Food Chemistry 232 (2017) 501–507

Contents lists available at ScienceDirect

Food Chemistry

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

First approach to assess the bioaccessibility of bisphenol A in canned seafood

Sara C. Cunha [a,*], Ricardo N. Alves [b], José O. Fernandes [a], Susana Casal [a], António Marques [b,c]

[a] LAQV – REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Rua Jorge de Viterbo Ferreira 228, 4050-313 Porto, Portugal
[b] Division of Aquaculture and Upgrading (DivAV), Portuguese Institute for the Sea and Atmosphere (IPMA), Lisboa, Portugal
[c] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Porto 11, Portugal

ARTICLE INFO

Article history:
Received 22 December 2016
Received in revised form 21 March 2017
Accepted 3 April 2017
Available online 6 April 2017

Keywords:
Bisphenol A
Bioaccessibility
Fish
Canned seafood
Contaminants

ABSTRACT

Human health risks due to bisphenol A (BPA) exposure through canned food consumption are an emerging safety concern worldwide. In this study, an *in vitro* digestion model was used to simulate human digestion and evaluate BPA bioaccessibility in canned seafood for the first time. BPA contents of canned tuna and sardine samples and their bioaccessible and non-bioaccessible fractions were determined by gas chromatography coupled to mass spectrometry (GC–MS). The 21 samples of canned tuna and sardines, all from the same producer but with different kind of sauces, showed BPA levels ranging from <1 μg kg^{-1} (limit of quantification, LOQ) to 62 μg kg^{-1}, with variable results within and between sample groups. BPA bioaccessibility was evaluated in six positive samples, with values ranging from 80 to 99%. The results suggest that BPA bioaccessibility was slightly lower in samples with higher lipid content.

1. Introduction

Bisphenol A (BPA) has been at the forefront of research in the last years due to its recognized estrogenic activity (EFSA, 2015). This organic chemical compound is used in large scale in the production of polycarbonate plastics and epoxy resins, which explains why the world production reaches nowadays an impressive value of >4.4 million tons annually (World Market, 2014).

BPA are often found in food and beverage containers, as well as in thermal paper, electronic devices, and medical supplies (EFSA, 2015). Owing to leaching during usage, due to e.g. high temperatures, acidic or basic conditions, or physical damage (Cao & Corriveau, 2008; Lim, Kwack, Kim, Kim, & Lee, 2009), BPA has a widespread presence in the environment and, likewise, in biota, and canned food and feed (Crain et al., 2007; Chen et al., 2016; Cunha & Fernandes, 2013).

Current knowledge indicates that the primary sources of human BPA exposure occurs through dietary sources, mainly from packaged food and beverages (Chen et al., 2016; Lorber et al., 2015). Two recent surveys performed by Liao and Kannan (2013, 2014) in the United States and China showed that canned food, usually contains higher concentrations of BPA than foods sold in glass, paper or plastic containers. Therefore, canned seafood represents an important source of BPA and other bisphenol analogues, like bisphenol B (BPB) (Cunha, Cunha, Ferreira, & Fernandes, 2012). Additionally, humans may be exposed to BPA from other sources, through diverse routes, including inhalation from aerosolized BPA, and dermal contact with thermal paper or medical supplies (Biedermann, Tschudin, & Grob, 2010; WHO, 2011). The accurate input of the human exposure to BPA through these different pathways is difficult to estimate, thus levels on the human organism, namely in urine, have been used to estimate total body burden in the population. It was estimated that human daily intake may vary from 0.1–1 μg kg^{-1} body weight (Vandenberg et al., 2010).

Currently, the tolerable daily intake (TDI) set by the European Commission is 4 μg kg^{-1} body weight (EFSA, 2015), while the maximum acceptable or "reference" daily dose established by the U.S. Environmental Protection Agency, is 50 μg^{-1} kg (FDA, 2008). The amount of BPA legally permitted to migrate from packaging into food, known as the specific migration limit, is based on TDI and it was set at 0.6 mg/kg by EU commission (Commission Regulation EU 10/2011).

In the last years, toxicological and epidemiological studies have established some links between relevant human BPA exposure and some disorders of endocrine origin (WHO, 2011). Exposure to BPA could result in a reduced ovarian response and *in vitro* fertilization success, reduced sperm quality and other male and female reproductive systems alterations (Rochester, 2013). BPA exposure in children could induce adverse neurodevelopmental effects

* Corresponding author.
E-mail address: sara.cunha@ff.up.pt (S.C. Cunha).

http://dx.doi.org/10.1016/j.foodchem.2017.04.006
0308-8146/

(Human Services, 2013; Rochester, 2013). Furthermore, exposure to BPA has also been associated to metabolic diseases such as type-2 diabetes, cardiovascular disease, altered liver function, obesity, albuminuria and oxidative stress (Mirmira & Evans-Molina, 2014; Rancière et al., 2015).

The relationship between a contaminant in a food and its presence in the human body may not be straightforward, because several factors such as the type of food matrix, manner in which food/feed is contaminated (internal or superficially), chemical properties of the contaminant, and cooking preparation, can influence its bioaccessibility – fraction released from the food matrix in the gastrointestinal tract which becomes available for absorption – and thus its bioavailability – fraction of the compound that reaches the systemic circulation (Hur, Decker, & McClements, 2009; Maulvault et al., 2011). Recently, studies on bioavailability indicated that when BPA is ingested with food, it is absorbed and consequently metabolized via two different mechanisms (NTP, 2013). The majority of BPA is quickly bound to glucuronic acid to produce BPA glucuronide, a metabolic process called glucuronidation that is carried out by enzymes primarily in the liver and gut (Thayer et al., 2015). However, a significant amount of BPA is absorbed directly in the mouth cavity (buccal absorption), thus surpassing the inactivation by the so-called first-pass metabolism, which takes place in the liver following intestinal absorption (Gayrard et al. 2013; Guignard et al., 2016).

Despite these advances, there are still many gaps to be filled in what respects BPA bioaccessibility and bioavailability. There is a clear need to study the influence of the food matrix and the possible interactions between BPA and other food components, the influence of the cooking process, and the effects of luminal factors (including pH and enzymes), etc., on the potential of BPA to be absorbed.

According to our knowledge, no studies were performed on the bioaccessibility of BPA in canned seafood so far. Therefore, the main aim of this study was to evaluate BPA bioaccessibility in canned seafood, in order to provide additional relevant information for BPA intake exposure. In order to achieve this objective, several canned tuna and sardine samples, with diverse type of sauces, were analyzed for BPA, and its bioaccessibility was ascertained in a model system simulating the different digestion steps.

2. Experimental

2.1. Reagents and materials

Bisphenol A (BPA, 99% purity) and d_{16}-bisphenol A $BPAd_{16}$ (98 atom% D), used as internal standard (I.S.), were purchased from Sigma (St. Louis, MO, USA). Individual stock solutions of BPA and $BPAd_{16}$ (20 mg/L) were prepared in methanol (Sigma, high purity solvents for HPLC). The derivatization reagent was acetic anhydride (AA; >99%) purchased from Sigma.

The reagents used for BPA extraction included: C18-bonded silica (Waters, particle size, 55–105 μm), Supelclean ENVI-Carb (Sigma, particle size, 120–400 mesh), anhydrous $MgSO_4$ (Sigma, 99.5%), NaCl (Merck, 99.5%), K_2CO_3 (Merck, 99%), acetonitrile (Sigma, MeCN high purity solvents for HPLC 99%), heptane (Merck, pro-analysis) and tetrachloroethylene (Sigma,T4CE high purity solvents for HPLC 99%). For lipid analysis, propanol, cyclohexane, KCl, KOH and BF3 (14% in methanol, Sigma).

The reagents used to prepare the digestion fluids solution were the following: $NaHCO_3$ (Merck, 99.5%), $CaCl_2{\cdot}2H_2O$ (Sigma, >99%), KCl (Merck, 99.5%), KSCN (Sigma, P2713), NaH_2PO_4 (Merck, 99.5%), Na_2SO_4 (Merck, 90%), NH_4Cl (Riedel-de Haen, 99.5%), KH_2PO_4 (Merck, 99.5%), $MgCl_2$ (Riedel-de Haen, 99.5%), HCl (Merck, 37%); urea (Sigma, 99–100.5%), glucose (Sigma, >99%), glucuronic acid (Sigma, ≥98%), D-(+)-glucosamine hydrochloride (Sigma, >99%), uric acid (Sigma, >99%), albumin from bovine serum (Sigma, pH7, ≥98%), α-amylase, from *Aspergillus oryzae* (Sigma, ~1.5 U/mg), mucin from porcine stomach (Sigma, type II), pepsin from porcine stomach mucosa (Sigma, ≥400 units/mg protein), lipase from porcine pancreas (Sigma, type II), pancreatin from porcine pancreas (Sigma, meets USP testing specifications), trypsin from porcine pancreas (Sigma, Proteomics Grade), α-chymotrypsin from bovine pancreas (Sigma, type II) and bile porcine extract (Sigma).

Ultra high purity Helium (99.999%) for GC–MS and GC-FID was obtained from Gasin (Maia, Portugal). The makeup gases nitrogen and hydrogen (99.99%) were also obtained from Gasin.

2.2. Sampling

A total of 21 canned seafood samples comprising 12 canned tunas (3 in water, 3 in oil, 3 with maize and red beans, and 3 with mayonnaise) and 9 canned sardines (3 in water, 3 in oil, and 3 in tomato sauce) were supplied by a seafood company from Oporto Metropolitan Area (North Portugal). Each sample was drained for 5 min, homogenized, evaluated for moisture content, and stored at −20 °C until further analysis. Total fatty acids content was evaluated by GC-FID, after organic solvent extraction, following the procedure of Cruz et al. (2013). In order to quantify BPA levels, samples were analyzed by GC–MS, according to the method developed by Cunha et al. (2012). From the 21 canned samples available, only 6 samples were used in the bioaccessibility assays, representing those where BPA amounts were higher within each group.

2.3. Lipid content and fatty acids analysis

Lipids were extracted according Cruz et al. (2013). Briefly, an accurate sample portion (300 mg) was extracted with a ternary mixture of propan-2-ol, cyclohexane and 0.9% (m/v) KCl, in the presence of the internal standard (triundecanoate), plus antioxidants (ascorbic acid and BHT). The extract was hydrolyzed with KOH (0.5 mol L^{-1} in methanol) at 100 °C (10 min) and further methylated with BF3 (14% in methanol) (30 min at 100 °C).

Fatty acids were analyzed by gas chromatography, on a Chrompack CP 9001 chromatograph (Chrompack, Middelburg, The Netherlands) equipped with a split – splitless injector, a flame-ionization detector, and a Chrompack CP-9050 autosampler. The temperatures of the injector and detector were 250 and 270 °C, respectively. Separation was achieved on a 50 m × 0.25 mm i.d. CP-Sil 88 column (0.19 μm film; Chrompack-Varian), using a temperature program from 140 °C to 220 °C. Helium was used as the carrier gas at an internal pressure of 120 kPa. Total fatty acids content was estimated on the basis of the total fatty acid methyl ester area counts in comparison with the undecanoic methyl ester, by converting the fatty acid methyl esters (FAMEs) to their respective fatty acid equivalents using the appropriate conversion factors.

2.4. In vitro digestion model

2.4.1. BPA bioaccessibility

Canned seafood samples were digested in duplicate with four digestion fluids (salivary, gastric, duodenal and bile) according to the *in vitro* digestion model described by Braga et al. (2016) for seafood (Fig. 1). Blanks were also prepared by containing the four digestion fluids without canned seafood. Briefly, for each sample, 1.5–2 g were digested in glass containers at 37 °C using a Rotary Tube Mixer with Disc (25 rpm; LSCI, Portugal) in an incubator (Genlab, UK). Simulated digestion included the following steps: oral phase (4 mL of saliva fluid for 5 min at pH 7.0 ± 0.2), gastric phase (8 mL of gastric fluid for 2 h at pH 2.0 ± 0.2) and intestinal phase (8 mL of duodenal fluid and 4 mL of bile fluid for 2 h at pH