www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 10: 351-355 (2010) DOI: 10.4194/trjfas.2010.0307

The Detection of Potential Carcinogenic PAH Using HPLC Procedure in Two Different Smoked Fish, Case Study: Istanbul/Turkey

Serden Basak[1,*], Gülgün F. Şengör[2], Fatma Telli Karakoç[3]

[1] Istanbul Technical University, Faculty of Civil Engineering, Department of Environmental Engineering, Maslak, Istanbul, Turkey.
[2] Istanbul University, Faculty of Fisheries, Department of Seafood Science and Technology, Laleli, Istanbul, Turkey.
[3] TUBITAK- Marmara Research Center, The Institute of Chemistry and Environment, P.O. Box: 21, Gebze, Kocaeli, Turkey.

* Corresponding Author: Tel.: +90.212 2853785; Fax: +90.212 2853781;
E-mail: basaks@itu.edu.tr

Received 09 April 2009
Accepted 13 April 2010

Abstract

In this study, 24 samples of smoked fish products were analysed in order to investigate the levels of the potently carcinogenic PAHs. Salmon and rainbow trout samples were tested with a liquid choromatographic (HPLC) method. In none of the smoked fish products benzo(a)pyrene, one of the potential carcinogenic PAH compounds limited in food codex alimentarious was detected. However, benzo(a)antrecene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, compounds which could be carcinogenic for humans, were detected in the smoked samples. There is a significant correlation between the fish fat and the total PAHs level. Consequently the average fat contents of the smoked salmon were significantly higher than that of the smoked rainbow trout. It was also detected that the total PAH amount variations of the smoked fish samples were due to the non-homogenous smoke dispersion in the traditional ovens and it was difficult to obtain homogenous smoked fish products from traditional ovens.

Keywords: Rainbow trout, salmon, polycyclic aromatic hydrocarbons, benzo(a)pyrene.

İki Farklı Füme Balıkta Örnek Olay İncelemesi İçin HPLC Prosedür Kullanmıyla Potansiyel Kanserojen PAH Tespiti: İstanbul / Türkiye

Özet

Bu çalışmada, 24 adet dumanlanmış balıkta potansiyel karsinojenik olan PAH'lar incelenmiştir. Salmon ve gökkuşağı alabalığı numuneleri likit kromatografi (HPLC) metoduyla analiz edilmiştir. Hiçbir dumanlanmış balık numunesinde güçlü karsinojenik ve gıda kodeksinde üst limiti belirtimiş bir PAH olan Benzo(a)pyrene'e rastlanmamıştır. Ancak yine karsinojenik potansiyeli bulunan benzo(a)antrecene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, dumanlanmış balık numunelerinde tespit edilmiştir. Balık yağı ve toplam PAH miktarları doğru orantılıdır. Dumanlanmış salmon balığının yağ oranı dumanlanmış gökkuşağı alabalığından fazladır. Ayrıca dumanlanmış balıkların toplam PAH miktarları arasında farklılıklar da saptanmıştır. Bu durum geleneksel fırında dumanlanmış örneklerin, dumanı homojen biçimde alamamasından kaynaklanmaktadır.

Anahtar Kelimeler: Gökkuşağı alabalığı, salmon, polisiklik aromatic hidrokarbonlar, benzo(a)pyrene.

Introduction

Smoking or smoke-curing as applied to fish is a method of preservation effected by a combination of drying and the deposition of naturally produced chemicals resulting from the thermal breakdown of wood (Kramlich *et al*., 1980). Wood smoke contains a wide range of chemicals such as phenols, aldehydes, acetic acid and a range of polycyclic hydrocarbons (Wilson, 1981). Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds that have been the subject of much concern in the recent years due to their toxic potential. They are known as highly stable contaminants of foodstuffs and included various sources such as contaminated soils, polluted air and water, made of cooking, food processing, pseudo-curing with smoking flavour agents or wood generation etc. reported previously (Derache, 1990; Karl *et al*., 1996; García Falcón *et al*., 1999; Simko, 2005; Reinik *et al*., 2007). Polycyclic aromatic hydrocarbons (PAH) constitute a large class of organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms. Hundreds of individual PAH may be formed and released during incomplete combustion or pyrolysis of organic matter, during industrial

processes and other human activities. PAHs are also formed in natural processes, such as carbonization. In food, PAH may be formed during processing and domestic food preparation, such as smoking, drying, roasting, baking, frying or grilling. Of the many hundreds of PAH, the most studied is benzo(a)pyrene, which is often used as a marker for PAH in ambient air and food (European Commission, 2002).

Smoke production is based on a process of incomplete combustion. Gilbert and Knowles (1975) reported, when wood is completely combusted only carbon dioxide (CO_2), water and ash are produced, but incomplete combustion via thermal pyrolysis of higher molecular organic compounds produces compounds of lower molecular weight, this is the case with PAHs, which are volatile in the gas phase.

The potentially carcinogenic PAH benzo(a)pyrene is often used as an indicator of the presence of PAHs in water and foodstuffs Derache (1990). It is well known that PAHs are present in smoke curing, and that they are easily deposited on and can penetrate the food surface during traditional smoking. In our country fish and meat is still flavored using smoke generated by combustion of wood. Wood smoke contains appreciable amounts of carcinogenic PAHs, which are the main cause of concern regarding its toxicity.

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 5 µg kg^{-1} for smoked fish and smoked fishery products, excluding bivalve molluscs. The Turkish Codex Regulation (2008) has permitted the maximum acceptable concentrations of benzo(a)pyrene at 2.0 µg kg^{-1} (wet weight) in smoked fish. It's essential that this limit should be controlled and applied to scientific laboratory analysis for smoked fish products which is prepared by liquid or traditional smoking.

The present study was undertaken to examine the PAH levels in smoked fish on the Turkish market. By using HPLC with UV detection it was possible to determine simultaneously 16 different PAH components including benzo(a)pyrene.

Materials and Methods

Preparation of Samples

Samples of cold smoked salmon (n=12) (*Salmo salar*) and hot smoked rainbow trout (n=12) (*Oncorhynchus mykiss*) were obtained from Ugurlu Seafood Inc, which is one of the leader company in terms of producing potentially smoked fish in the fish market, in Istanbul, Turkey. The bones and the skin of the smoked fish were removed, and the edible parts of fish were homogenized. The samples were stored at -20°C until the analysis.

Analysis of PAHs

Extraction: The whole glassware was rinsed with acetone before the analysis. Of the homogenate approximately 5 g was weighed accurately into a l00 ml round-bottomed flask, 5 ml KOH (50%) solution, 75 ml methanol, 1 ml internal standard solution (9, 10 dimethylantracene) and a few boiling chips were added. The mixture was boiled for 4 hours in soxhelet. The liquid phases were transferred to a separation funnel and extracted with 100 ml n-hexane with rigorous shaking for 3 min. The methanol KOH phase was drained and discarded. The combined n-hexane phases were rinsed twice, each time with 50 mL of methanol-water (8:1) and with 50 ml water at least. The methanol-water phase and water were drained and discarded. The organic phase was concentrated in a rotary evaporator (42°C, 19-21 kPa) to a volume of approximately 10 mL under reduced pressure. The concentrated extract was dried under nitrogen steam as defined by Telli-Karakoç *et al.* (2002).

Silica Gel Column Clean-up: A slurry of 15 g silica gel in n-hexane was transferred to a glass column (length 200 mm; diameter: 12 mm) and 1 g anhydrous sodium sulphate was added on top. The excess of solvent was allowed to drain to the level of sodium sulphate, the concentrated sample extract was transferred to the column and eluated with 1 ml n-hexane.

Stock Solutions: Stock solutions containing 0.1 mgm L^{-1} of 9-10 dimethylantrecene were dissolved in n-hexane were stored at 4°C, in volumetric flask (with glass stoppers) wrapped in aluminium foil to avoid possible light degradation. Working standard solutions were prepared from stock solution.

Chromatographic Conditions: For PAH compounds analysis, a Hewlett-Packard 1100 HPLC equipped with an Agilent-1100 fluorescence detector was used. Injection volume was 10 µl. The mobile phase (acetonitrile-water) gradient was: 80% acetonitrile +20% water with a flow rate 1 ml min^{-1} and a wavelength of 270 nm. The external standard mixture was used for calculation of the PAH concentrations, considering the recovery rate of the internal standard.

Fat and moisture Analysis

Each smoked fish samples was homogenized and analyzed in triplicate. Fat and moisture analysis of smoked fish fillets were done and reported previously (AOAC, 1990).

Statistical Analysis

Analysis of variance (ANOVA) was performed

on PAH concentration data using Statgraphics Plus 3.1 software (Statistical Graphics Corp., USA).The significant statistical level was set at $P<0.05$ (Açıkgöz, 1990). Each PAH quantification was the result of the mean of measurements carried out on twelve individual smoked mackerel fillets, smoked rainbow trout fillets and smoked salmon fillets.

Results

The PAH levels of smoked mackerel, rainbow trout and salmon are shown in Table 1. The minimum, maximum and mean values of individual compounds and the total PAHs are given for each species. The concentration of total PAH varied between 23.83-79.74 μg kg^{-1}.

The distribution of average total PAH amount, average moisture content and average fat content in the all smoked fish are shown in Figure 1.

As can be seen from Figure 1, the average total PAH level of smoked rainbow trout (23.83%) was lower than that of the smoked salmon (79.74%) because of rainbow trout had low fat content than the salmon. The average fat content of the smoked salmon (6.57%) was significantly higher than the average fat content of smoked rainbow trout (4.76%) ($P<0.05$ at 95% confidence level). The levels of the other carcinogenic compounds determined in this study, namely benzo(a)antrecene, benzo(b) flouranthene, benzo(k)flouranthene and benzo(g,h,i) perylene were between 0.44 μg kg^{-1} to 9.55 μg kg^{-1}.

Discussion

As a consequence, the total PAH level of smoked mackerel and smoked salmon were higher than that of smoked rainbow trout. Akpan *et al*. (1994) reported that strong correlation is found between fish lipids and PAH compounds. Especially, the PAH compounds are stored in fatty fish tissue. When fatty meat is grilled, a large amount of fat drops onto the coals and, due to the high temperature, PAHs are formed. As a consequence, smoke carries the PAHs onto the surface of the meat products. Lower concentrations of PAHs in sausages can be explained by shorter grilling time (Reinik *et al*., 2007). Our

Table 1. PAH-levels in edible part of smoked fish from traditional smoke oven as μg kg^{-1}

Fish	Naph	Acenaph	A	F	Phe	Ant	Flu	Pyr	BaA	Chr	BbF	BkF	BaP	DahA	BghiP	IPy	Σ PAH
RT, n=12																	
Min	0.47	nd	nd	nd	nd	2.45	nd	4.56	nd	nd	nd	0.45	nd	nd	nd	1.97	9.9
Max	14.00	0.22	nd	nd	nd	19.95	nd	nd	nd		nd	5.50	nd	nd	nd	nd	39.67
Mean	4.94	0.22	nd	nd	nd	10.31	nd	4.56	nd	nd	nd	1.83	nd	nd	nd	1.97	23.83
S, n=12																	
Min	2.24	2.09	0.10	nd	nd	8.57	1.14	0.34	nd	nd	9.55	0.16	nd	nd	0.10	nd	34.55
Max	27.06	11.36	0.44	nd	nd	92.06	nd	9.69	nd	nd	nd	5.10	nd	nd	0.78	nd	146.49
Mean	15.06	6.00	0.32	nd	nd	39.60	1.14	5.02	nd	nd	9.55	2.61	nd	nd	0.44	nd	79.74

RT: Rainbow Trout, Salmon: S, nd= not detected

Naphthalene = Naph, Acenaphtylene = Acenaph, Acenaphthene = A, Fluorene = F, Phenanthrene = Phe, Antracene = Ant, Fluoranthene = Flu, Pyrene = Pyr, Benzo(a)anthracene = BaA, Chrysene = Chr, Benzo(f)fluoranthene = BfF, Benzo(k)fluoranthene = BkF, Benzo(a)pyrene = BaP, Dibenzo(a,h)anthracene = DahA, Benzo(g,h,i)perylene = BghiP, Indeno(1,2,3-*cd*)pyrene = IPy.

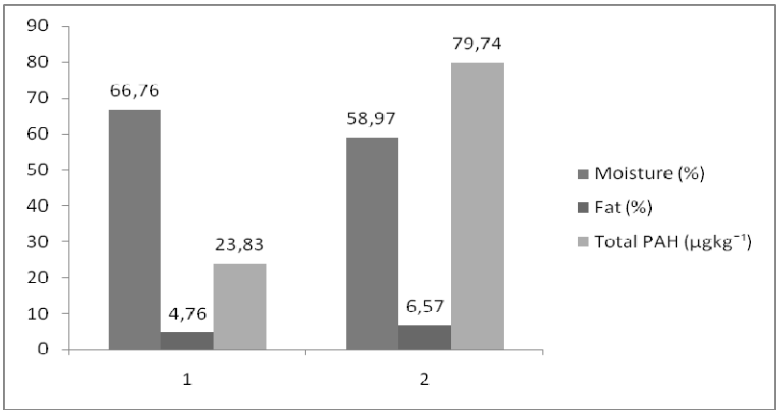

Figure 1. Average moisture (%), fat (%) and total PAH amounts in different smoked fishes.
1- Smoked rainbow trout, 2- Smoked salmon.

results are in agreement with the results obtained by Akpan *et al.* (1994) and Reinik *et al.* (2007). The content of PAH in the edible parts of smoked fish depends obviously on the concentration of these compounds in the wood smoke, smoking temperature and smoking time.

In this study, the amounts of the low molecular weight PAHs such as naphthalene, pyrene, acenaphthene were found higher than the high molecular weight PAHs such as benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene in smoked fish samples. In previous publications it was reported that the PAHs with higher molecular weight are more carcinogenic than the lower molecular weight PAHs (Potthast, 1979; García Falcón *et al.*, 1999; Moret *et al.*, 2000).

The carcinogenic PAH levels in smoked fish largely depend on the smoke generated by combustion of wood and vary with the fat level of fish. Data reported in the literature on PAH in smoked foods are highly variable (Karl *et al.*, 1996; Ova *et al.*, 1998; Simko, 2005; Reinik *et al.*, 2007; Varlet *et al.*, 2007). The main reason for such discrepancies is the differences in the procedures used for smoking. Such variables include: the type and composition of wood, type of generator (internal or external), oxygen accessibility, temperature of smoke generation, and smoking time, preparation of fish etc. Ova *et al.* (1998) reported that the PAH levels were significantly higher in the fish skins than in the edible parts, the PAH levels of the edible parts were also higher than the smoked fish as a whole in the smokehouse. Besides, they also stated that fish skins act like a barrier, and without this barrier the edible parts are directly exposed to smoke. The variation of PAH contents of smoked fish is directly connect with type of smoking oven (automatic smoking oven or traditional smoking oven). Karl *et al.* (1996) reported that the average benzo(a)pyrene concentration determined for the traditional ovens was 1.2 μg kg^{-1} and 0.1 μg kg^{-1} for the modern ovens. In this study, traditional smoking oven was used. The density of smoke was regularly controlled with stable combustion temperature of wood throughout the smoking process. None of samples were contained detectable benzo(a)pyrene. This also indicates the safety of smoked fish.

It seems evident from the results obtained that benzo(a)pyrene is not present in the smoked fish. In this study, the amounts of the low molecular weight PAHs were found higher than the high molecular weight PAHs in the samples. However as the high molecular weight PAHs are more carcinogenic at lower levels the tolerable limits of these compounds should also be established by food codex regulations.

Generally in Turkey, fish and meat are still flavoured using smoke generated by combustion of wood. Wood smoke contains appreciable amounts of carcinogenic PAHs, which are the main cause of concern regarding its toxicity. However, this process is controlled by the manual handling of the smoking conditions, which depends on the experience of the personnel.

Acknowledgements

We wish to acknowledge the contributions made to this work by Istanbul University, Research Foundation Project (T–1236/01112001) and Mr. Merdol Karaman of Ugurlu Seafood Inc., for providing smoked fish samples.

This article was prepared by the first author's master's thesis and the "National Water Day 2007" at the Symposium (16-18 May 2007, Antalya) was presented as a poster.

References

Açıkgöz, N. 1990. Tarımda Araştırma ve Deneme Metodları. Ege Üniversitesi, Ziraat Fakültesi Yayınları, Izmir, 478 pp.

Akpan, V., Lodovici, M. and Dolara, P. 1994. Polycyclic aromatic hydrocarbons in fresh and smoked fish samples from the three Nigerian cities. Bull. Environ. Contam. Toxicol., 53: 246-253.

AOAC. 1990. Official methods of analysis of the association of analytical chemists, 15th Ed., Kenneth Arlington, Virginia, USA.

Derache, R. 1990. Toxicologia y seguridad de los alimentos, edited by S.A. Omega, Barcelona: 296-317.

EC (European Commission), 2002. Opinion of the scientific committee on food on the risks to human health of polycyclic aromatic hydrocarbons in food. SCF/CS/CNTM/PAH/29 Final, Brussels, 74 pp.

García Falcón, M.S., Gonzáles Amigo, S., Lage Yusty, M.A. and Simal Lozano, J. 1999. Determination of benzo(a)pyrene in some spanish commercial smoked products by HPLC-FL. Food Additives and Contaminants, 1: 9–14.

Gilbert, J. and Knowles, M.E. 1975. The chemistry of smoked foods: a Review. Journal of Food Technology, 10: 245–261.

Karl, H. and Leinemann, M. 1996. Determination of polycyclic aromatic hydrocarbons in smoked fishery products from different smoking kilns. Z. Lebensm. Unters. Forsch., 202: 458-464.

Kramlich, W.E., Pearson, K.M. and Tauber, F.W. 1980. Processed meat. The AVI Publishing Comp. Inc., Westport- Connecticut: 61-67.

Moret, S. and Conte, L.S. 2000. Polycyclic aromatic hydrocarbon in edible fats and oils: Occurence and analytical methods. J. Chromatogr. A., 882: 245-253.

Ova, G. and Onaran, S. 1998. Polycyclic aromatic hydrocarbons contamination in salmon-trout and eel smoked by two different methods. Adv. Food Sci., 20(5/6): 168-172.

Potthast, K. 1979. Influence of smoking technology on the composition of polycyclic hydrocarbons in smoked meat products, smoke condensates and in waste gases from smoking plant. Fleischwirtschaft., 59(10): 1515–1523.

Reinik, M., Tamme, T., Roasto, M., Juhkam, K., Tenno, T. and Kus, A. 2007. Polycyclic aromatic hydrocarbons (PAHs) in meat products and estimated PAH intake by

children and the general population in Estonia. Food Additives and Contaminants, 24(4): 429–437.

Simko, P. 2005. Factors affecting elimination of polycyclic aromatic hydrocarbons from smoked meat foods and liquid smoke flavorings. Mol. Nutr. Food Res., 49: 637-647.

Telli-Karakoç, F., Tolun, L., Henkelmann, B., Klimm, C., Okay, O. and Schramm, K.W. 2002. Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) distributions in the Bay of Marmara Sea: Izmit bay. Environmental Pollution, 119(3): 383–397.

Turkish Food Codex. 2008. Turkish food codex communiqué on determining the maximum levels of certain contaminants in foodstuffs. The official gazette: 17.05.2008/26879.

Varlet, V., Serot, T., Monteau, F., Le Bizec, B. and Prost, C. 2007. Determination of PAH profiles by GC-MS/MS in salmon processed by four cold-smoking techniques. Food Additives and Contaminants, 24(7): 744–757.

Wilson, N.R.P. 1981. Meat and Meat Products Factors Affecting Quality Control, Applied Science Bub., London: 150–152.