EFFECT OF PROCESSING SMOKED SALMON ON CONTAMINANT CONTENTS

Sara C. Cunha[a*], D. Siminel[a], Guàrdia M.D.[b], Miren López de Alda[c], Ester López-Garcia[c], Muñoz I.[b], R. Ferreira[a], Ethel Eljarrat[c*], J.O. Fernandes[a]

[a)] LAQV, REQUIMTE, Departamento de Ciências Químicas, Laboratório de Bromatologia e Hidrologia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

[b)] IRTA-Food Technology Programme, Finca Camps i Armet, Monells, Girona E-17121, Spain

[c)] Water, Environmental and Food Chemistry, Dep. of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

Email: sara.cunha@ff.up.pt and eeeqam@cid.csic.es

Abstract

The influence of the type of smoking process (natural/liquid; hot/cold) and salt (NaCl or KCl) on the levels of polybrominated diphenyl ethers (PBDEs) and polycyclic aromatic hydrocarbons (PAHs) in smoked salmon was evaluated. One parent compound - BDE 47 - and two methoxylated forms - 2′-MeO-BDE-68 and 6-MeO-BDE-47 - were detected in all the samples. Among the 14 PAHs analysed, naphthalene was the most abundant followed by phenanthrene and fluorene. Only smoked salmon treated with NaCl presented quantifiable levels of chrysene and benzo[b]fluoranthene. Among the four smoking processes evaluated, natural smoke led to higher levels of PAHs. Risk characterization tools, such as hazard index (HI) and incremental lifetime cancer risk (ILCR), showed that the risk of both PBDEs and PAHs to human health through the consumption of smoked salmon was very low.

Keywords: Polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), GC-MS/MS, smoking process, risk exposure

Highlights

PBDEs and PAHs in smoked salmon produced using alternative salts and different types of smoking

Salting with KCl led to products less contaminated with both PBDEs and PAHs than those salted with NaCl

PBDEs and PAHs levels were not significantly different between the different types of smoking

1- Introduction

Smoking is an ancient method of fish preservation. This process increases the shelf life of the fish also promoting their flavour, colour and texture, due to the combined effects of a preliminary salting and the antimicrobial activity of some smoke components (formaldehyde, carboxylic acids, phenols). Smoked salmon is considered to be a luxury product, since it is much more expensive than fresh and frozen salmon (Xie et al., 2011). Cold, hot or liquid smoking are different ways to obtain high quality products with good consumer acceptance. Cold smoking is usually performed at temperatures ranging from 20 to 30 °C for 2 to 12 hours at a humidity rate of 60-75 % and is most often used with dry-salting (Birkeland et al., 2004). Hot smoking is performed for 2-4 h at temperatures above 60 ºC (usually 100-120 ºC). In both processes, the smoke is produced by smouldering shavings or sawdust of certain kind of wood (beech, hickory, oak) in the oven directly below the hanging fish. Furthermore, the smoke could be added in the smoking chamber from external smoke generators under controlled conditions of temperature and spring. Liquid smoking is similar to traditional smoking, but it is faster. The application of liquid smoke includes pressurization, drenching/showering, injecting directly into the filet, or using it as part of a marinade or seasoning. In general, both external smoke generators and liquid smoke reduce the presence of undesirable compounds such as the polycyclic aromatic hydrocarbons (PAHs) formed during the combustion. PAHs are associated to mutations and cancer in some animals and humans. The IARC has characterized 17 PAHs as priority toxics; among them, benzo[a]pyrene (BaP) is classified in group 1 (carcinogenic to humans) while dibenzo[a,h]anthracene (DBahA) and dibenzo[a,l]pyrene (DBalP) are categorized in group 2A (probably carcinogenic to humans). Other PAHs are classified in group 3 (not classifiable as to its carcinogenicity to humans) (IARC). Therefore, as safety measure, the European Union (EU) has established legal limits for the PAHs levels in ten food classes such as oils and fats, cacao and related products, smoked products, processed

food for infant and young children, and dietary food for special medical purposes, which range from 1.0 to 6.0 µg.kg^{-1} and from 1.0 to 35.0 µg.kg^{-1} for BaP and 4PAHs [sum of BaP, benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF) and chrysene (CHR)], respectively (European Comission 835/2011). Other deleterious compounds associated with salmon processing are persistent environmental contaminants such as brominated flame retardants (BFRs), and polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A and others that have recently raised concern with respect to bioaccumulation and human health.

Norway and Iceland are major EU supplying countries of seafood with the Northeast Atlantic Ocean, Baltic Sea and North Sea as main fishing regions (EUMOFA, 2016). These locations are known for their considerably higher BFRs contamination levels in comparison to southern regions (Xie et al., 2011). In addition, several studies have confirmed that the concentration of many classes of BFRs, including PBDEs, are significantly higher in seafood and seawater from aquaculture areas than in non-aquaculture areas, mostly due to the fish feed (Gu et al., 2017; Jacobs et al., 2002). Despite in the EU regulation, namely Directive 2013/39/EU which defines an environmental quality standard of 8.5 pg.g^{-1} wet weight for total PBDEs in biota, no legal limits exist for PBDEs or their metabolites in processed seafood. Therefore, it is crucial to verify if smoked salmon comprises a health concern for regular consumers.

In the framework of Seafood^{Tomorrow} project, smoked salmon with a reduced sodium content was developed, by replacing NaCl by KCl (25% and 50%) and combining different smoking procedures (natural-wood or liquid; cold or hot smoking).). A successfully healthy smoked salmon was obtained with replacement of sodium chloride by KCl 25%. The product obtained showed no significant differences compared to the control regarding microbiological and physicochemical parameters as well as their sensory properties (see more details in the publication of Muñoz et al., 2020). The aim of this work was to investigate the effect of sodium replacement (by 25% and 50% of

KCl) and smoking techniques on the levels of PAHs and PBDEs, in order to guarantee chemical safety of the produced smoked salmon. Based on the obtained results a risk assessment assay was conducted.

2. EXPERIMENTAL SECTION

2.1. Reagents and standards

2.1.1. PBDEs and MeO-PBDEs analyses

All standards (BDE-28, -37, -47, -77, -99, -100, -153, -154, -183 and -209, as well as 2-MeO-BDE-68, 6-MeO-BDE-47, 5-MeO-BDE-47, 4-MeO-BDE-49, 5-MeO-BDE-100, 4-MeO-BDE-103, 5'-MeO-BDE-99 and 4'-MeO-BDE-101) with >98% purity were acquired from Wellington Laboratories, Inc. (Guelph, Ontario, Canada). 5'-fluoro-3,3',4,4',5-pentabromodiphenyl ether (FBDE-126) and 4'-fluoro-2,2',3,3',4,5,5',6,6'-nonabromodiphenyl ether (FBDE-208) both >98% pure were obtained from AccuStandard, Inc. (New Haven, USA). Polybrominated diphenyl ethers, MeO-PBDEs and internal standards [BDE-37, BDE-77, FBDE-126, FBDE-208 and ^{13}C-6-MeO-BDE-47] mixtures were prepared in n-hexane (GC grade, Merck, Darmstadt, Germany) using individual standards. Toluene and acetonitrile, both HPLC grade organic solvents, were obtained from Honeywell, Riedel-de-Haën (Seetze, Germany). Trichloroethylene was purchased from Merck (Fontenay-sous-Bois, France). Sodium chloride was obtained from PanReac Quimica (Barcelona, Spain) and magnesium sulfate was acquired from Sigma-Aldrich (Japan). Supel™ QuE Z-Sep+ and QuEChERS dSPE EMR-Lipid, were purchased from Supelco (Bellefont, PA, USA) and Agilent Technologies (USA), respectively.

2.1.2 PAHs analysis

Studied PAHs included naphthalene, 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-c,d]pyrene. Standard mixtures of the 14 PAHs and isotopically labeled analogs used as surrogate standards (SS) (Table 1) were purchased from LGC (Middlesex, United Kingdom) as solutions in cyclohexane at a concentration of 100 $\mu g.mL^{-1}$. Working standard solutions containing the PAHs at concentrations between 0.1 and 2000 $ng.mL^{-1}$ and the SS at 100 $ng.mL^{-1}$ were prepared by appropriate dilution of the stock solutions in acetone. HPLC grade acetone, used as solvent for QuEChERS extraction, was purchased from J.T. Baker (Serviquimia, Barcelona, Spain). Magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl), used as extraction salts, and primary and secondary amine (PSA), C18e and MgSO4, used for clean-up, were obtained from Bekolut GmbH & Co (Hauptstuhl, Germany) as SALT KIT AC and PSA-KIT-04, respectively.

2.2. Instrumental analysis

2.2.1. PBDEs and MeO-PBDEs analyses

The analyses were performed in a gas chromatograph Agilent 7890B with an auto-sampler 7683 coupled to an Agilent 7000C triple quadrupole mass spectrometer (Agilent Technologies, USA) in electron ionization mode (EI). For PBDEs (except BDE-209) and MeO-PBDEs analysis, were performed in pulsed splitless mode (pulse pressure of 32 psi of 1 min and purge flow of 50 $mL.min^{-1}$) and the separation was performed in a 30 m × 0.25 mm × 0.25 mm DB-5ms capillary column (Agilent Technologies, USA). The BDE-209 analysis was performed in splitless mode (purge time of 1.85 min and purge flow of 75 $mL.min^{-1}$) and the separation was achieved using a 10 m × 0.25 mm × 0.10 mm DB-1 capillary column (Agilent Technologies, USA). MassHunter quantitative analysis software (v. B.02.03) was used for data processing. Other chromatographic and

106 detection specifications can be found in Cruz et al. 2018. Limits of detection and
107 quantification (LOD and LOQ, respectively) of the method were determined by the signal-
108 to-noise ratio as detailed in the previously cited work (LOD = 5 – 55 $pg.g^{-1}$ and LOQ =
109 15 – 165 $pg.g^{-1}$). For more details regarding analytical performance see supplementary
110 information.

111

112 2.2.2. PAHs analysis

113 PAHs analysis was performed with an Agilent 7890B gas chromatograph coupled to an
114 Agilent 7000C triple quadrupole mass spectrometer (Agilent, Santa Clara, CA, United
115 States). Chromatographic separation was carried out using a HP-5ms Ultra Inert column
116 (30 m x 0.25 mm, 0.25 µm) (Agilent), helium as carried gas at constant flow of 1.2
117 $mL.min^{-1}$, and the following GC temperature program: initial temperature of 70ºC held for
118 2 min, increased at a rate of 30 $ºC.min^{-1}$ to 200 ºC and held for 5 min, increased at a rate
119 of 5ºC.min^{-1} to 300ºC and held for 2 min, and return to initial conditions. The GC was
120 interfaced with the QqQ instrument via a transfer line heated at 280ºC (MassHunter
121 WorkStation Software). MS analyses were performed using electron ionization (EI). The
122 source temperature was set at 250ºC and mass acquisition was done in the SRM mode,
123 recording two SRM transitions per analyte. Optimum GC-EI-MS-MS conditions used for
124 analysis of the target compounds are listed in Table 1.

125

126 Table 1. GC-EI-MS-MS conditions used for analysis of the 14 target PAHs and their
127 corresponding isotopically labelled analogues, and method limits of detection (LOD)
128 and quantification (LOQ) expressed in wet weight (ww).

Analyte	Retention time (min)	SRM transition (quantification)	SRM transition (confirmation)	LOD ($ng.g^{-1}$)	LOQ ($ng.g^{-1}$)
4 - 7.3 min					

Naphthalene	5.4	128 >102	128 >77	9.0	31
Naphthalene-d_8		136 >108			
		7.3 – 11 min			
Fluorene	7.6	166 >165	166 >163	0.4	1.4
Fluorene-d_{10}		176 >174			
Phenanthrene	9.2	178 >152	178 >176	0.7	2.5
Phenanthrene-d_{10}		188 >184			
Anthracene	9.3	178 >176	178 >152	0.7	2.3
Anthracene-d_{10}		188 >184			
		11 – 22 min			
Fluoranthene	13.3	202 >200	202 >150	0.2	0.8
Fluoranthene-d_{10}		212 >208			
Pyrene	14.2	202 >200	202 >150	0.3	0.9
Pyrene-d_{10}		212 >208			
Benz[a]anthracene	19.9	228 >226	228 >224	0.1	0.5
Benz[a]anthracene-d_{12}		240 >236			
Chrysene	20.0	228 >226	228 >224	0.2	0.6
Chrysene-d_{12}		240 >236			
		22 – 28 min			
Benzo[b]fluoranthene	24.7	252 >250	250 >248	0.3	0.9
Benzo[b]fluoranthene-d_{12}		264 >260			
Benzo[k]fluoranthene	24.5	252 >250	250 >248	0.3	1.0
Benzo[k]fluoranthene-d_{12}		264 >260			
Benzo[a]pyrene	25.9	252 >250	250 >248	0.1	0.4
Benzo[a]pyrene-d_{12}		264 >260			
		28 – 33 min			
Indeno[1,2,3-cd]pyrene	30.1	276 >274	276 >272	0.2	0.8
Indeno[1,2,3-cd]pyrene-d_{12}		288 >284			
Dibenz[a,h]anthracene	30.3	278 >276	278 >274	0.2	0.7
Dibenz[a,h] anthracene-d_{14}		292 >288			
Benzo[g,h,i]perylene	30.9	276 >274	268 >272	0.2	0.7
Benzo[g,h,i]perylene-d_{12}		288 >284			

129

2.5. Smoked salmon samples

Salmon production was made according to the protocol described in detail in Muñoz et al (2020). Briefly, gutted salmons (*Salmo salar*) with 3.1±0.3 kg of weight were filleted and trimmed before salting with NaCl (Enisal, Barcelona, Spain) or KCl (Dead Seaworks LTD, Tel-Aviv, Israel). One fillet of each salmon was used as control treatment regarding salt content added (5 g NaCl.100 g^{-1} salmon) and the other fillet assigned to one specific treatment (25 or 50% molar substitution by KCl). Five samples of each salting treatment were smoked with the tested processes (natural-wood or liquid; cold or hot smoking). Smoking with natural-wood (beechwood) was performed using an electrical oven (DOLESCHAL Unimatic, Salzburg, Austria). Cold smoking was performed for 4 h at 18-19°C with a relative humidity of 65-75%. The hot smoking process was carried out in two steps: the first one lasted 7 min at 56 ºC (oven pre-heated at 56ºC; relative humidity 15–25%) and then, 3 h and 53 min at 18–19 ºC and relative humidity 65–75%. Smoking with liquid smoke (Smokez Supreme C&A; Red Arrow International LLC, Manitowoc, USA) was carried out by dipping the salmon fillets into a solution of 1:2 (liquid smoke:water) for 20–25 s. Then, the salmon fillets were allocated to cold or hot smoking treatments as those smoked with natural wood. After smoking, each fillet was cooled, vacuum-packed in plastic bags, and frozen until PAHs and PBDEs analyses. The PBDES, MeO-PBDES and PAHs analyses were performed for one fillet of NaCl and two fillet of both 25 and 50% of KCl. Fat and protein contents are presented in **Table S3**.

2.6. Sample extraction

2.6.1. PBDEs and MeO-PBDEs analyses

Samples were analysed according to a previously validated method (Cruz et al., 2018). Briefly, homogenized smoked fish (≈ 500 mg) was added with a solution of internal standards (15 µL) and 2.5 mL of acetonitrile:toluene (4:1, v/v). After extraction overnight with agitation, 2.5 mL of ultra-pure water and magnesium sulphate (1 g) plus sodium

chloride (0.25 g) were added and vials were shaken for 1 min. One milliliter of supernatant, obtained after centrifugation at 1690×g for 5 min., was cleaned-up using EMR-Lipid (200 mg) and 20 mg of magnesium sulphate plus 30 mg Z-Sep+. After centrifugation (1690×g, 5 min.), the supernatant was completely evaporated under a gentle nitrogen stream at 40 ºC, recovered with a total volume of 70 µL of trichloroethylene and 1 µL was analysed by GC-MS/MS.

2.6.2. PAHs analysis

Analysis of PAHs was performed with a methodology based on “Quick, easy, cheap, effective, rugged, and safe” (QuEChERS) extraction followed by GC-MS-MS. Briefly, 10 g of homogenized fish samples were weighted into a 50 mL polypropylene (PP) centrifuge tube, spiked with the surrogate standard mixture at a concentration of 100 $ng.g^{-1}$, vortexed at 15000 gs^{-1} for 1 min, and stored during 12 h at 4ºC to allow the interaction of the compounds with the matrix. For extraction, 10 mL of acetone was added to the centrifuge tube and manually shaken. Then the extraction salts (4 g $MgSO_4$, 1 g NaCl) were added, and the tube was manually shaken for 30 s, vortex-mixed at 15000 gs^{-1} for 1 min, and centrifuged at 21000 gs^{-1} for 10 min. The supernatant was then transferred to a PP centrifuge tube containing the clean-up sorbents (900 mg $MgSO_4$, 150 mg PSA, 150 mg C18e), manually shaken for 30 s, vortexed at 15000 gs^{-1} for 1 min, and centrifuged at 21000 gs^{-1} for 10 min. Two mL of the resulting supernatant was transferred into a vial and 2 µl were analyzed. Figures of merit of this analytical methodology are shown as supplementary information.

2.7. Human exposure and hazard risk

2.7.1. PBDEs

The estimated daily intake (EDI) of the PBDEs was obtained based on the sample levels, the recommended dose of fish portion for adults of 300 g of muscle per week according (EFSA, 2014) and a body weight of 70 kg for adults (EFSA, 2012). The EDI was calculated by the following equation:

$$\mathrm{EDI} = \frac{C_i \text{ x } B_f}{BW_i}$$

Where: EDI is the total daily exposure of an individual i (μg.kg^{-1} body weight/day); Ci is the recommended dose of fish intake by an individual i per day (g.day^{-1}); Bf is the PBDEs concentration in the smoked salmon (μg.kg^{-1}); BWi is the body weight of the individual (kg).

Target hazard quotient

The target hazard quotient (THQ) is defined as "the ratio between exposure to a chemical and the respective reference values (i.e. acceptable daily intake or tolerable daily intake)" (EFSA, 2019). In this study EDI was compared with the Provisional Tolerable Weekly Intake (PTWI) of 0.7 μg of PBDEs per kg of body weight per week, as defined by EFSA (EFSA, 2005).

The THQ was calculated by the following equation:

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{TDI}$$

Where: EDI is the estimated daily intake obtained from equation 1; TDI was the PTWI (i.e. 0.7 μg.kg^{-1} of body weight (BW) per week).

If the THQ is < 1 then non-carcinogenic health effects are not expected. If, however, the THQ is > 1 then there is a possibility that adverse health effects could be experienced.

205 Hazard index

206 The hazard index (HI) is equal to the sum of each chemical component's Hazard Quotient
207 (HQ = Exposure ÷ Safe Dose) (EFSA, 2019). The HI assumes that the consumption of
208 a particular food type would result in simultaneous exposure to several potentially toxic
209 elements. When HI < 1 the exposed person is unlikely to experience evident harmful
210 health effects, while when HI > 1 there is the possibility that non-carcinogenic effects
211 may occur. The HI was calculated as:

212 $$HI = \sum_{i=k}^{n} \mathrm{THQ_i} \qquad (3)$$

213

214 Where: HI is the sum of individual THQi values obtained from equation 2.

215

216 2.7.2. PAHs

217 The individual PAHs have different ability to produce a toxic effect, so the concentrations
218 of PAHs congeners were calculated as BaP equivalents, to express the relative toxic
219 potency compared to BaP (Nisbet & LaGoy, 1992). The BaP toxic equivalent (TEQBaP)
220 concentration was obtained by multiplying the concentration of each PAH by its TEQ, as
221 follows (FAO/WHO, 2005):

222 $$TEQBaP = \sum[Ci] * TEFi$$

223 where Ci is the mean concentration of the individual congeners of PAHs in the smoked
224 salmon and TEFi is the toxic equivalency factor of the individual congeners of PAHs, as
225 suggested by Nisbet and LaGoy (1992).

226 The incremental lifetime cancer risk (ILCR) associated with dietary exposure of PAHs in
227 smoked salmon was calculated as follows:

228 $$ILCR = (TEBaP * IR * Ef * ED * SF * CF)/(Bw * AT)$$

229

230 where ILCR = the incremental lifetime cancer risk of dietary exposure; IR = the ingestion
231 amount of fish products (300 g by day). SF = the oral cancer slope factor of BaP, which
232 obeys lognormal distribution with a geometric mean of 7.3 mg kg^{-1} day^{-1} (USEPA, 2003).
233 ED = the exposure duration (year) (for adults: ED = 43). BW = average body weight (70
234 kg for adults); AT = life expectancy (equal to 81 years based on Eurostat 2020). EF =
235 the exposure frequency (365 days/year); CF = the conversion factor (10^{-6} $mg.ng^{-1}$).

236

237 2.8. Statistical analyses

238 All statistical analyses were performed using SPSS software, version 22.0 (IBM
239 Corporation, New York, USA) at 5% significance level. Analyse of variance was
240 performed by Kruskal-Wallis test, since normal distribution of the residuals was not
241 confirmed by Shapiro-Wilk's test. Furthermore, if a statistically significant difference was
242 verified, Mann–Whitney U test was applied for means of comparison of more than two
243 independent samples.

244

245 **3. RESULTS AND DISCUSSION**

246 **PBDEs levels**

247 Levels of PBDEs and their methoxylated metabolites (MeO-PBDEs) found in processed
248 smoked salmon are shown in Table 2, on a ww basis. Regarding PBDEs, BDE-47 was
249 the only compound detected, being present in all samples at levels ranging from 1.2 to
250 2.8 ng. g^{-1} ww. Concerning MeO-PBDEs, 2'-MeO-BDE-68 and 6-MeO-BDE-47 were
251 found in all the different smoked salmon samples in levels ranging between 9.4 and 16.0
252 and from 13.2 to 28.6 $ng.g^{-1}$ ww, respectively.

In general, BDE-47 is frequently found in greater amounts in smoked fish (Cruz et al., 2018; Carlsson et al., 2014). Levels found here were somewhat similar to those found in commercialized smoked salmon samples from Norway, Greenland, Poland and Belgium (0.56 ng.g $^{-1}$ ww of BDE-47, Knutsen et al., 2008; 0.69 to 3.96 ng.g $^{-1}$ ww of total PBDEs, Usydus et al., 2007; 1.02 ng.g $^{-1}$ ww of total PBDEs, Voorspoels et al., 2009).

In all the samples 6-MeO-BDE-47 was found in higher levels than 2'-MeO-BDE-68. Similar findings were obtained by Cruz et al. (2018) analysing 30 commercial smoked fish samples, with levels ranging from 1.3 to 125.8 ng.g $^{-1}$ ww for 6-MeO-BDE-47 while 2'-MeO-BDE-68 ranged from 1.2 to 29.8 ng.g $^{-1}$ ww. The natural presence of ortho-substituted MeO-PBDEs in fish (as 2'-MeO-BDE-68 and 6-MeO-BDE-47) could be explained by their high capacity of bioaccumulation (high Log K_{ow}) (Vandermeersch et al. 2015).

In general, the residue levels of PBDEs in aquatic organisms depend on contamination of the habitat and their ability to metabolize these compounds (Vandermeersch et al., 2015). Many studies show that the aquatic ecosystem is contaminated by these compounds that could be found in seafood (Vandermeersch et al., 2015, Aznar-Alemany et al., 2017).

Table 2- Levels of polybrominated diphenyl ethers and their methoxylated derivatives found in smoked fish samples salted with NaCl (one fillet analysed), 25% and 50% KCl (two fillets analysed).

Salt	**Smoking**	**BDE-47**	**2'-MeO-BDE-68**	**6-MeO-BDE-47**	**∑MeO-PBDEs**	**∑PBDEs+ MeO-PBDEs**
NaCl	Cold smoked with natural smoke	1.4	10.9	26.0	**36.9**	**38.3**
25%KCl		1.6	9.4	27.0	**36.4**	**38.0**
		1.3	10.8	26.0	**36.8**	**38.1**

50%KCl		1.9	10.3	20.0	**30.3**	**32.2**
		1.5	10.1	21.0	**31.1**	**32.6**
NaCl	Hot smoked with natural smoke	1.8	19.0	21.9	**41.0**	**42.8**
25%KCl		2.8	12.1	28.6	**40.7**	**43.5**
		2.6	15.8	23.2	**39.0**	**41.6**
50%KCl		1.4	17.7	20.7	**38.4**	**39.8**
		2.4	14.5	20.5	**35.0**	**37.4**
NaCl	Cold smoked with liquid smoke	1.3	12.1	22.8	**34.8**	**36.1**
25%KCl		2.3	12.6	13.2	**25.8**	**28.1**
		2.6	15.1	13.9	**29.0**	**31.6**
50%KCl		2.7	14.8	16.6	**31.4**	**34.1**
		2.2	16.2	17.6	**33.7**	**35.9**
NaCL	Hot smoked with liquid smoke	1.5	17.5	23.7	**41.2**	**42.7**
25%KCl		2.2	10.5	25.9	**36.4**	**38.6**
		2.5	15.3	23.0	**38.2**	**40.7**
50%KCl		1.7	13.7	21.3	**35.0**	**36.6**
		1.2	11.9	26.5	**38.4**	**39.6**

Results are expressed in $\mu g.kg^{-1}$ on a wet weight basis.
Limit of detection: 5 $pg.g^{-1}$ ww for PBDEs and 40 $pg.g^{-1}$ ww for MeO-PBDEs: Limit of quantification 15 $pg.g^{-1}$ ww for PBDEs and 80 $pg.g^{-1}$ ww for MeO-PBDEs.
BDE, brominated diphenylether; MeO, methoxylated.

273

274 The impact of type of smoking or salt on PBDE and MeO-PBDE levels was evaluated.
275 Significant differences ($p < 0.0001$) between hot and cold smoked were observed for
276 ∑MeO-PBDEs and ∑PBDEs+MeO-PBDEs (Table 3). Regarding salting, differences
277 were observed for 50% KCl substitution when compared to the control and 25% KCl
278 substitution samples.

279

280 **Table 3.** Polybrominated diphenyl ethers and their methoxylated derivatives according
281 to salt and smoking process

Salt and smoking type	∑MeO-PBDEs	∑PBDEs+MeO-PBDEs
NaCl	38.950^{A}	40.500^{A}
25% KCl	36.600^{A}	38.350^{A}
50% KCl	34.350^{B,C}	36.250^{B,C}
Hot Smoke	38.400^{D}	40.250^{D}
Cold Smoke	32.550^{E}	35.000^{E}
Natural Smoke	36.850^{F}	38.200^{F}
Liquid Smoke	34.900^{F}	36.350^{F}

Results are expressed in ng.g^{-1} median on a wet weight basis.

Different letters in a column show significant differences (p < 0.0001) from the given median.

PBDEs, polybrominated diphenylethers; LOD, limit of detection; MeO-PBDEs, methoxylated polybrominated diphenylethers.

282

283 PAHs levels

284 The levels of PAHs in smoked salmon are shown in Table 4, on a ww basis. Among the
285 14 analytes studied, naphthalene, fluorene, phenanthrene, chrysene and
286 benzo[b]fluoranthene were found at levels above the LOQ. Whatever the settings of the
287 parameters, all tested processes (type of salt and smoking) led to higher levels of PAHs
288 of low molecular-weight (≤ 178). Thus, the concentrations of the PAHs naphthalene,
289 fluorine and phenanthrene varied between 0.6 and 56.2 ng.g^{-1}. In contrast, the heavier
290 PAHs measured, chrysene and benzo[b]fluoranthene were below 0.7 ng.g^{-1}. These
291 outcomes are in accordance with those already reported in the literature for smoked
292 salmon processed with smouldering, thermostated plates; friction liquid smoke (Varlet et
293 al., 2007). Similar results were also reported by Visciano et al. (2008) for rainbow trout
294 fillets processed by traditional flue gas smoking and by liquid smoke flavourings. The
295 prevalence of light PAHs can be attributed to the smoke composition itself, independently

of the smoking procedure, since these low molecular weight compounds are usually found in higher amounts (Hokkanen et al., 2018). Herein, PAHs with 2 or 3 aromatic rings represented >99% of the total detected PAHs, regardless of smoking type and salt type which agrees with the findings of Hokkanen et al. (2018).

The levels of benzo[a]pyrene as well as the 4PAHs [sum of BaP, benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF) and chrysene (CHR)] are below, or slightly above, their corresponding LODs (between 0.1 and 0.3 ng. g^{-1}); not exceeding the 2.0 and 12.0 ng g^{-1} maximum levels in smoked fish for BaP and 4PAHs, respectively, established by the EU (European Commission 835/2011). These results suggest that the smoked salmon produced with the different types of smoking and salting processes can be commercialized.

The sum of the 14 PAHs ranged from 4.34 to 67 ng g^{-1}. These levels are somewhat underestimated as all samples have several PAHs at levels <LOQ, which are not included in the sum.

Table 4- Levels of polycyclic aromatic hydrocarbons (ng.g^{-1}, ww) found in smoked fish samples salted with NaCl (one fillet analysed), 25% and 50% KCl (two fillets analysed). . (LOD= limit of detection, see table 1)

Salt	**Smoking**	Naphthalene	Fluorene	Phenanthrene	Chrysene	Benzo[b]fluoranthene	**∑PAHs**
NaCl	Cold smoked with natural smoke	53.3	1.5	3.0	0.5	0.7	**59.0**
25%KCl		37.6	0.8	2.5	<0.2	<0.3	**40.9**
		46.1	0.9	2.4	<0.2	<0.3	**49.4**
50%KCl		53.0	<0.4	2.0	<0.2	<0.3	**55.0**
		33.5	5.0	2.7	<0.2	<0.3	**41.2**
NaCl		57.8	1.4	3.0	<0.2	<0.3	**62.3**

25%KCl	Hot smoked with natural smoke	32.5	<0.4	2.9	<0.2	<0.3	**35.4**
		40.5	0.8	2.7	<0.2	<0.3	**44.0**
50%KCl		44.5	0.6	2.7	<0.2	<0.3	**47.7**
		47.2	6.1	3.2	<0.2	<0.3	**56.4**
NaCl	Cold smoked with liquid smoke	31.2	0.6	2.3	<0.2	<0.3	**34.1**
25%KCl		<9	2.5	1.9	<0.2	<0.3	**4.34**
		34.1	<0.4	2.4	<0.2	<0.3	**36.5**
50%KCl		32.1	2.0	2.0	<0.2	<0.3	**36.1**
		49.3	2.3	2.0	<0.2	<0.3	**53.6**
NaCl	Hot smoked with liquid smoke	42.9	1.1	2.3	<0.2	<0.3	**67.0**
25%KCl		56.2	<0.4	2.3	<0.2	<0.3	**58.5**
		42.8	3.4	2.5	<0.2	<0.3	**48.6**
50%KCl		27.1	1.6	1.0	<0.2	<0.3	**29.6**
		33.3	1.2	3.3	<0.2	<0.3	**37.7**

The impact of the type of smoking or salt substitution level on PAHs was evaluated. No significant differences between hot and cold smoked and between natural and liquid smoke were observed for ∑PAHs (Table 5). Even so, slightly higher PAHs levels were obtained for hot smoking than for cold smoking as well as for natural smoking than for liquid smoking. In the literature it is described that the replacement of natural smoking by liquid smoking may reduce PAH contamination (Codex, 2009). Other critical factors to consider in the smoking process are temperature and time which should be as low and short as possible, from a point of view of food safety and product shelf life; a higher temperature and prolonged smoking time increases the PAHs of the product (Essumang et al., 2013, Racovita et al., 2020). Regarding salting, significant differences ($p < 0.0001$) were observed between control samples and those with reduced sodium content (with

25 and 50% KCl). In general, the total PAHs levels were lower in salmon salting treated with KCl.

Table 5. Polycyclic aromatic hydrocarbons (median $ng.g^{-1}$, ww) derivatives according to salt and smoking process

Salt and smoking type	∑PAHs
NaCl	60.650[A]
25% KCl	42.450[B]
50% KCl	44.450[B]
Hot Smoke	48.150[c]
Cold Smoke	41.050[c]
Natural Smoke	48.550[d]
Liquid Smoke	37.100[d]

Different letters in a column show significant differences ($p < 0.0001$) from the given median.

Human exposure and hazard risk to PBDEs and PAHs

The exposure of consumers to PBDEs by smoked fish consumption was assessed. The THQs of individual PBDEs and MeO-PBDE, as well as HI values, are given in **Table 6**. To calculate the THQ values, the average concentrations of PBDEs and MeO-PBDE were used. All THQ values were lower than 1 as well as HI, thus the smoked salmon produced using different types of salt and smoking processes was safe for adult consumption. However, EFSA emphasizes that this PTWI is derived from a restrict database and that it is not considered to be sufficiently robust.

Smoked foodstuffs are known to be a main source of human dietary exposure to PAHs (e.g., benzo[a]pyrene) that have adverse health effects, such as immunosuppression, induction of oxidative stress, and tumour promotion. Therefore, considering the mean

PAHs levels found with the different smoking processing the dietary exposure expressed as TEQBaP and ILCR was evaluated (**Table 7**). The highest TEQBaP was obtained with NaCl salting while cold smoking leads to the lowest. This variation is related with presence of high-molecular PAHs in NaCl smoked salmon. According to the guidelines for carcinogen risk assessment, the values of ILCR range from 10^{-6} to 10^{-4} implied potential cancer risk, while the practical safety was expressed with an ILCR of 10^{-6} or less and a potential high risk was evaluated by an ILCR of higher than 10^{-4} (EPA, 1991). In this study the ILCR of adults predicted by the TEQBaP of the different smoke salmon products were lower than the US EPA baseline.

354 **Table 6 –** A) Estimated daily intake (EDI), B) target hazard quotient (THQ) and C) hazard index (HI) of PBDEs in different smoked salmon

355 produced

356 **A)**

Salt and smoking type	EDI				
	BDE47	2′-MeO-BDE-68	6-MeO-BDE-47	∑MeO-PBDEs	∑PBDEs+MeO-PBDEs
NaCl	0.918	9.107	14.449	23.556	24.474
25% KCl	1.370	7.776	13.837	21.604	22.974
50% KCl	1.148	8.357	12.566	20.916	22.056
Hot Smoke	1.231	9.061	14.406	23.467	24.692
Cold Smoke	1.151	7.488	12.496	19.971	21.122
Natural Smoke	1.102	7.996	14.382	22.384	23.529
Liquid Smoke	1.237	8.553	12.520	21.055	22.286

357

B)

Salt and smoking type	THQ		
	BDE47	2′-MeO-BDE-68	6-MeO-BDE-47
NaCl	0.013	0.130	0.206
25% KCl	0.020	0.111	0.198

50% KCl	0.016	0.119	0.180
Hot Smoke	0.018	0.129	0.206
Cold Smoke	0.016	0.107	0.179
Natural Smoke	0.016	0.114	0.205
Liquid Smoke	0.018	0.122	0.179

358

C)

Salt and smoking type	**HI**	
	∑MeO-PBDEs	**∑PBDEs+MeO-PBDEs**
NaCl	0.337	0.350
25% KCl	0.309	0.328
50% KCl	0.299	0.315
Hot Smoke	0.335	0.353
Cold Smoke	0.285	0.302
Natural Smoke	0.320	0.335
Liquid Smoke	0.301	0.319

359

Table 7 - BaP toxic equivalent (TEQBaP) and incremental lifetime cancer risk (ILCR) associated with dietary exposure of PAHs in smoked salmon

Salt and smoking type	TEQ_{BAP}	ILCR
NaCl	0.125	1.14821E-05
25% KCl	0.040	3.6461E-06
50% KCl	0.045	4.10383E-06
Hot Smoke	0.047	4.31475E-06
Cold Smoke	0.041	3.75395E-06
Natural Smoke	0.049	4.49923E-06
Liquid Smoke	0.039	3.54009E-06

360

4- CONCLUSIONS

In this study, the occurrence of PBDEs and PAHs in smoked salmon produced using three alternative treatments (100% NaCl, 75% NaCl + 25% KCl, and 50% NaCl + 50% KCl) and different types of smoking (natural/liquid, cold/hot) were assessed. In general, salmon salted with KCl (25 and 50% of NaCl replacement with KCl) led to products less contaminated with both PBDEs and PAHs compared to 100% NaCl smoked salmon. Among the smoking processes tested, natural smoke, which is the most common in the industry, led to products with higher PAHs levels. Despite the higher levels of PBDEs were obtained from hot salmon processed, the limited number of samples analyzed does not allow to related that difference to the smoking process. The replacement of NaCl by KCl despite affecting the profile of PAHs did not contribute to the increase in TEQ_{BPA}. Risk characterization tools, such as HI and ILCR, showed that the risk of both PBDEs and PAHs to human health through smoked salmon consumption is very low independently of the type of NaCl content or smoking process used.

ACKNOWLEDGEMENTS

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 773400 (SEAFOOD ^{TOMORROW}). This output reflects only the view of the author(s) and the Research Executive Agency (REA) cannot be held responsible for any use that may be made of the information contained therein. This work was also supported by the Spanish Ministry of Science and Innovation (Project CEX2018-000794-S), the Generalitat de Catalunya (Consolidated Research Group Water and Soil Quality Unit 2017 SGR 1404), and through FEDER (Programa Operacional Competitividade e Internacionalização - COMPETE 2020), from PIDDAC through FCT/MCTES project POCI-01-0145-FEDER-028708, from UID/QUI/50006/2020

with funding from FCT/MCTES. Sara C. Cunha also acknowledges FCT for the IF/01616/2015 contract. Bekolut is acknowledged for providing QuEChERS kits.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

References

Aznar-Alemany, O., Trabalón, L., Jacobs, S., Barbosa, V. L., Fernández-Tejedor, M., Granby, K., Kwadijk, C., Cunha, S. C., Ferrari, F., Vandermeersch, G., Sioen, I., Verbeke, W., Vilavert, L., Domingo, J. L., Eljarrat, E., Barceló, D., 2007. Occurrence of halogenated flame retardants in commercial seafood species available in European Markets. Food Chem. Toxicol. 104, 35-47. https://doi.org/10.1016/j.fct.2016.12.034.

Birkeland, S., Røra, A. M. B., Skåra, T., Bjerkeng, B., 2004. Effects of cold smoking procedures and raw material characteristics on product yield and quality parameters of cold smoked Atlantic salmon (Salmo salar L.) fillets. Food Res. Int. 37(3), 273-286. https://doi.org/10.1016/j.foodres.2003.12.004.

Carlsson, P., Herzke, D., Kallenborn, R., 2014. Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and perfluorinated alkylated substances (PFASs) in traditional seafood items from western Greenland. Environ. Sci. Pollut. Res. 21, 4741-4750. https://doi.org/10.1007/s11356-013-2435-x.

Codex Alimentarius Commission, 2009. Code of practice for the reduction of contamination of food with polycyclic aromatic hydrocarbons (PAH) from smoking and direct drying processes, CAC/RCP 68-2009. http://www.fao.org/fao-who-codexalimentarius/codex-texts/codes-of-practice/en/ (accessed 28 January 2021).

Cruz, R., Marques, A., Casal, S., Cunha, S.C., 2019. Fast and environmental-friendly methods for the determination of polybrominated diphenyl ethers and their metabolites in fish tissues and feed. Sci. Total Environ. 646, 1503-1515. doi:10.1016/j.scitotenv.2018.07.342

Essumang, D. K., Dodoo, D. K., Adjei, J. K., 2013. Effect of smoke generation sources and smoke curing duration on the levels of polycyclic aromatic hydrocarbon (PAH) in different suites of fish. Food Chem. Toxicol. 58, 86–94. https://doi.org/10.1016/j.fct.2013.04.014.

EU Market Observatory for Fisheries and Aquaculture products, 2016. The EU Fish Market.https://www.eumofa.eu/documents/20178/77960/The+EU+fish+market+-

+2016+Edition.pdf/ca1e7801-c4da-4799-aa00-f3d1784a3021 (accessed 29 January 2021).

European Commission, 2011. Commission regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. Off. J. Eur. Union. 215(4), 4-8.

European Food Safety Authority, 2005. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to the safety assessment of wild and farmed fish. The EFSA J., 22, 236. doi: 10.2903/j.efsa.2005.236.

European Food Safety Authority, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA J., 10 (3), 2579. doi:10.2903/j.efsa.2012.2579.

European Food Safety Authority, 2014. Scientific Opinion on health benefits of seafood (fish and shellfish) consumption in relation to health risks associated with exposure to methylmercury. EFSA J., 12 (7), 3761. doi: 10.2903/j.efsa.2014.3761.

Eurostat 2020. https://ec.europa.eu/eurostat/web/products-eurostat-news/-/EDN-20200930-1 (accessed in April 2021).

European Food Safety Authority, 2019. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA J., 17 (3), 5634. doi: 10.2903/j.efsa.2019.5634.

Food and Agriculture Organization of the World Health Organization, 2006. Safety evaluation of certain contaminants in food (64th : 2005: Rome, Italy). WHO FOOD ADDITIVES SERIES: 55.

Gu, S.-Y., Ekpeghere, K.I., Kim, H.-Y., Lee, I.-S., Kim, D.-H., Choo, G., Oh, J.-E., 2017. Brominated flame retardants in marine environment focused on aquaculture area: Occurrence, source and bioaccumulation. Sci. Total Environ. 601-602, 1182-1191. doi:10.1016/j.scitotenv.2017.05.209.

Hokkanen, M., Luhtasela, U., Kostamo, P., Ritvanen, T., Peltonen, K., Jestoi, M., 2018. Critical Effects of Smoking Parameters on the Levels of Polycyclic Aromatic Hydrocarbons in Traditionally Smoked Fish and Meat Products in Finland. J. Chem. 1–14. doi:10.1155/2018/2160958

Jacobs, M.N., Covaci, A., Schepens, P., 2002. Investigation of Selected Persistent Organic Pollutants in Farmed Atlantic Salmon (Salmo salar), Salmon Aquaculture Feed, and Fish Oil Components of the Feed. Environ. Sci. Technol. 36(13), 2797-2805. doi: 10.1021/es011287i.

Knutsen, H.K., Kvalem, H.E., Thomsen, C., Frøshaug, M., Haugen, M., Becher, G., Alexander, J., Meltzer, H.M., 2008. Dietary exposure to brominated flame retardants correlates with male blood levels in a selected group of Norwegians with a wide range of seafood consumption. Mol. Nutr. Food Res. 52(2), 217-227. doi: 10.1002/mnfr.200700096.

Muñoz, I., Guàrdia, M. D., Arnau, J., Dalgaard, P., Bover, S., Fernandes, J. O., Monteiro, C., Cunha, S. C., Gonçalves, A.,Nunes, M. L., Oliveira, H.,2020. Effect of the sodium reduction and smoking system on quality and safety of smoked salmon (Salmo salar). Food Chem. Toxicol.143, 111554. doi:10.1016/j.fct.2020.111554.

Nisbet, I.C.T., LaGoy, P.K., 1992. Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). Regul. Toxicol. Pharm. 16, 290–300. https://doi.org/10.1016/0273-2300(92)90009-X.

Racovita R. C., Secuianu C., Ciuca M. D., Israel-Roming F., 2020. Effects of Smoking Temperature, Smoking Time, and Type of Wood Sawdust on Polycyclic Aromatic Hydrocarbon Accumulation Levels in Directly Smoked Pork Sausages. J. Agric. Food Chem. 68 (35), 9530-9536. https://doi.org/10.1021/acs.jafc.0c04116.

United States Environmental Protection Agency, 1991. Risk Assessment Guidance for Superfund: Volume 1, Human Health Evaluation Manual (Part B, Development of Risk-Based Preliminary Remediation Goals) (EPA/540/R-92/003). Washington, DC 20460.

United States Environmental Protection Agency, 2003. 2001 Toxics Release Inventory Executive Summary (EPA 260-S-03-001). Washington, D.C. 20460.

Usydus, Z., Szlinder-Richert, J., Polak-Juszczak, L., Komar, K., Adamczyk, M., Malesa-Ciecwierz, M., Ruczynska, W., 2009. Fish products available in Polish market – Assessment of the nutritive value and human exposure to dioxins and other contaminants. Chemosphere. 74, 1420-1428. doi: 10.1016/j.chemosphere.2008.12.023.

Vandermeersch, G., Van Cauwenberghe, L., Janssen, C. R., Marques, A., Granby, K., Fait, G., Kotterman, M. J., Diogène, J., Bekaert, K., Robbens, J., Devriese, L., 2015. A critical view on microplastic quantification in aquatic organisms. Environ. Res. 143, 46–55. doi:10.1016/j.envres.2015.07.016.

Varlet, V., Serot, T., Monteau, F., Le Bizec, B., Prost, C., 2007. Determination of PAH profiles by GC–MS/MS in salmon processed by four cold-smoking techniques. Food Addit. Contam. 24(7), 744-757. doi: 10.1080/02652030601139946.

Visciano, P., Perugini, M., Conte, F., Amorena F., 2008. Polycyclic aromatic hydrocarbons in farmed rainbow trout (Oncorhynchus mykiss) processed by traditional flue gas smoking and by liquid smoke flavourings. Food Chem. Toxicol. 46, 1409-1413. doi: 10.1016/j.fct.2008.01.001.

513 Voorspoels, S., Covaci, A., Neels, H., Schepens, P., 2007. Dietary PBDE intake: A
514 market-basket study in Belgium. Environ. Int. 33(1), 93-97. doi:
515 10.1016/j.envint.2006.08.003.

516
517 Xie, J., Myrland, Ø., 2011. Consistent Aggregation in Fish Demand: A Study of French
518 Salmon Demand. Mar. Resour. Econ. 26(4), 267-280. doi:10.5950/0738- 1360-26.4.267.

519
520 Xie, Z., Möller, A., Ahrens, L., Sturm, R., Ebinghaus, R., 2011. Brominated Flame
521 Retardants in Seawater and Atmosphere of the Atlantic and the Southern Ocean.
522 Environ. Sci. Technol. 45, 1820-1826. https://doi.org/10.1021/es103803t.

523

524