Levels of Benzo(a)pyrene (BaP) in Fish, Smoked According to Different Procedures
Benzo(a)pirēna (BaP) saturs zivīs, tās kūpinot dažādos apstākļos

Ilze Stumpe-Vīksna, Andris Morozovs
LLU Pārtikas tehnoloģijas fakultāte
Faculty of Food Technology, LLU
e-mail: ilze_st@inbox.lv
Vadims Bartkevičs, Agnese Kukāre
Nacionālais diagnostikas centrs
The National Diagnostic Centre
e-mail: vadims.bartkevics@ndc.gov.lv

Abstract. Polycyclic aromatic hydrocarbons (PAH) are widespread environmental contaminants representing an important group of carcinogens that have been detected in smoked fish. Benzo(a)pyrene (BaP) is the most well-known and studied representative of these compounds because it is one of the most potent carcinogens for animals. Occurrence of BaP in smoked fish and canned sprats in vegetable oil has been studied. Raw fish, vegetable oil, smoked fish as well as canned smoked fish in oil and its fish and oil fractions separately were analyzed using method of gas chromatography-mass spectrometry (GC-MS). Generally, the level of BaP in smoked fish samples varied (from 0.8 up to 13.9 µg kg^{-1}) depending on the disposition of fish in the smoking chamber and on the method used for smoke production. The results suggest that another factor affecting the concentration of BaP in finished canned fish in oil is a diffusion of benzo(a)pyrene from fish fraction in oil fraction, which could reduce the level of benzo(a)pyrene in fish. Oil from canned smoked fish cannot be recommended as edible constituent.
Key words: smoked fish, benzo(a)pyrene, smoking procedures, gas chromatography–mass spectrometry.

Introduction

Foodstuffs such as meat and fish and some types of cheese have been smoked in many countries for centuries. Originally the purpose was to preserve the food, partly by reducing the moisture content and partly through the transfer of anti-microbiological components, such as aldehydes and phenols, from the smoke to the food. Now the smoking is primarily used to achieve the characteristic taste and appearance of smoked food, with preservation playing a minor role. Nevertheless, smoking can still influence the shelf life of food because components of the smoke may inhibit growth of some microorganisms. Smoking in uncontrolled technological conditions, characteristic for traditional smoking process, results in high levels of polycyclic aromatic hydrocarbons (PAH) (Alonge, 1987, 1988; Afolabi et al., 1983; Šimko, 2002).

Polycyclic aromatic hydrocarbons constitute a large class of organic compounds that have the carcinogenic activity. PAH have been found in water, air, soil, and in different foods, including oil, fat, smoked/grilled products, plants, marine products, smoking flavourings, coffee, tee, beverages (Gomaa et al., 1993). In food, PAH may be present as a result of processing and cooking, such as smoking, drying, roasting, baking, frying, or grilling. Vegetables may be contaminated by the deposition of airborne particles or by growth in contaminated soil (Guillen et al., 1997).

To simplify the problems that have been related with PAH variety and content multiplicity, benzo(a) pyrene (BaP) has been accepted as indicator for PAH content in food products. The European Commission has introduced maximum levels for PAH in certain foodstuffs via Commission Regulation No. 1881/2006. The maximum level of benzo(a)pyrene (5 ng g^{-1}) was set for smoked meat and smoked fish products.

In this work we have studied the influence of the type of smokehouse and fish disposition into the smoking chamber during smoking on the BaP content in the product.

Materials and Methods

Materials and Smoking Equipment

The fish, used for this research, was from the Baltic Sea and Riga Gulf region. The vegetable oil (rapeseed and soya oil) was obtained from commercial suppliers.

Technological process of canned sprats in oil involves hot smoking including drying (50-70 ºC, 10-20 min), cooking (100-135 ºC, 15-20 min), and smoking (60-80 ºC, 25-35 min), with subsequent sorting, packing, filling of oil, and sterilization. The total level of benzo(a)pyrene in canned sprats consists of the content of BaP in raw fish, BaP level emerging from smoking process, and benzo(a)pyrene – from oil used for canning.

Smoke generators were used for smoking of fish. The temperature of smoke was from 60 to 80 ºC. Alder chips were used as firewood; moisture content of alder chips was 35-45%. Burning temperature of woodchips and the grating reached 400-450 ºC.

In general, a conveyor-tunnel type smoking chamber consists of a welded foundation and welded vertical frames covered on either side and on the top with steel plates to form a double wall space. The chamber consists of two symmetrical parts forming two tunnels. Each compartment of the chamber consists of several sections. There are two intermediate sections in the chamber (entrance and exit) that divide the chamber in three areas: 1) drying, 2) cooking and smoking, and 3) chilling.

Hot air, which is brought inside from the heat generator by means of the ventilator through the supply pipeline in drying area, flowing through containers with fish is further brought out through the exit pipeline. The mixture of smoke and air brought inside by means of the ventilator is supplied to the cooking and smoking area through the supply pipeline and is flowing through containers with fish in a similar manner.

In this research, five different smoking ovens were used:

A – an oven with two-tunnel conveyor-type smoking chambers with a smoke generator, produced by JSC "Miiduranna Texas", Estonia;

B – an oven with two-tunnel conveyor-type smoking chambers with a smoke generator, produced by "Peruza" Ltd, Latvia;

C – a Kverner-Bruk oven with smoke chambers with a ventilator and underneath location of a smoke generating furnace;

D – a Kverner-Bruk oven with tunnel-type modified smoking chambers with a smoke generator;

E – an electrical oven with a smoke chamber with a smoke generator.

Sprats in the smoking chamber were hanged up on frames. Frames in the chamber were placed at different height.

Sampling

After the smoking process, approximately a sample of 500 g of fish was taken according to the sampling procedure (from specific frame or from different places on several frames). The sample was cooled at +4 ºC and then sent to the laboratory. Afterwards it was homogenated in laboratory using sample mills.

Chemicals

For the sample treatment, cyclohexane (*ECD* tested), N,N-dimethylformamide, methanol (HPLC grade), and sodium chloride (ACS) were purchased from Acros, ethanol – from J.T. Baker, sodium sulphate (ACS) – from Fluka, potassium hydroxide – from Avsista, and silica solid phase extraction (SPE) tubes (500 mg) – from Phenomenex. Ultra pure water was produced by a MilliQ filter system. Standards of benzo(a)pyrene and deuterated standard benzo(a) pyrene-d_{12} were purchased from Dr. Ehrenstrofer.

Analysis of BaP

Homogenized samples were hydrolyzed with the solution of potassium hydroxide in ethanol, and the extracted with cyclohexane. The cyclohexane solution was washed with water, afterwards – with a mix of methanol and water, then re-extracted with N,N-dimethylformamide/water (9:1) blend, and repeatedly extracted from it with cyclohexane. After sample cyclohexane solution purification on Silica SPE column, it was concentrated and analyzed on Agilent Model 6890 gas chromatograph equipped with the mass selective detector Model 5973 (Stumpe-Vīksna et al., 2007).

Results and Discussion

In this study, raw fish, vegetable oil, smoked fish, as well as canned smoked fish in oil, and fish and oil fractions separately were analyzed using method of gas chromatography-mass spectrometry (GC-MS).

Fish and marine invertebrates may naturally contain small amounts of different PAH absorbed from the environment (Stolyhwo, Sikorski, 2005). The edible parts of fish from unpolluted seas generally do not contain detectable amounts of BaP (Rainio et al., 1986). We found that levels of BaP in the raw fish were insignificant. BaP content was under the limit of detection (<0.1 µg kg^{-1}) in 30 different samples of sprats used for canned fish production.

The main amount of PAH in smoked foods comes from the wood smoke. Cold- and hot-smoked fish contain much more PAH than raw fish, depending on the properties of the fish, method and parameters of smoking, composition of the smoke, and exposure of the edible parts to the smoke. The concentration of

BaP found in different studies varies from about 0.05 to 60 μg kg^{-1} (Lawrence, Weber, 1984).

The BaP levels found in smoked fish from commercial smokehouses are shown in Figure 1. Depending on the used construction of smoking equipment and disposition of fish into the smoking chamber, the average level of benzo(a)pyrene in smoked semi-finished fish (after the smoking stage and before canning) ranged from 0.7 up to 13.9 μg kg^{-1}. Fish products which were smoked in smokehouses with a ventilator and underneath location of a smoke generating furnace (C), contained the highest BaP levels in comparison to those with a smoke generator or to the electrical smokehouse (Figure 1).

The content of benzo(a)pyrene in smoked semi-finished fish products differed substantially depending not only on the type of a smokehouse, but also on fish disposition into the smoking chamber in vertical plane (Figure 1).

Comparison of BaP concentration in smoked fish by sampling in different places in smokehouses with a smoke generator and with upper input of smoke showed that samples smoked on upper frame contained highest amount of BaP due to its full concentration in incoming smoke. The greatest differences in benzo(a)pyrene content, depending on placement of fish samples, were found in fish samples smoked in the older generation smokehouse (A) with a smoke generator in comparison with the modified smokehouse (B) and Kverner-Bruck smokehouse (D), although between them greater concentrations of BaP in fish smoked in such type of smokehouse were found in fish samples using smokehouse (B); however, that might be explained by differences in the smoking methods. A higher amount of BaP was also found in fish samples smoked on upper frame of the electrical smokehouse. Similarly, BaP concentration distribution between smoked fish sampled in different places of the smokehouse (C) with a ventilator was higher than in samples from other smokehouses.

We compared the concentration of BaP in smoked fish by sampling in different points (places located at similar height) on the same frame. Results showed relatively great differences between BaP concentrations in fish samples smoked in smokehouse A with a smoke generator (see Figure 2). Obviously, smoke flow distribution in that smoking chamber is greatly uneven.

Although we observed general tendency towards dependency of BaP content in smoked fish on the construction and operation of a smokehouse as well as on the position of fish in the flux of smoke, the results of samples analysis showed high dispersion of benzo(a)pyrene level in the smoked product even in the same equipment and at the same fish location.

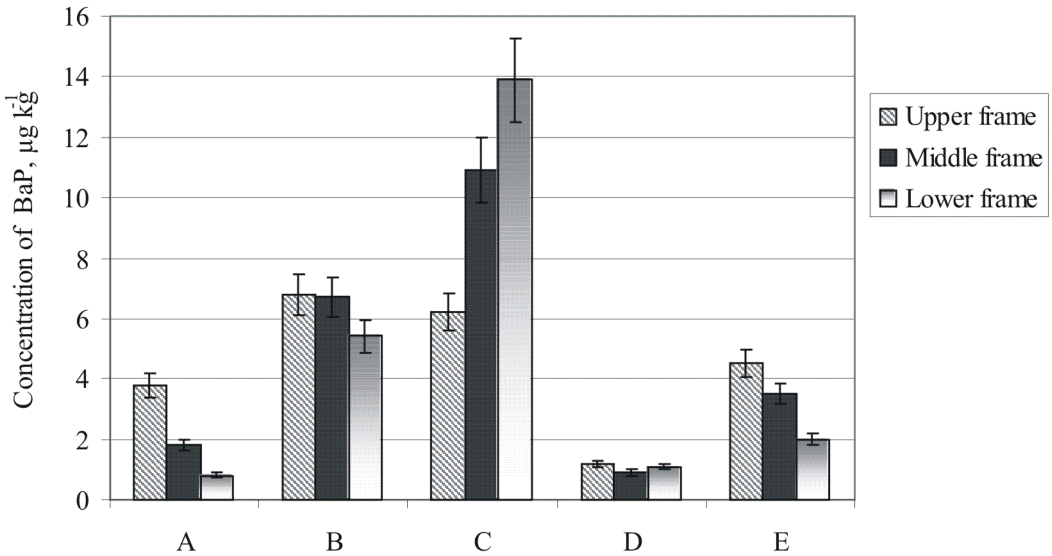

Fig. 1. Average benzo(a)pyrene levels in fish samples smoked in different smokehouses and in different places in a smoking chamber: A – a smokehouse with two-tunnel conveyor-type smoking chambers with a smoke generator, produced by JSC "Miiduranna Texas", Estonia, and upper smoke inflow; B – a smokehouse with two-tunnel conveyor-type smoking chambers with a smoke generator, produced by "Peruza" Ltd, Latvia, and upper smoke inflow; C – a Kverner-Bruk smokehouse with a ventilator and underneath location of a smoke generating furnace, and smoke inflow from below; D – a Kverner Bruck smokehouse with modified smoking chambers with a smoke generator and upper smoke inflow; E – an electrical smokehouse with a smoke generator and upper smoke inflow.

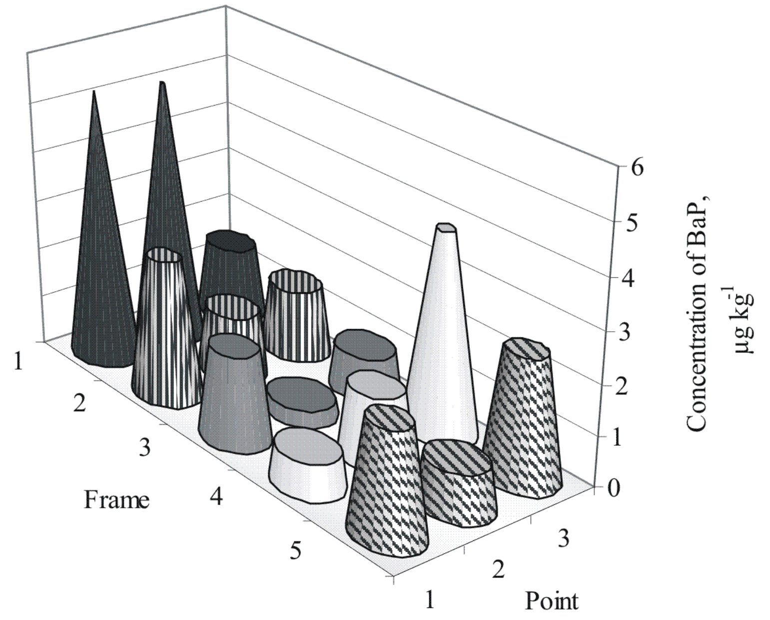

Fig. 2. Benzo(a)pyrene levels in fish samples taken from different points on the same frame in smoking chamber A.

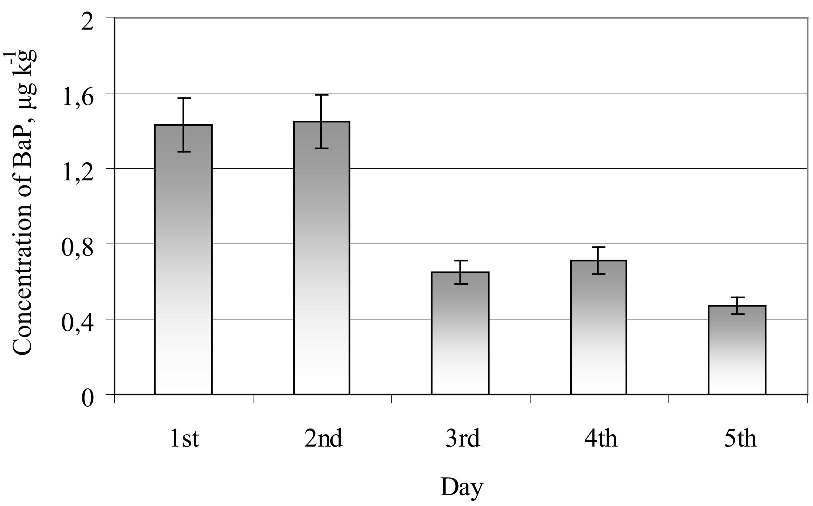

Fig. 3. Changes in benzo(a)pyrene concentration during a week in fish samples smoked in smokehouse A with a smoke generator.

This dispersion could be provoked by differences in fat content in raw fish or by changes in smoke chemical composition during the smoking process. Influence of other unknown factors on the content of contaminants can not be excluded.

Since the smokehouses were cleaned once a week, it can be assumed that the product has been fouled by tar residues from the previous processing. The changes in BaP levels in smoked fish during a week were investigated to verify such hypothesis. Analysis of results showed that the level of carcinogen on 3rd-5th day after smokehouse cleaning had diminished approximately by half (see Figure 3). The reduction in BaP levels in smoked fish produced at the end of the week can be explained by the PAH sorption in tar deposits on the walls of the smoking chamber or by some unknown factors.

However, in canned smoked fish in oil, the part of contamination with PAH might have originated from the vegetable oil used. The average BaP level found in the vegetable oil used for canned, smoked sprats production was 0.38 µg kg^{-1}.

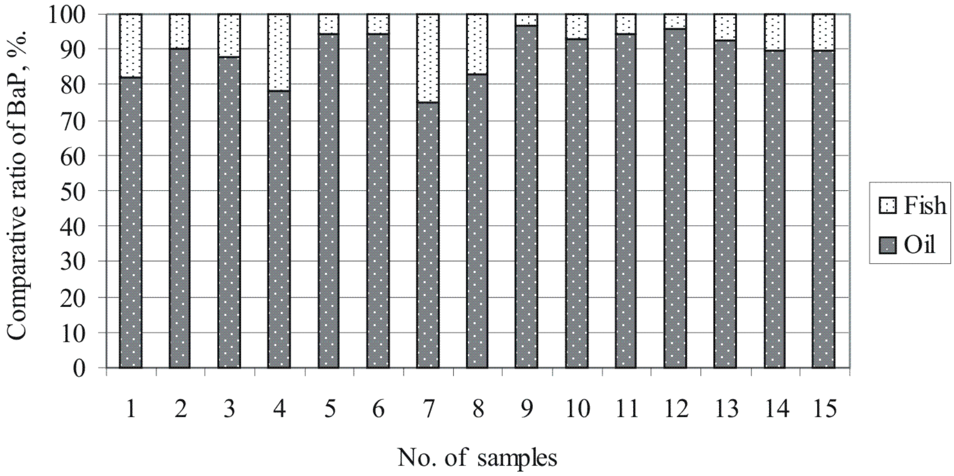

Fig. 4. Comparative ratio of benzo(a)pyrene (%) in fish and oil fractions.

Another factor that affects the concentration of BaP in finished canned fish in oil is a diffusion of benzo(a) pyrene from fish fraction in oil fraction, which could reduce the level of benzo(a)pyrene in fish. By separate detection of BaP concentration in oil and fish fraction of canned fish in oil as well as undivided sample, a decrease by about 73 up to 95 percent of the initial BaP amount in fish was found. For the BaP content in percentage in fish and oil fractions see Figure 4. If a person consumes only a fish fraction of canned smoked fish in oil, he becomes by 56±8 percent less contaminated than if he consumes the same amount of fish and oil together. By comparison, an equivalent mass of only an oil fraction produces approximately a 355±108 percent higher dose of BaP. The change in the level of contamination with BaP in fish fraction in comparison with the total contamination level in the whole product was calculated according to equation 1. In the same way it was done for oil fraction according to equation 2:

$$C(\%)_f = \frac{c_f - c_t}{c_t} \cdot 100, \qquad (1)$$

$$C(\%)_o = \frac{c_o - c_t - c_{av}}{c_t} \cdot 100, \qquad (2)$$

where

$C(\%)_f$ – alteration in benzo(a)pyrene content in fish fraction relative to BaP content in an undivided sample of canned fish in oil, %;

$C(\%)_o$ – alteration in benzo(a)pyrene content in oil fraction relative to BaP content in an undivided sample of canned fish in oil, %;

c_f – concentration of BaP in fish fraction, µg kg^{-1};

c_o – concentration of BaP in oil fraction, µg kg^{-1};

c_{av} – average concentration of BaP in the vegetable oil used in production, µg·kg^{-1};

c_t – concentration of BaP in an undivided sample, µg kg^{-1}.

The research suggests that the consumer, using smoked fish as food, ingests less content of BaP than its total content in the product, therefore the oil from canned smoked fish cannot be recommended as an edible constituent.

Conclusions

Generally, the level of BaP in smoked fish samples varies depending on the disposition of fish in the smoking chamber and on the method used for smoke production. In order to obtain characteristic and favourite properties of canned sprats, it is very difficult to ensure the level of benzo(a)pyrene below 5.0 µg kg^{-1} in all stages of the technological procedure of a batch.

After sterilization and storage, part of BaP from canned sprats is transferred to oil which is not usually consumed. This diminishes the risk of contamination. Oil from canned smoked fish cannot be recommended as an edible constituent.

Treatment of smoked fish with oil can be used for diminishing the PAH level in the final product.

Literature

1. Afolabi, A.O., Adesulu, E.A., Oke, O.L. (1983) Polynuclear aromatic hydrocarbons in some Nigerian preserved freshwater fish species.

Journal of Agricultural and Food Chemistry, 31, pp. 1083-1090.
2. Alonge, D.O. (1987) Factors affecting the quality of smoke-dried meats in Nigeria. *Acta Aliment*, 16, pp. 263-270.
3. Alonge, D.O. (1988) Carcinogenic polycyclic aromatic hydrocarbons (PAH) determined in Nigerian kundi (smoke-dried meat). *J. Sci. Food Agric*., 43, pp. 167-172.
4. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. (2006) *Official Journal of European Union*, L 364/5.
5. Gomaa, E.A., Gray, J.I., Rabie, S., Lopez-Bote, C., Booren, A.M. (1993) Polycyclic aromatic hydrocarbons in smoked food products and commercial liquid smoke flavourings. *Food Additives and Contaminants*, 10, pp. 503-521.
6. Guillen, M.D., Sopelana, P., Partearroyo, M.A. (1997) Food as a source of polycyclic aromatic carcinogens. *Rev. Environ. Health*, 12, pp. 133-146.
7. Lawrence, J.F., Weber, D.F. (1984) Determination of polycyclic aromatic hydrocarbons in some Canadian commercial fish, shellfish, and meat products by liquid chromatography with confirmation by capillary gas chromatography–mass spectrometry. *Journal of Agricultural and Food Chemistry*, 32, pp. 789-794.
8. Rainio, K., Linko, R.R, Routsila, L. (1986) Polycyclic aromatic hydrocarbons in mussel and fish from the Finnish archipelago sea. *Bulletin Environmental Contamination and Toxicology*, 37, pp. 337-343.
9. Stolyhwo, A., Sikorski, Z.E. (2005) Polycyclic aromatic hydrocarbons in smoked fish – a critical review. *Food Chemistry*, 91, pp. 303-311.
10. Stumpe-Vīksna, I., Kukāre, A., Bartkevičs, V. (2007) Policiklisko aromātisko ogļūdeņražu noteikšana pārtikas produktos ar masspektrometrijas metodēm. *Latvijas Ķīmijas žurnāls,* Nr. 2, 138.-146. lpp.
11. Šimko, P. (2002) Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *Journal of chromatography B*, 770, pp. 3-18.

Anotācija

Policikliskie aromātiskie ogļūdeņraži (PAO) ir viena no lielākajām organisko savienojumu klasēm, kam piemīt kancerogēna aktivitāte un kurus atrod arī kūpinātās zivīs. Benzo(a)pirēns (BaP) ir viens no visvairāk pētītajiem savienojumiem, un to izmanto kā marķieri PAO noteikšanā. Šajā darbā mēs veicām pētījumu par BaP saturu zivīs, kas kūpinātas dažādos apstākļos. Nozīmīgi benzo(a)pirēna koncentrāciju ietekmējoši faktori ir dūmu ģenerators, atrašanās vieta kūpināšanas kamerā, kā arī citi faktori. Kūpinātu zivju konservos lielākā daļa BaP pāriet eļļas frakcijā, tādējādi patērētāji, uzturā lietojot kūpinātu zivju produktus eļļā, uzņem mazāku BaP daudzumu nekā produktā kopumā.