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EFFECT OF PROCESSING SMOKED SALMON ON CONTAMINANT CONTENTS

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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 °C (usually 100-120 °C). 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 (DBaH) 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 $\mu\text{g.kg}^{-1}$ and from 1.0 to 35.0 $\mu\text{g.kg}^{-1}$ for BaP and 4PAHs [sum of BaP, benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF) and chrysene (CHR)], respectively (European Commission 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 ¹³C-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 (Bellefonte, 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 $\mu\text{g}.\text{mL}^{-1}$. Working standard solutions containing the PAHs at concentrations between 0.1 and 2000 $\text{ng}.\text{mL}^{-1}$ and the SS at 100 $\text{ng}.\text{mL}^{-1}$ 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 MgSO_4 , 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 $\text{mL}.\text{min}^{-1}$) and the separation was performed in a 30 m $\times$ 0.25 mm $\times$ 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 $\text{mL}.\text{min}^{-1}$) and the separation was achieved using a 10 m $\times$ 0.25 mm $\times$ 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

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

2.2.2. PAHs analysis

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

Table 1. GC-EI-MS-MS conditions used for analysis of the 14 target PAHs and their corresponding isotopically labelled analogues, and method limits of detection (LOD) 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			

2.5. Smoked salmon samples

Salmon production was made according to the protocol described in detail in Muñoz et al (2020). Briefly, gutted salmon (*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 ($5 \text{ g NaCl} \cdot 100 \text{ g}^{-1}$ 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 °C (oven pre-heated at 56 °C; relative humidity 15–25%) and then, 3 h and 53 min at 18–19 °C and relative humidity 65–75%. Smoking with liquid smoke (Smoke 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 ($\approx 500 \text{ mg}$) was added with a solution of internal standards ($15 \mu\text{L}$) 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 °C, 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 4°C 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 MgSO₄, 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 MgSO₄, 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:

$$EDI = \frac{C_i \times B_f}{BW_i}$$

Where: EDI is the total daily exposure of an individual *i* ($\mu\text{g} \cdot \text{kg}^{-1}$ body weight/day); C_i is the recommended dose of fish intake by an individual *i* per day ($\text{g} \cdot \text{day}^{-1}$); B_f is the PBDEs concentration in the smoked salmon ($\mu\text{g} \cdot \text{kg}^{-1}$); BW_i 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:

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

Where: EDI is the estimated daily intake obtained from equation 1; TDI was the PTWI (i.e. 0.7 $\mu\text{g} \cdot \text{kg}^{-1}$ 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.

Hazard index

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

$$HI = \sum_{i=k}^n THQ_i \quad (3)$$

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

2.7.2. PAHs

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

$$TEQ_{BaP} = \sum [Ci] * TEF_i$$

where C_i is the mean concentration of the individual congeners of PAHs in the smoked salmon and TEF_i is the toxic equivalency factor of the individual congeners of PAHs, as suggested by Nisbet and LaGoy (1992).

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

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

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

2.8. Statistical analyses

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

3. RESULTS AND DISCUSSION

PBDEs levels

Levels of PBDEs and their methoxylated metabolites (MeO-PBDEs) found in processed smoked salmon are shown in Table 2, on a ww basis. Regarding PBDEs, BDE-47 was the only compound detected, being present in all samples at levels ranging from 1.2 to 2.8 ng. g⁻¹ ww. Concerning MeO-PBDEs, 2'-MeO-BDE-68 and 6-MeO-BDE-47 were found in all the different smoked salmon samples in levels ranging between 9.4 and 16.0 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	ΣMeO-PBDEs	ΣPBDEs+MeO-PBDEs
NaCl	Cold smoked	1.4	10.9	26.0	36.9	38.3
25%KCl	with natural smoke	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 $\mu\text{g.kg}^{-1}$ 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 $\Sigma\text{MeO-PBDEs}$ and $\Sigma\text{PBDEs+MeO-PBDEs}$ (Table 3). Regarding salting, differences

277 were observed for 50% KCl substitution when compared to the control and 25% KCl

278 substitution samples.

279

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

Salt and smoking type	Σ MeO-PBDEs	Σ 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.

PAHs levels

The levels of PAHs in smoked salmon are shown in Table 4, on a ww basis. Among the 14 analytes studied, naphthalene, fluorene, phenanthrene, chrysene and benzo[b]fluoranthene were found at levels above the LOQ. Whatever the settings of the parameters, all tested processes (type of salt and smoking) led to higher levels of PAHs of low molecular-weight (≤ 178). Thus, the concentrations of the PAHs naphthalene, fluorine and phenanthrene varied between 0.6 and 56.2 ng.g⁻¹. In contrast, the heavier PAHs measured, chrysene and benzo[b]fluoranthene were below 0.7 ng.g⁻¹. These outcomes are in accordance with those already reported in the literature for smoked salmon processed with smouldering, thermostated plates; friction liquid smoke (Varlet et al., 2007). Similar results were also reported by Visciano et al. (2008) for rainbow trout fillets processed by traditional flue gas smoking and by liquid smoke flavourings. The 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	ΣPAHs
NaCl	Cold	53.3	1.5	3.0	0.5	0.7	59.0
25%KCl	smoked	37.6	0.8	2.5	<0.2	<0.3	40.9
	with	46.1	0.9	2.4	<0.2	<0.3	49.4
50%KCl	natural	53.0	<0.4	2.0	<0.2	<0.3	55.0
	smoke	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	32.5	<0.4	2.9	<0.2	<0.3	35.4
	smoked	40.5	0.8	2.7	<0.2	<0.3	44.0
50%KCl	with	44.5	0.6	2.7	<0.2	<0.3	47.7
	natural smoke	47.2	6.1	3.2	<0.2	<0.3	56.4
NaCl	Cold	31.2	0.6	2.3	<0.2	<0.3	34.1
25%KCl	smoked	<9	2.5	1.9	<0.2	<0.3	4.34
	with liquid	34.1	<0.4	2.4	<0.2	<0.3	36.5
50%KCl	smoke	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	42.9	1.1	2.3	<0.2	<0.3	67.0
25%KCl	smoked	56.2	<0.4	2.3	<0.2	<0.3	58.5
	with liquid	42.8	3.4	2.5	<0.2	<0.3	48.6
50%KCl	smoke	27.1	1.6	1.0	<0.2	<0.3	29.6
		33.3	1.2	3.3	<0.2	<0.3	37.7

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316 The impact of the type of smoking or salt substitution level on PAHs was evaluated. No
317 significant differences between hot and cold smoked and between natural and liquid
318 smoke were observed for Σ PAHs (Table 5). Even so, slightly higher PAHs levels were
319 obtained for hot smoking than for cold smoking as well as for natural smoking than for
320 liquid smoking. In the literature it is described that the replacement of natural smoking
321 by liquid smoking may reduce PAH contamination (Codex, 2009). Other critical factors
322 to consider in the smoking process are temperature and time which should be as low
323 and short as possible, from a point of view of food safety and product shelf life; a higher
324 temperature and prolonged smoking time increases the PAHs of the product (Essumang
325 et al., 2013, Racovita et al., 2020). Regarding salting, significant differences ($p < 0.0001$)
326 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	ΣPAHs
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	ΣMeO-PBDEs	ΣPBDEs+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

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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

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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

c)

Salt and smoking type	HI	
	$\sum \text{MeO-PBDEs}$	$\sum \text{PBDEs+MeO-PBDEs}$
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

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Table 7 - BaP toxic equivalent (TEQ_{BaP}) 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

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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.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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