Journal of Food Composition and Analysis 73 (2018) 39–46

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Levels of polycyclic aromatic hydrocarbons (PAHs) in smoked and sun-dried fish samples from areas in Lake Victoria in Mwanza, Tanzania

John Andrew Marco Mahugija[a,*], Emmanuel Njale[b]

[a] Chemistry Department, University of Dar es Salaam, P.O. Box 35061 Dar es Salaam, Tanzania
[b] Mwalimu Julius K. Nyerere University of Agriculture and Technology, P.O. Box 976 Musoma, Tanzania

ARTICLE INFO

Chemical compounds studied in this article:
Acenaphthylene
Anthracene
Benzo[a]anthracene
Benzo[b]fluoranthene
Benzo[k]fluoranthene
Benzo[a]pyrene
Benzo[ghi]perylene
Chrysene
Dibenz[a,h]anthracene
Indeno[1,2,3-cd]pyrene
Fluorene
Phenanthrene
Pyrene

Keywords:
Food analysis
Food composition
PAHs
Levels
Smoked fish
Sun-dried fish
Lake Victoria
Tanzania

ABSTRACT

This study investigated the concentrations of 13 polycyclic aromatic hydrocarbons (PAHs) in smoked and sun-dried fish samples of three species (*Synodontis victoriae*, *Haplochromis* spp and *Lates niloticus*) from areas in Lake Victoria in Mwanza region, Tanzania. The PAHs in cleaned extracts were determined using gas chromatography-mass spectrometry (GC–MS). The concentrations of total PAHs in sun-dried fish samples were up to 0.13 mg/kg in *S. victoria* and *Haplochromis* spp and ranged from 0.08 to 0.3 mg/kg in *L. niloticus*. The concentrations of total PAHs in smoked fish samples ranged from 12.1 to 31.4, 21.8 to 27.4 and 19.5 to 33.9 mg/kg in *S. victoriae*, *Haplochromis* spp and *L. niloticus*, respectively. The concentrations of benzo[a]pyrene in all the smoked fish samples exceeded the European Union (EU) maximum permissible level (0.002 mg/kg) and the sum of the concentrations of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene in all the smoked fish samples exceeded the EU maximum permissible level (0.012 mg/kg), but were generally below these limits in the sun-dried fish samples. The concentrations of the PAHs in smoked fish samples were significantly greater than in the sun-dried samples.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs, also called polynuclear aromatic hydrocarbons) constitute a class of organic compounds containing two or more aromatic rings made up of hydrogen and carbon atoms only. PAHs are formed by incomplete combustion processes during the burning of petroleum, coal, wood or other materials. PAHs are also emitted from natural sources such as natural fires and thermal geological processes (*e.g.*, volcanic activity) (Benner et al., 1990; Howsam and Jones, 1998; Pointet and Milliet, 2000). Humans are exposed to PAHs through different pathways. Most individuals are primarily exposed to PAHs through food sources (Forsberg et al., 2012). PAHs have received much attention because of their environmental and public health effects. Studies have shown that exposure to the PAHs can lead to the development of cancers, mutation, reproductive defects, immunosuppression, retarded growth and damage to the nervous system and vital organs, such as the liver and kidney. They therefore affect the survival of organisms (Agency for Toxic Substances and Disease Registry (ATSDR), 1995; International Agency for Research on Cancer (IARC), 2010; European Commission (EC), 2011a,b).

In order to avoid spoilage of fish by microbial activity on prolonged storage at high ambient temperature and for other preservation purposes, fish are usually frozen or dried by smoking, or other techniques, such as sun-drying. Smoke has antibacterial, antioxidant and dehydrating properties and therefore it enhances preservation of fish or other food substances. In addition, the smoke gives a good taste, colour and aroma to the food (Mičulis et al., 2011). One of the significant sources of PAHs in the human food chain is smoking of food substances,

* Corresponding author.
E-mail address: mahugija@udsm.ac.tz (J.A.M. Mahugija).

https://doi.org/10.1016/j.jfca.2018.07.010
Received 26 March 2018; Received in revised form 25 June 2018; Accepted 18 July 2018
Available online 19 July 2018
0889-1575/

such as fish and meat. Smoking of food substances is an ancient technology that has been used for many years. It is the process of exposing the food products to the smoke for the purpose of preserving them and increasing their palatability by adding flavour and imparting rich brown colour (Šimko, 2002). Traditional fish smoking involves salting and treating whole eviscerated filleted fish with wood or biomass smoke. The smoke is produced by smouldering firewood and grasses or other materials such as sawdust in an oven or kiln and are placed directly below the hanging fish or fillets that are laid out on a mesh tray. Traditional smoking methods can either be direct or indirect. In direct smoking method, the smoke is generated in the same chamber where the products are processed, while in indirect smoking, the smoke is generated in a different chamber and the products are placed in separate heated sections. Direct smoking exposes the products to higher PAH contents than indirect smoking (Akpambang et al., 2009). Apart from smoking as a preservative method, some people use sunlight to dry the fish in which the pre-salted fish fillets are dried (exposed) under the sun.

The levels and compositions of PAHs in dried food substances vary greatly depending on the materials and techniques used for the drying and hence the compositions in different areas/countries are variable. Although PAHs have received much attention in developed countries, studies in developing countries are scarce. To the best of our knowledge, there has been no study conducted on PAHs in fish in Tanzania. With rapid population growth in the area, the multiple activities in the Lake Victoria basin including waste and industrial discharges, transportation, oil spillage, accidental oil spills from water vessels and the use of firewood, grasses and other materials for fish smoking processes, serious contamination of fish by PAHs could be expected. The most common fish drying techniques used in the study areas are smoking and sun-drying and are usually used without appropriate control. These drying techniques can have different significant effects on the contents of PAHs in the dried products. Therefore, the aim of this study was to determine the concentrations of PAHs in smoked and sun-dried fish samples from areas in Lake Victoria in Mwanza region, Tanzania.

2. Materials and methods

2.1. Sampling

Fish samples were collected from four sites on 18th to 19th June 2017. Two sites (Mchangani and Ntama) were located in Kome Island in Lake Victoria in Sengerema district and the other sampling sites were Ibanda and Kirumba, located in Ilemela district in Mwanza region. Smoked fish samples were collected directly from the smoking premises. The unsmoked sun-dried fish samples were collected from areas where sun drying is done. The smoking techniques used in the areas include smoking over a firewood kiln at Ntama, smoking over open fire using grasses at Ibanda and firewood smoking kiln at Mchangani (Fig. 1). Smoked fish samples were randomly collected from the smoking sites at Mchangani, Ibanda and Ntama. The smoked fish samples comprised of three species, namely *Lates niloticus* (Nile perch), *Synodontis victoriae* (Lake Victoria squeaker) and *Haplochromis* spp (Haplochromine). Sun-dried (unsmoked) fish samples were collected from Kirumba (*Lates niloticus*), Ibanda (*Haplochromis* spp) and Ntama (*Synodontis victoriae*). A total of 38 smoked fish samples and a total of 22 sun-dried fish samples were collected from the sites. The samples were collected depending on the fish species available at each site. Each fish sample was separately packed in aluminium foil, then placed in a polyethylene bag and kept in an icebox for transportation to the laboratory (Chemistry Department, University of Dar es Salaam) where the samples were kept deep frozen prior to preparation and analysis.

2.2. Chemicals

The chemicals used included methanol (99.8% purity, certified ACS reagent grade), potassium hydroxide (> 85.0% purity, certified ACS grade), acetone (99.6% purity, ACS reagent grade), cyclohexane (99.99% purity, HPLC grade) and anhydrous sodium sulfate (99.5% purity, analytical reagent grade) purchased from Fisher Scientific, Loughborough, United Kingdom (UK); silica gel (ultrapure, 60–200 µm, 60 A, for column chromatography, Acros Organics, New Jersey USA and Geel, Belgium), and PAH standards (> 99% certified purity, analytical standard grade, for HPLC and GC from Sigma-Aldrich, Gillingham, UK).

2.3. Sample preparation

Before extraction, the fish samples were deboned and cut into small pieces and then homogenised in a blender. The skin was not removed and therefore it was included in the processing and analysis due to the fact that dried fish is usually cooked and consumed/eaten with its skin in Tanzania. To avoid cross-contamination, the blender was thoroughly cleaned after use for every sample. The homogenised samples were kept in separate containers ready for extraction.

2.4. Extraction and clean up

Extraction of the fish samples was performed according to the procedures described by Mittendorf et al. (2010) with minor modifications. The homogenised sample (15 g) was placed into a 250-mL E-flask and mixed with anhydrous sodium sulfate (20 g) for drying any water present in the sample. Then, 4 M methanolic potassium hydroxide solution (60 mL) was added for saponification and the sample was shaken in an ultrasonic bath (Branson 2510, Marshall Scientific) for 30 min in a sealed flask. The saponified sample was filtered through glass wool into a 250-mL E-flask; cyclohexane (100 mL) was added to the sample, which was then shaken for about 5 min and allowed to stand to let the layers separate. After separation, the upper layer containing cyclohexane was placed in an E-flask. The cyclohexane layer was washed with a mixture of methanol:water (4:1, 50 mL) and allowed to separate, and then, the organic phase was drawn into the E-flask. The extract was dried with anhydrous sodium sulfate (10 g). The cyclohexane fraction was transferred to a round-bottomed flask and concentrated to about 2 mL in a rotary evaporator (Büchi rotavapor R-114 with water bath B-480, Heto cold bath/circulator CBN-8-30, vacuum pump & controller; Tamro Med-Lab AB, Mölndal, Sweden) under reduced pressure at 40 °C.

Clean-up of the extracts was done using adsorption column chromatography. A glass column (10 mm i.d. × 30 cm) was packed with silica gel (15 g) and topped up with anhydrous sodium sulfate (5 g) and the column was rinsed with cyclohexane (10 mL). Then, the extract (2 mL) was slowly introduced into the column and eluted with cyclohexane (30 mL). Eluates were concentrated in a rotary evaporator to 2 mL ready for gas chromatography-mass spectrometry (GC–MS) analysis.

2.5. Gas chromatographic analysis

The cleaned sample extracts were analysed for PAHs using a GC system coupled to a mass spectrometer (GC–MS QP2010 Ultra; Shimadzu Corporation, Kyoto, Japan). Separation was accomplished with a non-polar capillary column (Rtx-5MS, 30 m × 0.25 mm i.d. × 0.25 µm film thickness; Restek, Bellefonte, PA). The column gas flow rate was maintained at 1.0 mL/min. Ultra-pure helium gas (99.99%) was used as a carrier gas. The following temperature programme was used: initial oven temperature of 90 °C held for 2 min, then ramped at a rate of 5.0 °C/min to the final temperature of 320 °C and held for 5 min. The injection port temperature was maintained at 250 °C and the internal pressure was set at 150 kPa. Samples (1 µL) were injected in splitless mode using an auto-sampler with a septum purge flow of 3 mL/min. The GC interface temperature was maintained at 230 °C. The mass spectrometer was operated in electron ionization (EI) mode