Environmental Research 164 (2018) 165–172

Contents lists available at ScienceDirect

Environmental Research

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

Polycyclic aromatic hydrocarbons bioaccessibility in seafood: Culinary practices effects on dietary exposure

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ARTICLE INFO

Keywords:
Polycyclic aromatic hydrocarbons
Seafood
Bioaccessibility
Daily intake
In vitro digestion

ABSTRACT

This work aimed to determine the effect of culinary practices on the contamination level and bioaccessibility of polycyclic aromatic hydrocarbons (PAHs) in seafood. The selected farmed seafood species (marine shrimp, clams and seaweed) were commercially available in Portugal. The mean concentrations of PAHs varied between 0.23 and 51.8 μg kg^{-1}, with the lowest value being observed in raw shrimp and the highest in dried seaweed. The number of compounds detected in seaweed and clams (naphthalene, acenaphthene, fluorene, phenanthrene, benzo(b)fluoranthene and benzo(j)fluoranthene) were higher than in shrimp (fluorene and pyrene). Among the PAHs measured, fluorene was the predominant one. There was a significant interaction effect between species and culinary treatment ($p < 0.05$), thus boiled and dried seaweed samples presented the lowest and the highest levels of fluorene (0.13 and 1.8 μg kg^{-1}), respectively. The daily intake of PAHs decreased with bioaccessibility, varying from 22% for benzo(k)fluoranthene (in raw clam) to 84% for phenanthrene (in steamed clam). According to the potency equivalent concentrations, screening values and bioaccessibility of PAHs, the consumption of marine shrimp, clam and seaweed is considered as safe for consumers.

1. Introduction

Seafood is an important source of proteins, healthy lipids, vitamins and minerals in the Portuguese's diet, which recorded the highest annual consumption rate in the EU 52.2 kg by person $year^{-1}$ (Food and Agriculture Organization, 2016). However, some seafood can accumulate organic lipophilic nonpolar pollutants such as polycyclic aromatic hydrocarbons (PAHs), from the aquatic environment (Nasher et al., 2016), representing a potential risk for consumers.

PAHs are ubiquitous and persistent compounds with two or more benzene rings fused in various arrangements (Yu et al., 2012), that are formed during pyrolysis or incomplete combustion of organic material (Veiga et al., 2014). Man-made sources of PAHs include motor-vehicle exhausts, emissions from industry, commercial and household heating with coal, wood or other biomass fuels, indoors tobacco smoke (Li et al., 2014) and cooking processes (Singh et al., 2016). The pyrolysis of organic matter, such as fat, carbohydrate and protein, at temperatures above 200 °C promotes PAH formation, as well as the yield of lipids dripping in direct contact over the flame at intense heat (Hamidi et al., 2016).

These compounds are organic lipophilic, non-biodegradable, environmentally persistent, toxic and categorized as carcinogen (Ledesma et al., 2014). Because of their toxicity, mutagenic and/or carcinogenic properties, the US Environmental Protection Agency listed 16 PAHs as priority compounds (EPA, 2005). Benzo(a)pyrene is the only known carcinogen (group 1; IARC, 2010) whereas naphthalene, benz(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, chrysene, and indeno(1,2,3-cd)pyrene are considered as possible carcinogens to humans (group 2B; IARC, 2002, 2010); dibenzo(a,l)pyrene and dibenz(a,h)anthracene are considered probable carcinogens to humans (group 2A; IARC, 2010). They are easily and rapidly absorbed by organisms, passing into the marine food chain (Martinez et al., 2004), and consequently promoting seafood contamination.

The human exposure to PAHs occurs mainly through ingestion

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https://doi.org/10.1016/j.envres.2018.02.013
Received 30 September 2017; Received in revised form 25 January 2018; Accepted 11 February 2018

(88–98%), followed by inhalation (2–12%) (Alomirah et al., 2011). Oral bioaccessibility testing has been adopted for measuring contaminants fraction released from the food matrices that can be absorbed by the human gastrointestinal tract after ingestion and digestion (Koch et al., 2013).

Bioaccessibility tests can be carried out using in vitro models, which are simple, easy, cost-effective, provide accurate results in a short time and reduce the need of animal experimentation (Hamidi et al., 2016). Despite already validated for PAHs, only few studies employed this tool to assess these compounds bioaccessibility in seafood and its relationship with culinary practices (Dosunmu et al., 2016; Soriano et al., 2007; Wang et al., 2010; Yu et al., 2012). The bioaccessibility of PAHs from Shanghai seafood were determined in shrimp, clam, carp and croaker with shrimp presenting the lowest levels of PAHs and clam the highest levels and, revealing 47.2% of bioaccessibility on average (Yu et al., 2012). Shrimps can bioaccumulate contaminants from water and sediment (Dosunmu et al., 2016), while filter feeding organisms like bivalves can absorb contaminants from water and plankton (Soriano et al., 2007). Thus, these species can be used as sentinel organisms for monitoring PAHs in the environment and may, simultaneously, be an important tool to assess human exposure to contaminants (Mercogliano et al., 2016).

In order to gather more data on contamination of PAHs in seafood and to characterize the effects of different culinary practices on dietary exposure of this contaminant group, PAHs bioaccessibility was determined in several seafood species (shrimp, clams and seaweed) from different geographic origins (Equator, Vietnam and Portugal).

2. Material and methods

2.1. Reagents and materials

The reference mixture of PAHs (EPA 610) (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, ben-zo(a)pyrene, dibenz(a,h)anthracene, benzo(g,*h*,i)perylene and indeno(1,2,3-cd)pyrene) and individual standards of each compound, benzo(j)fluoranthene and dibenzo(a,l) pyrene were purchased from Supelco (Bellefonte, PA, USA). Mixed standard solutions containing all PAHs were prepared by dilution of the stock solutions with acetonitrile and stored at − 20 °C in the 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, cooking and proximate chemical composition

Samples of marine shrimp (*Litopenaeus vannamei*; n = 270, 3.0 kg) and clams (*Meretrix lyrata;* n = 240, 3.3 kg) originated from Equator and Vietnam aquaculture farms were purchased from Portuguese markets in Lisbon. A total of 5.0 kg of marine seaweed (*Ulva* sp.) were caught in aquaculture ponds situated in Olhão city, Algarve region, South of Portugal. All samples were transported to the laboratory in clean polyethylene bags according to the recommendations of EFSA (2008) and processed immediately upon arrival.

Raw shrimp specimens were carefully cleaned, headed and peeled; clams were washed and if necessary opened. Only the edible tissues of shrimps and clams were preserved; a sample for analysis consisted of a minimum mass of 200 g. A portion of each fresh sample (raw) was kept without any cooking treatment. Each treatment was prepared, in duplicate, with 30 shrimps and 40 clams each. Different portions of shrimp and clam samples were steamed at 105 °C during 5 and 7 min, respectively. Shrimp fillets were prepared and spiced over 15 min with salt (1.5% w/w) and garlic cloves (1% w/w) and fried in extra virgin olive oil (2% w/v after removing garlic from the fillets) during 5 min at 180 °C. Portions of fresh seaweed samples were boiled in distilled water (1:20 w/v) during 15 min, drained, cooled and, when reached room temperature, weighed in order to define the uptake of water during boiling (Maehre et al., 2016). Dried seaweed samples were prepared by drying portions of the fresh samples during 48 h at 50 °C. Both raw and cooked samples were homogenized with a blender (800 × *g*, 10 min) and stored at − 20 °C until further analysis.

Moisture was evaluated according to the Portuguese Standard NP 2282-1991 and the official AOAC method (AOAC, 2007). Total ash content was assessed through the complete combustion of samples over 16 h at 500 °C, until a constant weight was achieved (AOAC, 2005). Crude protein and fat contents were determined according to the methodologies described by Saint-Denis and Goupy (2004) and Folch et al. (1957), respectively.

2.3. Bioaccessibility assays

Bioaccessibility was assessed using a static in vitro human digestion protocol adapted from Versantvoort et al. (2005), being calculated according to the following equation:

$$\text{Bio}\ (\%) = ([\text{PAHs}]_{\text{bio}} \times 100)/[\text{PAHs}]_{\text{fresh sample}} \quad (1)$$

where Bio (%) is the bioaccessibility of a specific compound, $[\text{PAH}]_{\text{bio}}$ is the concentration of the PAH on the bioaccessible fraction, and $[\text{PAHs}]_{\text{fresh sample}}$ is the concentration of the compound in the fresh sample (adapted from Manita et al., 2017).

The simulated gastro-intestinal (GI) digestion was performed in three consecutive phases: saliva, gastric and intestinal by using saliva (pH 7.0), gastric juice (pH 2.0), duodenal juice and bile (pH 7.0) digestion fluids, respectively. Briefly, 1.5 g of shrimp (raw, fried, and steamed) and clam (raw and steamed) samples was stirred during 5 min with 4.0 ml of artificial saliva. Then, 8.0 ml of artificial gastric juice and 2 drops of pure hydrochloric acid (A.C.S., 37%, Sigma-Aldrich, Saint Louis, USA) were added, followed by 2 h of incubation at 37 °C with constant rotation. Additionally 8 ml of artificial duodenal juice, 4 ml of artificial bile and 1.3 ml of sodium bicarbonate (A.C.S., ≥ 99.7%, Sigma-Aldrich, Saint Louis, USA) were added to the extract and submitted to a second incubation (2 h at 37 °C). Digestion was stopped by immersion of samples on ice during 5 min. The digested and non-digested fractions of samples were separated through centrifugation at 2750 × *g* (4 °C, 10 min). The simulated GI digestion for seaweed was performed according to Maehre et al. (2016), namely reducing the enzymes (amylase, pepsin, and pancreatin) by 50% due to the lower protein content (2–3%) in algae samples. Approximately 1 g of boiled and 0.5 g of raw and dried seaweed samples was used.

To confirm the in vitro digestion efficiency, total protein levels were determined in shrimp, clam, and seaweed raw and cooked samples before digestion and in the non-bioaccessible (NBIO) fractions by-using a combustion method of analysis with the FP-528 DSP LECO nitrogen analyser (LECO, St. Joseph, MI, USA) calibrated with EDTA according to the Dumas method (Saint-Denis and Goupy, 2004).

2.4. Extraction and chromatographic analysis of PAHs

Microwave-assisted extractions were performed in a MARS-X 1500 W (Microwave Accelerated Reaction System for Extraction and Digestion, CEM, Mathews, NC, USA) and according to the validated conditions previously described by Ramalhosa et al. (2012a, 2012b). Briefly, 1 g of fresh and 0.5 g for lyophilized samples were extracted with 10 ml of acetonitrile at 110 °C during 20 min with a medium stirring speed. The solvent selection (acetonitrile), its volume, the extraction temperature and time were previously optimized and validated by Ramalhosa et al. (2012b), with quantitative extraction rates over 70% for all PAHs. After cooling, extracts were completely dried using a rotary evaporator (Buchi Rotavapor, R-200) at 20 °C, being the residue re-dissolved in 250 μL of acetonitrile.