic separation for PAH analysis in foods. Sample preparation is an important stage in the analysis of PAHs (Warzecha et al., 2002). Common extraction methods are liquid-liquid or solid-phase extraction methods (Carabias-Martinez et al., 2000). The analysis involves identification of the compounds using a variety of techniques following a solvent extraction. By using reference standards, the

peaks determined in the chromatograms are defined and the efficiency of the extraction method is determined. The extract from the food does not only contain PAHs, but may also contain other different hydrophobic and slightly polar compounds. To facilitate separation and identification of PAHs, these compounds must be removed in further stages of analysis (Stolyhwo and Sikorski, 2005; Öz and Yüzer, 2016). Therefore, various solvents can be used for the clean-up stage. The extraction efficiency of PAHs from foods depends on polarity of solvent, content of foods and sample preparation (Moret et al., 1999, Wang et al., 1999).

In the analysis of PAHs, column chromatography, paper chromatography and thin layer chromatography (TLC) have been replaced by GC and HPLC due to some advantages of them (Moret and Conte, 2000, García-Falcón et al., 2005). High yield columns are required to identify most of the PAHs by using GC. The use of HPLC in analysis has significant advantages (Simko, 2002).

PAH contents in smoked fish and the factors affecting of PAHs formation

Unprocessed fish may contain low levels of PAH due to the contaminated water. The PAH contents of cold and hot smoked fishes are higher than the PAH content of raw fishes and the PAH concentrations in smoked fish can reach dangerous levels for human health, especially once smoking is carried out under uncontrolled conditions.

Many studies about PAHs in smoked fish have been published. However, a comparison of these data is difficult due to the difference of fish type, smoking technique and analysis methods. Some published quantitative data on PAHs in smoked fish were shown in Table 2.

Fish smoked under modern and controlled conditions generally contains <1 ppb of benzo[a]pyrene (0.1-0.5 ppb), but this value increases in intense smoked fish (Moret et al., 1999). On the other hand, the characteristics of the fish, the method and parameters of the smoking, the composition of the smoking and the exposure of the edible parts of fish to smoking affect the amount of PAH formed in smoked fish (Stolyhwo and Sikorski, 2005). Hokkanen et al. (2018) reported that some process such as indirect smoking, low smoking duration, optimized smoke generation temperature and longer distance from the smoke source caused lower PAH

concentrations formed in smoked products. In addition, Duedahl-Olesen et al. (2015) indicated that Σ PAH25 content of hot smoking was higher than that of cold smoking for salmon and direct smoking caused higher Σ PAH25 compared to indirect smoking for other fish species. Afolabi et al. (1983) found that fish species dried in oven contained significantly lower levels of PAH, while traditional smoked products always contained much higher PAHs. The same researchers reported that the concentrations of carcinogenic and mutagenic hydrocarbons in the smoked products were 2-10 times higher than those of the products in other tested protection methods.

Fats are one of the most important sources of PAHs in foods due to their lipophilic character (Moret and Conte, 2000). Although PAHs in meat and fish preferentially accumulate in lipid tissues, they are distributed to the muscles where they can bind with structural elements (Stolyhwo and Sikorski, 2005). Duedahl-Olesen et al. (2015) revealed that the PAH concentration decreased in the order fish skin >> outer layer of the fish muscle > inner part of the fish muscle. Zabik et al. (1996) found that the contents of benzo[a]pyrene and total PAH in lean trouts warm-smoked at 82°C for 30 min as 5.12 ppb and 154.4 ppb, respectively, while the contents of benzo[a]pyrene and total PAH in fatty trouts warm-smoked at 82°C for 30 min as 8.43 ppb and 270.9 ppb, respectively. While benzo[a]pyrene could not be detected in any of the raw fishes, total PAH concentrations were determined as 1.52 ppb in lean trout and as 6.34 ppb in fatty trout by Zabik et al. (1996). In a study conducted by Moret et al. (1999) PAH contents of packaged trout were investigated and up to 49.5 ppb of PAH were determined in different smoked samples. In addition, significant differences were observed in terms of PAH contents of skin and meat of trout samples stored in plastic packages. It was determined that the skin layer contained much more of these compounds and that the skin was an effective barrier to the entry of PAHs in the meat, in particular of the light PAHs. On the other hand, the researchers stated that the heavy PAHs such as DahA and BghiP could easily enter the interior of the meat. Although the PAH concentrations of trout skins were very variable, the PAH concentrations in trout meat were generally homogenous.

Table 2. Quantitative data on PAHs in smoked fish

| Fish | Smoking technique | BaP µg/kg | BaA ng/kg | BbFA µg/kg | BkFA µg/kg | Chr 0.4-0.6 µg/kg | DahA ND | BghiP ND | Σ4PAHs | References |
|-------------------|-----------------------------|--------------|--------------|---------------|---------------|----------------------|--------------|--------------|--------|-----------------------------|
| Salmon | Cold | 0.03-0.06 | ND-0.1 | 0.1-0.2 | 0.03-0.06 | 0.4-0.6 | ND | ND | | Karl and Leinemann (1996) |
| Salmon (Denmark) | Commercial | ND | 1.2±0.01 | | | | ND | | | |
| | | | wet wt | | | | | | | |
| Salmon (Scotland) | Commercial | 0.7±0.01 | 23.2±0.03 | | | | ND | | | |
| | | wet wt | ng/g | | | | | | | |
| Salmon (Norway) | Commercial | ND | 0.5±0.01 | | | | ND | | | Storelli et al. (2003) |
| | | | wet wt | | | | | | | |
| Herring (French) | Commercial | ND | 1.2±0.01 | | | | ND | | | |
| | | | wet wt | | | | | | | |
| Herring (Norway) | Commercial | 0.5±0.01 | 1.8±0.02 | | | | ND | | | |
| | | wet wt | ng/g | | | | | | | |
| Herring(Denmark) | Commercial | ND | 1.0±0.01 | | | | ND | | | |
| | | | wet wt | | | | | | | |
| Mackerel | Commercial | ND | ND | | | | | ND | | |
| | (Cold) | | | | | | | | | |
| Mackerel | Commercial | 0.70 | 2.64 | | | | | 0.30 | | |
| | (Hot) | µg/kg | µg/kg | | | | | µg/kg | | |
| Salmon | Commercial | 0.40 | 0.94 | | | | | ND | | Yurchenko and Mölder (2005) |
| | (Cold) | µg/kg | µg/kg | | | | | | | |
| Salmon | Commercial | 0.81 | 1.12 | | | | | ND | | |
| | (Hot) | µg/kg | µg/kg | | | | | | | |
| Herring | Commercial | ND | ND | | | | | ND | | |
| | (Cold) | | | | | | | | | |
| Herring | Commercial | 0.71 | 3.41 | | | | | 0.30 | | |
| | (Hot) | µg/kg | µg/kg | | | | | µg/kg | | |
| Salmon | Cold | 3.20±2.05 | 72.48±12.70 | 2.36±2.43 | 0.52±0.50 | 6.45±5.36 | | 4.11±3.75 | | Visciano et al. (2006) |
| | | ng/g dry wt | ng/g dry wt | ng/g dry wt | ng/g dry wt | ng/g dry wt | | ng/g dry wt | | |
| Mackerel | Laboratory smoked/grilled | 0.9 | 2.5 | 2.0 | 0.9 | 5.8 | 0.9 | 1.4 | | |
| | | µg/kg dry wt | µg/kg dry wt | µg/kg dry wt | µg/kg dry wt | µg/kg dry wt | µg/kg dry wt | µg/kg dry wt | | |
| Mackerel | Commercially smoked/grilled | 6.6 | 12.6 | 10.0 | 6.3 | 20.0 | 1.3 | 8.9 | | Akpambang et al. (2009) |
| | | µg/kg dry wt | µg/kg dry wt | µg/kg dry wt | µg/kg dry wt | µg/kg dry wt | µg/kg dry wt | µg/kg dry wt | | |
| Tuna | Commercial | 1.3 | ND | ND | 0.3 | | 0.5 | | | |
| | (Cold) | ng/g | | | ng/g | | ng/g | | | |
| Swordfish | Commercial | 0.1 | ND | ND | 0.1 | | 1.1 | | | Visciano et al. (2009) |
| | (Cold) | ng/g | | | ng/g | | ng/g | | | |
| Salmon | Commercial | 0.4 | 1.2 | | 0.2 | | ND | | | |
| | | ng/g | ng/g | | ng/g | | | | | |

Table 2 continue

| | | | | | | | | | |
|------------------------------------|--------------------|--------------------|--------------------|------------------|--------------------|-----------------|-----------------|--------------------|-------------------------|
| Salmon | Commercial (Cold) | ND | ND | ND-9.55 µg/kg | 0.16-5.10 µg/kg | ND | ND | 0.10-0.78 µg/kg | Basak et al. (2010) |
| Rainbow trout | Commercial (Hot) | ND | ND | ND | 0.45-5.50 µg/kg | ND | ND | ND | |
| Herring | Direct | 0.4-14.4 µg/kg | | | | | | | |
| Salmon | Direct | 1.4-8.4 µg/kg | | | | | | | |
| Salmon | Indirect | ND<0.3 µg/kg | | | | | | | Wretling et al. (2010) |
| Mackerel | Direct | <0.3-3.7 µg/kg | | | | | | | |
| Cod | Commercial | | | | | | | 7.05 µg/kg | |
| Salmon | Commercial | | | | | | | 4.34 µg/kg | Miculis et al. (2011) |
| Trout | Commercial | | | | | | | 3.80 µg/kg | |
| Salmon | Commercial | ND-2.87±0.51 µg/kg | | | | | | | |
| Squid | Commercial | ND-0.40±0.07 µg/kg | | | | | | | Cho and Shin (2012) |
| <i>Hypophthalmichthys molitrix</i> | Commercial | 1.86±0.06 µg/kg | 1.43±0.01 µg/kg | 1.72±0.03 µg/kg | 1.03±0.04 µg/kg | 1.40±0.01 µg/kg | 0.42±0.08 µg/kg | | |
| <i>R. frisitikatum</i> | Commercial | 0.30±0.05 µg/kg | 0.21±0.01 µg/kg | 0.72±0.05 µg/kg | 0.54±0.02 µg/kg | 0.57±0.04 µg/kg | 0.59±0.03 µg/kg | | |
| <i>Liza saliens</i> | Commercial | 1.64±0.07 µg/kg | 0.56±0.04 µg/kg | 1.55±0.03 µg/kg | 1.60±0.09 µg/kg | 1.18±0.02 µg/kg | 0.51±0.03 µg/kg | | Mohammadi et al. (2013) |
| <i>Alosa kessleri</i> | Commercial | 1.48±0.02 µg/kg | 0.76±0.03 µg/kg | 1.03±0.04 µg/kg | 1.00±0.04 µg/kg | 1.18±0.01 µg/kg | 0.57±0.03 µg/kg | | |
| Shrimp | Hot (Chorkor kiln) | 5.43-107.01 µg/kg | 7.17-102.38 µg/kg | 5.06-56.87 µg/kg | 10.35-127.93 µg/kg | | | 28.02-394.19 µg/kg | |
| Shrimp | Hot (Barrel kiln) | 11.23-91.76 µg/kg | 12.94-142.24 µg/kg | 8.01-54.54 µg/kg | 21.50-193.13 µg/kg | | | 53.67-481.67 µg/kg | Kpoclou et al. (2014) |

The type of wood is another factor that affecting the PAH content of smoked products. Stumpe-Viksna et al. (2008) reported that softwood used in smoking caused higher PAH levels in smoked meat products due to the chemical compounds and natures of the wood. Softwoods can promote intensive origination of soot due to the high content of resin. Therefore, softwoods can cause high concentrations of PAH in smoked products (Stumpe-Viksna et al., 2008). Also, the smoke generation temperatures of the wood chips used for the smoking can be different. It was reported that the smoke generation temperatures of wood chips varied depending on the total cellulose and hemicellulose content of the wood chips (Malarut and Vangnai, 2018). Duedahl-Olesen et al. (2015) reported that the PAH content of fish smoked with alder was higher than those fish smoked with beech.

Legal Limits about PAHs in Smoked Fish

BaP and PAH4 (BaP, Chry, BaA and BbF) that are carcinogenic or possibly carcinogenic to humans have been widely used as the markers for the presence of PAHs in foods (EFSA, 2008). The European Commission has declared several regulations over the last decades in order to protect consumers against PAH intake from diet (Ledezma et al., 2016). According to European Commission, the legal limits of BaP and Σ PAH4 were 2 $\mu\text{g}/\text{kg}$ and 12 $\mu\text{g}/\text{kg}$ for smoked fishes, respectively (Commission Regulation (EU), 2011). In Turkey, while the legal limits in smoked fishes were determined as 5 $\mu\text{g}/\text{kg}$ for BaP and as 30 $\mu\text{g}/\text{kg}$ for Σ PAH4 (BaA, Chry, BbF and BaP) before 2014, the new legal limits in smoked fishes have been reported as 2 $\mu\text{g}/\text{kg}$ for BaP and as 12 $\mu\text{g}/\text{kg}$ for Σ PAH4 by Turkish Food Codex Regulation on Contaminants since 01.09.2014 (Turkish Food Codex, 2014).

CONCLUSION

Fish plays a crucial role in the human nutrition due to its high biological value protein, fatty acid composition, mineral and vitamin content. Smoking is a commonly used technique to preservation of fish and to achieve particular sensorial profiles. However, polycyclic aromatic hydrocarbons that are mutagenic and/or carcinogenic are formed during the smoking of fishes. Therefore, it is necessary to reduce the exposure to PAHs. Thus, much more attention should be paid to the factors affecting the formation of PAHs in smoked fishes. If detailed information is known about PAHs formed in smoked fishes, once fishes are smoked, the exposure to PAHs could be reduced.

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