Food and Chemical Toxicology 104 (2017) 69–78

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Preliminary assessment on the bioaccessibility of contaminants of emerging concern in raw and cooked seafood

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ARTICLE INFO

Article history:
Received 9 August 2016
Received in revised form
13 January 2017
Accepted 29 January 2017
Available online 13 February 2017

Keywords:
Seafood
Perfluorinated compounds
Brominated flame retardants
Pharmaceuticals and personal care products
Bioaccessibility
Steaming

ABSTRACT

A preliminary assessment of the bioaccessibility of contaminants of emerging concern (CeCs), including perfluorinated compounds (PFCs; i.e. PFOS and PFUnA), brominated flame retardants (BFRs; i.e. BDE47, BDE100, α-HBCD) and pharmaceuticals and personal care products (PPCPs; i.e. venlafaxine, methylparaben and UV-filter OC) was performed in seafood species available in the European markets. Additionally, the effect of steaming on CeCs bioaccessibility was also investigated for the first time. Overall, steaming affected differentially contaminants' concentrations, for instance, decreasing PFOS levels in flounder, but increasing both BDE47 and BDE100. CeCs bioaccessibility varied according to seafood species and contaminant group, i.e. in general, lower bioaccessibility values were obtained for PBDEs (<70%, except for mackerel), while PFCs and PPCPs revealed higher bioaccessibility percentages (between 71 and 95%). The lowest bioaccessibility value was obtained for α-HBCD (mussel; 14%), whereas the highest percentage was observed in venlafaxine (mullet; 95%). Our preliminary study reports also, for the first time, the effects of steaming on CeCs bioaccessibility. In most cases, bioaccessibility was not affected by cooking, however, a decrease was observed in PBDEs and venlafaxine bioaccessibility in steamed mussels and mullet, respectively, thus lowering the potential health risks associated with seafood consumption.

Abbreviations: α-HBCD, hexabromocyclododecane; BD, sample before digestion; BDE47, 2,2′,4,4'-tetrabromodiphenyl ether; BDE99, 2,2′,4,4′,5-pentabromodiphenyl ether; BDE100, 2,2′,4,4′,6-pentabromodiphenyl ether; BFRs, brominated flame retardants; BIO, bioaccessible fraction after *in vitro* digestion; CeCs, contaminants of emerging concern; K_{ow}, hydrophobicity parameter, octanol/water partition coefficient; LOD, limit of detection; LOQ, limit of quantification; NBIO, non-bioaccessible fraction after *in vitro* digestion; OC, octocrylene; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; PFCs, perfluorinated compounds; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFUnA, perfluoroundecanoic acid; pp, poly propylene; PPCPs, pharmaceuticals and personal care product.

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http://dx.doi.org/10.1016/j.fct.2017.01.029
0278-6915/

1. Introduction

A balanced seafood diet has been widely recommended due to the health benefits associated with several constituents, such as biologically valuable proteins, long chain polyunsaturated n-3 fatty acids (LC n-3 PUFA), vitamins (E, A, D and B), macro and trace elements. For example, LC n-3 PUFA have been considered as key players in the prevention of cardiovascular diseases (He et al., 2004; Larsen et al., 2011). Nevertheless, seafood can also accumulate high levels of a broad range of chemical contaminants that raise human health-related concerns through consumption (Boucher et al., 2010; Calatayud et al., 2012; Haug et al., 2010; Maulvault et al., 2015; Moon and Choi, 2009; Vazquez et al., 2015). Until the present date, the European Commission regulation (ECR) 1881/2006 described maximum levels of lead, cadmium, mercury, some dioxins and PCBs and benzo(*a*)pyrene for several types of seafood (ECR, 2006, 2008, 2011a, 2011b, 2014). Despite these well-known monitored harmful contaminants, information regarding contaminants of emerging concern (CeCs) is still scarce. CeCs include perfluorinated compounds (PFCs), pharmaceuticals and personal care products (PPCPs), brominated flame retardants (BFRs), microplastics and toxins from harmful algal blooms (Vandermeersch et al., 2015).

For almost all CeCs, maximum permissible concentrations (MPCs) are not described in the EU legislation. However, regulatory authorities and the scientific community have been focusing their attention on these contaminants as accurate data are needed to assess risks associated with the presence of CeCs in seafood (Cardoso et al., 2010, 2013; Maulvault et al., 2013; Maulvault et al., 2011; reviewed by Vandermeersch et al., 2015). For example, high levels of the psychiatric drug venlafaxine (up to 36.0 ng g^{-1} dry weight (dw)) and the antibiotic azithromycin (up to 13.3 ng g^{-1} dw) have been observed in mussels from Po delta (Álvarez-Muñoz et al., 2015). These authors also reported caffeine, bisphenol A, triclosan, methylparaben, propylparaben, tris(2-butoxyethyl)phosphate (TBEP), which are known for their endocrine disruptive effects (Kaneko et al., 2008; Ma et al., 2016; Pinto et al., 2013), as the main endocrine disrupting compounds (EDCs) present in bivalves and fish from several coastal areas in Europe (e.g. Álvarez-Muñoz et al., 2015).

Levels of a specific contaminant detected in seafood do not reflect the quantity that will be released from food during the gastro-intestinal digestive process becoming available for absorption at the intestinal epithelium, and entering into the blood stream, i.e. the bioaccessible fraction of a contaminant. Therefore, assessing the bioaccessible fraction of a contaminant that can potentially reach the systemic circulation is essential to accurately assess consumer's health exposure (Collins et al., 2015; Versantvoort et al., 2005). Several *in vitro* models have been developed to simulate the gastrointestinal digestion process (Cardoso et al., 2015; Collins et al., 2015; Marques et al., 2011; Minekus et al., 2014; Versantvoort et al., 2005). Contrasting the most realistic but time demanding and costly *in vivo* methodologies, *in vitro* models are reproducible, fast, low cost, energy saving, and independent from physiological factors (reviewed by Cardoso et al., 2015). Nowadays, *in vitro* methodologies used to simulate human digestion are well-established and considered as one of the most suitable tools for food safety authorities. *In vitro* digestion methodologies have been used to evaluate nutrients and contaminant (particularly toxic metals) bioaccessibility in seafood, mainly in molluscs and fish (e.g. Amiard et al., 2008; Gao and Wang, 2014; He et al., 2010; He and Wang, 2013; Maulvault et al., 2011), and within the actual methods, the procedure described by Versantvoort et al. (2005) has been intensively described as realistic and reliable (Braga et al., 2016; Gao and Wang, 2014; He et al., 2010; He and Wang, 2013; Maulvault et al., 2011; Metian et al., 2009; Wang et al., 2014).

Most bioaccessibility studies and exposure assessment of chemical contaminants have been performed with raw products, despite most seafood products are commonly consumed after being processed (Cardoso et al., 2010; Maulvault et al., 2013). Additionally, for the majority of CeCs, little bioaccessibility information exists for seafood (polybrominated diphenyl ethers (PBDEs), Li et al., 2015; e.g. perfluorooctanoic acid (PFOA), Yu et al., 2010).

In this context, the main purpose of this study was to undertake a preliminary assessment of CeCs (i.e. PFCs, BFRs and PPCPs (including pharmaceuticals, parabens and UV-filters)) bioaccessibility in nine seafood samples (including fish and molluscs) available in different European markets, using an *in vitro* digestion protocol. The effect of culinary treatment (steaming) in CeCs bioaccessibility was also evaluated.

2. Material and methods

2.1. Sampling species and culinary treatment

Nine seafood samples were collected in different European markets, including mackerel (*Scomber scombrus*; Atlantic and Adriatic Sea), mullet (*Liza aurata*; Tagus estuary), flounder (*Platichthys flesus*; Western Scheldt), plaice (*Pleuronectes platessa*; North Sea), farmed seabream (*Sparus aurata*; Greece), canned tuna (unknown species and origin) and mussels (*Mytilus galloprovincialis*; Adriatic Sea, Po delta). All samples were collected between April 2014 and November 2015. For each species, specimens were of commercial sizes, with uniform sizes and weights. Origin, market country, number of specimens, total length (mm), weight (g) and moisture (%) are described in Table 1.

For fish, muscle tissue was collected from fillets without skin of 25 specimens, whereas for bivalves the edible part with the intervalvar liquid was collected from 50 individuals. Additionally, to investigate the effect of culinary treatment in CeCs bioaccessibility, some seafood samples were divided into two portions, one for raw assessment and the other one for cooking assessment (steaming at 105 °C during 15 min for fish and during 5 min for bivalves, wrapped in aluminium foil). The selected contaminants were the following: PFCs (perfluorooctanesulfonic acid, PFOS; perfluoroundecanoic acid, PFUnA); BFRs (2,2′,4,4'-tetrabromodiphenyl ether, BDE47; 2,2′,4,4′,6-pentabromodiphenyl ether, BDE100; hexabromocyclododecane, α-HBCD); and PPCPs (venlafaxine; methylparaben; UV-filter octocrylene, OC). The levels of targeted contaminants were assessed in all seafood samples, but only those presenting levels at least 7× times higher than LOD of a specific contaminant were selected for the study (see Table 1 for more details).

Finally, raw and steamed samples were homogenised with a grinder (Retasch Grindomix GM200, Germany) using polypropylene cups and stainless steel knives at 5000 rpm until complete visual disruption of the tissue, and further kept at −20 °C until *in vitro* digestion.

2.2. In vitro human digestion model

2.2.1. Reagents

The components used to prepare the digestive solutions were the following: Inorganic: NaCl (Merck, 99.5% m/v), $NaHCO_3$ (Merck, 99.5% m/v), $CaCl_2 \cdot 2H_2O$ (Sigma, C3881), KCl (Merck, 99.5% m/v), KSCN (Sigma, P2713), NaH_2PO_4 (Merck, 99.5% m/v), Na_2SO_4 (Merck, 90% m/v), NH_4Cl (Riedel-de Haen, 99.5% m/v), KH_2PO_4 (Merck, 99.5%), $MgCl_2$ (Riedel-de Haen, 99.5% m/v), HCl (Merck, 37% m/v); Organic: urea (Sigma, U5128), glucose (Sigma, G5400), glucuronic