Food and Chemical Toxicology 59 (2013) 46–54

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

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

Polycyclic aromatic hydrocarbons in commercial squids from different geographical origins: Levels and risks for human consumption

Filipa Gomes [1], Marta Oliveira [1], Maria João Ramalhosa, Cristina Delerue-Matos, Simone Morais *

REQUIMTE, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 431, 4200-072 Porto, Portugal

ARTICLE INFO

Article history:
Received 24 February 2013
Accepted 21 May 2013
Available online 30 May 2013

Keywords:
Squids
Polycyclic aromatic hydrocarbons
Atlantic
Indic and Pacific Oceans
Health risks for human consumption

ABSTRACT

The concentrations of 18 polycyclic aromatic hydrocarbons (PAHs) were determined in five commercially valuable squid species from different geographical origins (Atlantic, Indic and Pacific Oceans). Out of the 18 quantified PAHs (the 16 PAHs considered by US EPA as priority pollutants, dibenzo(a,l)pyrene and benzo(j)fluoranthene) only dibenz(a,h)anthracene was not detected. The total concentrations of PAHs varied by a factor of more than 100-fold, from 0.22 (*Loligo gahi*) to 60.9 μg/kg ww (*Loligo reynaudii*). Intra- and inter-specific variability of PAH levels was statistically assessed. Nine carcinogenic (probable/possible) PAHs accounted for 1% (*L. reynaudii*) to 26% (*Loligo opalescens*) of the total PAHs content being the main contributors naphthalene (in *Loligo duvaucelii*, *L. reynaudii* and *Loligo vulgaris* species), chrysene (in *L. opalescens*) and indeno(1,2,3-cd)pyrene (in *L. gahi*). PAHs source analysis indicated that four of the five zones of capture of the different squid species are significantly affected by both petrogenic and pyrolytic sources. Assessment of the target carcinogenic risks, established by the US EPA, suggested that *L. gahi* (Atlantic Ocean) and *L. opalescens* (from Pacific Ocean) may pose additional risks for consumers, if not eaten in moderation, derived from benzo(a)pyrene ingestion.

1. Introduction

Among seafood species, cephalopods represent one of the ecologically and commercially most interesting classes. The share of cephalopods (squid, cuttlefish and octopus) in world fish trade was 4% in 2008 (FAO, 2011). In Portugal, they represented *ca.* 10% of landings, but the values in terms of auction transaction corresponded up to 26% of the wholesale market registered for marine species (European Commission, 2010; Lourenço et al., 2009). In terms of trade and landing, *Loligo duvaucelii*, *Loligo gahi*, *Loligo reynaudii*, *Loligo opalescens* and *Loligo vulgaris* represent the major squid species of three distinct oceans, namely, the Atlantic Ocean, Indic Ocean and Pacific Ocean (FAO, 2010, 2011). Like several other cephalopods, squids are part of the traditional diet of Japan, Korea, Argentina, Taiwan and China, and of coastal communities of southern Europe, such as Spain, Portugal, Morocco, Mauritania, Greece and Italy (FAO, 2012). Despite the health benefits of a seafood diet, an issue of concern related to frequent seafood consumption is the potential risk arising from exposure to aquatic pollutants (Kalogeropoulos et al., 2012; Martorell et al., 2010; Ramalhosa et al., 2012a; Storelli, 2008; Vieira et al., 2011). Furthermore, the cephalopods capacity to accumulate contaminants and to grow and reproduce in polluted milieus is well described in the literature (Raimundo et al., 2004, 2010; Pierce et al., 2008; Semedo et al., 2012). Polycyclic aromatic hydrocarbons (PAHs) are a group of over 500 different ubiquitous compounds that are usually found in complex mixtures (Kazerouni et al., 2001; McGrath et al., 2007; WHO, 2013). Some PAHs are classed as persistent organic pollutants (WHO, 2013) and sixteen are regarded as priority pollutants by US EPA (2005). Several of the PAHs are referred to as endocrine disrupting chemicals, with the most well-known marker of PAHs being benzo(a)pyrene (WHO, 2013). PAHs may affect a variety of biological processes and can be potent cell mutagens and carcinogens (IARC, 2010; Karacik et al., 2009; WHO, 2013). In non-smokers and non-occupationally exposed populations, diet is the primary source of human to PAHs, contributing to more than 70% of the total exposure (Martorell et al., 2010, 2012; Moon et al., 2010). According to The European Food Safety Authority (EFSA), the compound benzo(a)pyrene cannot be maintained as the only PAH marker in seafood. On September 2012 and following EFSA advice, the European Commission published Regulation 835/2011 (European Commission, 2011) with new maximum permitted levels of benzo(a)pyrene and the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene.

Ingestion of lower trophic level organisms is one of the most important pathways for contaminants accumulation in squids (Croxton et al., 2012). Both environmental and biological factors may also be relevant (Raimundo et al., 2004; Maioli et al., 2010).

* Corresponding author. Tel.: +351 228340500; fax: +351 228321159.
E-mail address: sbm@isep.ipp.pt (S. Morais).
[1] These authors contributed equally to this work.

0278-6915/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2013.05.034

In aquatic fauna, mollusks (which include cephalopods) have the poorest metabolic capacity to metabolize PAHs (van der Oost et al., 2003; Varanasi, 1989). In addition to their important commercial role, cephalopods have the potential to act as bioindicator and sentinel species for ecological risk assessment and marine environment monitoring studies (Semedo et al., 2012). They display high growth rates and short life spans (6 months–2 years; Boyle and Rodhouse, 2005; Ceriola and Milone, 2007; FAO, 2010). For these reasons, cephalopods are of major interest for monitoring variations of pollutant concentrations in the environment as the accumulated concentrations in their tissues reflect the bioavailability and the pollutant variations in their immediate environment over a relatively short time scale (Raimundo et al., 2010; Kojadinovic et al., 2011; Semedo et al., 2012).

Information on PAH levels in squids is almost inexistent (Morais et al., 2012; Perugini et al., 2007a, 2007b; Unger et al., 2008). To the best of our knowledge, no study was found concerning the PAH levels in four of the five selected species, *L. duvaucelii*, *L. gahi*, *L. reynaudii*, *L. opalescens* and *L. vulgaris*. Only data obtained in several surveys performed in Catalonia, Spain concerning *L. vulgaris* were found (Llobet et al., 2006; Martí-Cid et al., 2008; Martorell et al., 2010).

Thus, the aim of this work was to characterize five commercially valuable squid species, captured in three distinct oceans, regarding their PAH levels in order to assess their status of contamination and their intra and inter-specific variability. Analysis was focused on 18 PAHs (the 16 PAHs considered by US EPA as priority pollutants, dibenzo(a,l)pyrene and benzo(j)fluoranthene corresponding to 10 PAHs included in Commission Regulation 1881/2006 (European Commission, 2006)) which includes the four PAHs recently indicated as the most appropriate indicators of the presence of carcinogenic and genotoxic PAHs in foodstuffs (European Commission, 2011). Based on per capita cephalopod consumptions for low and high consumer populations (FAO, 2012), the daily PAHs intake, non-carcinogenic and carcinogenic risks were also estimated.

2. Experimental

2.1. Reagents and materials

The reference mixture of PAHs (EPA 610) containing naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene was from Supelco (Bellefonte, PA, USA). Individual standards of each compound, benzo(j)fluoranthene and dibenzo(a,l)pyrene were also purchased from Supelco (Bellefonte, PA, USA). Working mixed standard solutions containing all the PAHs were prepared by dilution of the stock solutions with acetonitrile and stored at −20 °C in darkness to avoid volatilization and photodegradation.

Acetonitrile was purchased from Sigma–Aldrich (Steinheim, Germany) and ultrapure water was obtained from a Milli-Q simplicity 185 system (Millipore, Bedford, MA, USA).

2.2. Sample collection and characterization

Squids from the different geographical origins available to Portuguese consumers were purchased randomly from the markets in NW region of Portugal during 2010 and 2011. The species collected were *L. duvaucelii* and *L. vulgaris* from the Indic Ocean, *L. reynaudii* and *L. gahi* from the Atlantic Ocean, and *L. opalescens* from the Pacific Ocean (Table 1). Specimens were transported to the laboratory in clean polyethylene bags, stored in ice and processed immediately after collection.

Sample collection, biometric characterization and preparation were performed in accordance with EPA Guide No 823-B-00-07 and EC Regulation No. 333/2007. Specimens were carefully weighted, biometrically characterized (tentacle, mantle and total lengths; Table 1) and manually eviscerated. Each sample for further analysis consisted of an equal amount of the edible parts of, at least, four individuals and had a minimum mass of 200 g. For each sample, three independent assays were performed. Homogenization was performed mechanically with a blender until a smooth paste was obtained. Samples were kept frozen in 50 mL polycarbonate containers at −20 °C until analysis.

Moisture was evaluated according to the Portuguese Standard NP 2282-1991 and the official AOAC method (AOAC, 2007). For total fat content determination, the recommended AOAC method (AOAC, 2007) was applied.

2.3. Microwave-assisted extraction

Microwave-assisted extractions were performed accordingly with Ramalhosa et al. (2012a, 2012b) with a MARS-X 1500 W (Microwave Accelerated Reaction System for Extraction and Digestion, CEM, Mathews, NC, USA) configured with a 14 position carousel. Briefly, extractions were performed at the optimal conditions: glass extraction vessels, 1 g of sample, 20 min at 110 °C with 10 mL of acetonitrile and medium stirring speed. The extracts were reduced to a small volume using a rotary evaporator (Buchi Rotavapor, R-200) at 20 °C. Then, a gentle stream of nitrogen was used to evaporate the extracts and immediately before chromatographic analysis, the residue was redissolved in 1.0 mL of acetonitrile.

2.4. Liquid chromatography analysis

Extracts were analyzed according to Ramalhosa et al. (2009, 2012a, 2012b) using a LC system (Shimadzu Corporation, Kyoto, Japan) equipped with a LC-20AB pump (high-pressure gradient solvent delivery module equipped with two dual-plunger tandem-flow pumps), DGU-20AS degasser and photodiode array SPD-M20A (PAD) and fluorescence RF-10AXL (FLD) detectors on line. The equipment is placed in a temperature-controlled room (set to 20 ± 1 °C). Separation of the compounds was performed in a C18 column (CC 150/4 Nucleosil 100-5 C18 PAH, 150 × 4.0 mm; 5 μm particle size; Macherey–Nagel, Duren, Germany).

Each compound was detected at its optimum excitation/emission wavelength pair: 260/315 nm (naphthalene, acenaphthene and fluorene), 260/366 nm (phenanthrene), 260/430 nm (anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b + j)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene and dibenzo(a,l)pyrene,) and 290/505 nm (indeno(1,2,3-cd)pyrene). Acenaphthylene, which shows limited fluorescence, was analyzed at 254 nm in PAD.

The overall MAE-LC procedure for analysis of PAHs was previously validated by systematic recovery experiments and analyzing the certified reference material SRM 2977 (Mussel tissue) (Ramalhosa et al., 2012b). Recoveries ranged from 70.2 ± 4.8% (benzo(g,h,i)perylene) to 101.2 ± 4.2% (pyrene).

External calibrations with PAH mixed matrix matched standards using 6 calibration points were performed (Ramalhosa et al., 2009, 2012a,b). Analytical blanks and PAH mixed matrix matched standards were included in each batch of samples to check instrument performance. The results were accepted if the relative standard deviation of the standards were lower than 10%. Each analysis was run at least in triplicate. Detection limits between 0.04 ng/g wet weight (ww) for benzo(k)fluoranthene and 8.1 ng/g ww for acenaphthylene were obtained. PAH concentrations were determined on wet (ww) and dry (dw) weight basis but to simplify the discussion of the results only concentrations in ww are presented.

2.5. Estimation of potential public health risks

The methodology for estimation of non-carcinogenic and carcinogenic risks was applied in accordance with that provided in the US EPA Region III Risk-based Concentration table (US EPA, 2010). One age-category ⩾ 21 years adult (70 kg) was used. The exposure duration (ED) value used in the intake calculations was 21 years.

The non-carcinogenic risks for naphthalene, acenaphthene, fluorene, anthracene, fluoranthene and pyrene (detected PAHs for which an oral reference dose is established) through squid consumption were assessed by the non-carcinogenic target hazard quotient (THQ; US EPA, 2010).

For carcinogens, risks (TR) were estimated as the incremental probability of an individual to develop cancer, over a lifetime, as a result of exposure to that potential carcinogen (i.e., incremental or excess individual lifetime cancer risk; US EPA, 1989).

THQ and TR approaches were calculated using Eqs. (1) and (2), respectively:

$$THQ = (EFr \times ED \times IR \times C)/(RfD \times BW \times AT) \quad (1)$$

$$TR = (EFr \times ED \times IR \times C \times CSFo)/(BW \times AT) \quad (2)$$

where THQ and TR are dimensionless; EFr is the exposure frequency (350 days per year); ED is the exposure duration (years); IR is the food ingestion rate (1.37×10^{-3} and 1.12×10^{-2} kg per day per person for the world and for Portugal, respectively; FAO, 2012); C is the PAHs concentration in squid samples (mg/kg); RfD is the oral reference dose (2.0×10^{-2} mg/kg/day for naphthalene, 6.0×10^{-2} mg/kg/day for acenaphthene, 3.0×10^{-1} mg/kg/day for anthracene, 3.0×10^{-2} mg/kg/day for pyrene, 4.0×10^{-2} mg/kg/day for fluorene and fluoranthene; US EPA, 2010); BW is the body weight (kg) and AT is the number of days over which the exposure is averaged (365 days per year × ED for non-carcinogenic effects and 25,500 days (70 years × 365 days/year) for carcinogenic effects); CSFo is the oral carcinogenic slope factor from the Integrated Risk Information System (US EPA, 2010) database (7.30 $(mg/kg/day)^{-1}$ for benzo(a)pyrene; 7.30×10^{-1} $(mg/kg/day)^{-1}$