ECsafeSEAFOOD

Priority environmental contaminants in seafood: safety assessment, impact and public perception

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Deliverable D2.7

Effect of processing seafood on levels and profiles of priority contaminants

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1. Summary

Environmental contaminants of emerging concern in seafood are gaining increasing interest by the scientific community and regulatory authorities due to the possible harmful effects on ecosystems and human health. These contaminants include brominated flame retardants (BFRs), perfluorinated compounds, toxic element species (inorganic arsenic, cadmium and organic mercury), endocrine disruptors, polycyclic aromatic hydrocarbons, pharmaceutical and personal care products, microplastics, toxins and others. Indeed, reduced data is available about the levels of these contaminants in seafood, there is still a lack of monitoring programmes targeting the assessment of these contaminants in seafood, and the effect of culinary processing on the levels of these contaminants in seafood is mostly unknown.

Knowing that seafood is a major dietary route for human exposure to these widespread contaminants, one of the main purposes of ECSafeSEAFOOD was the monitoring of the priority environmental contaminants in seafood taking into account the effect of culinary processing. Thus, the purpose of Deliverable 2.7 is to present the results of the analysis of contaminants of emerging concern in seafood subjected to culinary processing.

The contaminants monitored in selected cooked commercial species were the following:

- Metals (iAs & MeHg)
- Per Fluorinated Compounds (PFCs)
- Endocrine Disruptors (EDCs)
- Brominated Flame Retardants (BFRs)
- Pharmaceuticals (PhACs)
- Polycyclic aromatic hydrocarbons (PAH)
- Musks
- UV-filters
- Biotoxins

The samples were selected from commercial species sampled in round II, taking into account the species showing the highest levels of contaminants in round I. The same samples were analysed as raw and cooked, according to the procedures defined in D2.1.

2. Objective

Evaluate the effect of culinary processing on levels of environmental contaminants of emerging concern in seafood.

3. Background

The Human health risks and benefits associated with seafood consumption have been widely addressed. The well-established seafood low cholesterol levels and source of high quality proteins with all essential amino acids, polyunsaturated n-3 fatty acids, vitamins and essential elements (e.g. selenium) are associated with health benefits for consumer by reducing the risk of coronary heart diseases, hypertension and diabetes (Bayen et al., 2005). However, like any other source of food, it has also risks. Seafood can indeed accumulate persistent organic pollutants (POPs), toxic elements (e.g. Hg, Cd, Pb and As) and microbiological contaminants through environmental exposure that can be very dangerous for human health (Domingo, 2010; Marques et al., 2011).

So far, several studies assessing the levels of chemical contaminants in seafood enabled the establishment of dietary intake levels of such contaminants by consumers (Domingo, 2010). Moreover, despite most seafood products are cooked before consumption, the risk assessment and limits set by authorities are mainly based in the analysis of uncooked/raw products (Marques et al., 2011). However, it is known that the nutritional quality of seafood products can considerably change with the cooking process (Maulvaut et al., 2011). Despite few studies already assessed the effects of cooking on the levels chemical contaminants in seafood, as far as contaminants of emerging concern are concerned this information is still scarce. Strong variations in the concentration of chemical contaminants in cooked/processed seafood products have been highlighted according to the cooking procedure and species (Marques et al., 2011). Indeed, Maulvault et al. (2011) and Perugini et al. (2013) reported increased Hg levels in fried, grilled and steamed black scabbard fish and in Norway lobster after boiling, compared to raw products, whereas Schmidt et al. (2015) registered significant Hg losses in six fish species (Bearded brotula, Yellowfin tuna, Bluewing searobin, Pirarucu, Flatfish and Salmon), mainly after frying. Concerning Cd, the levels decreased in mollusks after boiling (Amiard et al., 2008), whereas the cooking treatments (frying and microwaving) increased significantly the Cd levels in seabass (Ersoy et al., 2006), as well as in mussels after boiling (Houlbrèque et al., 2011). In contrast, for As, all studies revealed increased levels regardless of the culinary treatment or species (Devesa et al., 2001; Ersoy et al., 2006; Maulvault et al., 2011). Concerning persistent organic pollutants, boiling resulted in lower concentrations of PBDEs, PCBs

and dioxins in salmon and mackerel (Hori et al., 2001; Bayen et al., 2005). Additionally, Bhavsar et al. (2014) reported increased levels of Per Fluorinated compounds (PFCs) lake trout in after boiling, but decreased levels in common carp after baking and frying. These variations may be due to: (1) decrease in weight of samples due to the loss of water, volatiles, lipids, carbohydrates and proteins; (2) decrease of contaminant contents in the edible part as a result of drip loss from volatilization or solubilisation of contaminants, once the heat from cooking melts fat in seafood; and (3) migration of chemical contaminants, such as PAH in canned bivalves into the vegetable oil used during processing (Marques et al., 2011).

Despite the mechanisms involved in the transfer and/or degradation of chemical contaminants during the cooking process are not totally clear, it seems that the concentration of a contaminant is directly linked to the tissue in which it accumulates (Bayen et al., 2005; Bhavsar et al., 2014). In the case of neutral organic compounds, such as PCBs and dioxins, which have higher affinity for fatty tissues, the decrease of contaminant content is mainly due to the loss of fat by cooking or skin removal (Bayen et al., 2005). On the other hand, heavy metals, such as As and Hg, have higher affinity for tissue proteins, and therefore, are less affected by culinary treatments.

Worldwide, the diversity of cooking and industrial processing methods used has hampered the utilization of these factors in risk assessment by food safety authorities. Indeed, cooking methods can vary according to the food product, within geographical regions and at regional level. Also, for each culinary treatment, different temperatures and cooking duration can be implemented together with diverse ingredients and food items (Marques et al., 2011).

Nevertheless, the absence of data targeting the effect of cooking procedures in food contaminants levels in food risk assessment may lead to the over/underestimation of the risks for consumers health and lead to misleading dietary messages (Houlbrèque et al., 2011; Marques et al., 2011).

4. Sampling Plan

4.1 Sampling plan for chemical pollutants and micro-plastics

In order to assess the levels of contaminants of emerging concern in the most relevant commercially available seafood species, two sampling periods were undertaken. The species selection was based on the following criteria: a) most common species consumed in Europe; b) species that potentially accumulate high concentrations of contaminants; c) species wide geographic distribution; d) species

from different habitats; e) species from extra-EU origin or from EU production; and f) species from wild and farmed origin (see D2.1 Sampling Plan).

The commercial species selected to assess the effect of culinary processing in contaminants concentration were chosen taking into account the results of contaminants levels in the first sampling round (see D2.4). The effect of cooking was only analysed in the worst case scenarios and therefore, commercial species with the highest concentration of contaminants detected in the first sampling round were selected (table 1).

Table 1. List of species accessed in the second commercial sampling round

Sample ID	Sample	Origin
AEIF0007b	sole, large	Goro, IT
AEIF0008	mackerel, fresh	Goro, IT
AEIF0009	seabream	Other origin
AEIF00010	Mussels	Goro, IT
IMAR0009a	Plaice, small	North Sea
IMAR0009b	Plaice, large	North Sea
IMAR0010	Mackerel, fresh	North Sea
IMAR0012	Mussels	Netherlands
IMAR0013	Mussels	Ireland
IMAR0014	Brown crab	Netherlands
IRTA0007	Mussels	Spain
IRTA008a	Octopus, small	Mediterranean
IRTA008b	Octopus, large	Mediterranean
IRTA0009	mackerel fresh	Spain
DTU0005	Mussels	Limfiord, Denmark
DTU0006	Norwegian salmon (farmed)	Farmed (DanSalmon)
DTU0007	Atlantic Cod	North Sea, Denmark
DTU0008	mackerel	North Sea, Denmark
ICETA0006a	Monkfish, small	Portugal
ICETA0006b	Monkfish, largel	Portugal
ICETA0007	Canned Tuna	Portugal
ICETA0008	Canned mackerel	Portugal
ILVO0005a	Plaice, small	Channel
ILVO0005b	Plaice, large	Channel
ILVO0006	Mussels	France
IPMA0011a	Pacific hake, small	South America
IPMA0011b	Pacific hake, large	South America
IPMA0012a	Atlantic hake, small	South Africa
IPMA0012b	Atlantic hake, large	South Africa
IPMA0013a	Imported Tuna, small	Pacific
IPMA0013b	Imported Tuna, large	Pacific

A minimum of 25 specimens from each species were prepared using common household practices, according to the procedures described in D2.1. For each species, the edible tissue was divided in 3 portions and the culinary treatment was performed only in one portion. The cooking procedure consisted in adding culinary salt to each sample (2% w/w of edible meat), followed by cooking through steaming (105ºC wrapped in aluminium foil).

The priority contaminants (according to WP1 and WP2 decision) screened in commercial species were analysed by procedures already described by each responsible partner (table 2).

Table 2. List of priority contaminants analysed and respective techniques.

Contaminants	Partner	Techniques
MeHg	IPMA	Atomic Absorption Spectrometry
iAs	DTU	HPLC coupled Inductively Coupled Plasma Mass Spectrometry
PFCs	IMARES	Liquid-chromatography-ion trap tandem mass spectrometry (LC-IT-MS/MS)
EDCs	ICRA/CSIC	Ultra performance liquid chromatography–triple quadrupole mass spectrometry (
BFRs	DTU	Liquid-Chromatography tandem mass spectrometry
	ICRA/CSIC	Gas-chromatography coupled to mass spectrometry
PhACs	ICRA	Ultra-high-performance liquid chromatography tandem mass spectrometry for fish (Huerta et al., 2013); ultra-performance liquid chromatography–triple quadrupole mass spectrometry (UHPL–MS/MS) for bivalves
PAHs	ILVO	Gas chromatography-mass spectrometry (GC-MS)
Musks	URV	Gas chromatography ion trap tandem mass spectrometry (GC–IT-MS/MS)
UV-filters	ICETA	Gas chromatography mass spectrometry (GC-MS)
Microplastics	ILVO	Acid mix Method with combination of nitric acid and perchloric acid
	UGent	Nitric acid Method using only nitric acid

4.2 Sampling plan for biotoxins

In order to assess the levels of biotoxins in commercially available seafood species two types of samples were chosen (puffer fish and mussels) to assess the effect of culinary processing. The species were selected taking into account previous results where high concentrations of tetrodotoxin in puffer fish gonads (*Lagocephalus sceleratus*) were detected, as well as azaspiracids and okadaic acid in mussels. The cooking procedure for puffer fish was performed by steaming at 100ºC during 10 min and the extraction was done as described in Reverté et al. (2015).

Mussels were shucked and homogenized before extraction. Tissue samples were weighed (2 g) in duplicate into 50 mL centrifuge tubes with one set placed in a water bath, heated to 90 °C for 10 min and then allowed to cool. The samples were extracted by vortex mixing for 1 min with 9 mL of MeOH, centrifuged at 950 *g* (5 min) and the supernatants were decanted into 25 mL volumetric flasks. The remaining pellet was further extracted using an Ultra Turrax for 1 min with 9 mL of MeOH, centrifuged at 3950 *g* (5 min) and the supernatants were decanted into the same 25 mL volumetric flasks, which were brought to volume with MeOH. The samples were then passed through Whatman 0.2 µm cellulose acetate filters into HPLC vials for analysis by LC-MS/MS.

5. Results

The concentration of contaminants in cooked samples was compared with the same raw samples. When an increase or decrease was observed in the levels of cooked samples, the respective value was highlighted according with the following colour scale:

increase 10-20%	decrease 10-20%
increase 20-50%	decrease 20-50%
increase >50%	decrease >50%

5.1 Metals (iAs & MeHg)

Most seafood samples revealed an increase in contaminant levels of heavy metals after cooking. Indeed, in all analysed cooked samples, methyl-mercury (Me-Hg) and total mercury (T-Hg) levels were detected, with the highest levels of Me-Hg (365.89 µg/kg) and T-Hg (553.20 µg/kg) observed in the large cooked octopus (table 3).

Table 3 – Methylmercury (Me-Hg) and Total mercury (T-Hg) content (µg/kg ww) obtained in raw and cooked seafood samples. Bold letters represent cooked samples where the highest increase percentage in contaminant levels above 50% was registered compared to raw samples.

Sample ID	Species	Sampling site	Me-Hg		T-Hg	
			raw	cooked	raw	cooked
AEIF0007b	Sole, large	Goro, IT	17.09	**34.02**	27.67	**58.33**
AEIF0008	Mackerel, fresh	Goro, IT	159.72	173.32	234.63	275.83
AEIF0009	Seabream	Other origin	65.03	83.81	97.57	122.39
IMAR0009a	Plaice, small	North Sea	48.97	51.05	68.17	74.49
IMAR0009b	Plaice, large	North Sea	48.62	43.21	66.38	69.79
IRTA008a	Octopus, small	Mediterranean	50.41	**97.32**	81.36	**164.77**
IRTA008b	Octopus, large	Mediterranean	266.07	365.89	375.79	553.20
IRTA0009	Mackerel fresh	Spain	78.90	99.07	147.67	182.51
ICETA0006a	Monkfish, small	Portugal	127.53	166.90	178.86	238.40
ICETA0006b	Monkfish, largel	Portugal	174.78	207.02	241.32	280.85
ILVO0005a	Plaice, small	Channel	53.32	65.27	81.06	92.73
ILVO0005b	Plaice, large	Channel	67.78	85.90	95.06	123.29
IPMA0011a	Pacific hake, small	South America	130.67	142.17	164.83	196.04
IPMA0011b	Pacific hake, large	South America	216.11	200.57	288.14	288.66
IPMA0012a	Atlantic hake, small	South Africa	84.58	91.73	110.99	130.42
IPMA0012b	Atlantic hake, large	South Africa	187.05	153.80	236.62	291.80
IPMA0013a	Imported Tuna, small	Pacific	120.28	125.30	181.37	183.80
IPMA0013b	Imported Tuna, large	Pacific	147.97	191.68	209.61	287.83

From the 18 samples analyzed after cooking, 10 samples revealed an increase in at least 10% of Me-Hg concentration, of which 9 revealed at least a 20% increase. The highest increase of Me-Hg contamination levels were observed in cooked sole (99.06%), followed by small octopus (93.06%). Only 2 samples (large plaice from North Sea and large Atlantic hake from South Africa) showed a decrease in Me-Hg concentration (between below 20%). Contrarily, all samples presented an increase in T-Hg levels. Fourteen cooked samples showed an increase in T-Hg levels above 10%,

whereas 9 had levels above 20% of increase and only 2 was above 50%. Like for Me-Hg the highest increases were observed in cooked samples of sole (110.84%), followed by small octopus (102.51%) (table 3).

Concerning Arsenic, detectable levels of total arsenic (T-As) and inorganic arsenic (iAS) were found in all analysed species. The highest T-As level was found in cooked brown crab (13.41 mg/kg), while the highest iAs level was found in cooked mussels from Netherlands (0.12 mg/kg) (table 4).

Table 4 – Inorganic arsenic (iAS) and Total arsenic (T-As) content (mg/kg ww) obtained in raw and cooked seafood samples. Bold letters represent cooked samples where the highest increase percentage in contaminant levels above 50% was registered compared to raw samples.

Sample ID	Species	Sampling site	iAS		T-As	
			raw	cooked	raw	cooked
AEIF00010	Mussels	Goro, IT	0.01	0.02	1.48	**2.17**
IMAR0012	Mussels	Netherlands	0.11	0.12	1.87	2.00
IMAR0013	Mussels	Ireland	0.06	0.11	1.93	2.54
IMAR0014	Brown crab	Netherlands	0.03	0.03	13.50	13.41
IRTA0007	Mussels	Spain	0.02	0.03	2.91	3.25
ILVO0006	Mussels	France	0.02	**0.05**	3.63	4.30

Cooked brown crab did not reveal an increase in iAs concentration level even revealed a slight decrease in T-As. In contrast, all cooked mussels showed increased iAs levels compared to raw samples. Mussels from France, Spain and Ireland even revealed an increase of iAs levels above 50% after cooking. In contrast, for T-As levels, four out of five cooked mussel samples revealed an increase above 10%, with the cooked samples from Goro and Ireland revealing an increase above 20%. The highest increases were observed in cooked samples of mussels from France for iAs (221.42%) and in cooked samples of mussels from Goro, Italy, for T-As (46.02%) (table 4).

5.2 Per Fluorinated Compounds (PFCs)

In the 9 samples analyzed after cooking, no detectable contamination levels were observed for PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFBS, PFHxS, PFHpS and PFDS. Detectable contamination levels were observed in 4 samples after cooking: small plaice, mussels, and small and large imported tuna. The highest contamination levels were observed for PFTrA (16 µg/kg) and PFUnA (7.4 µg/kg) in large and small tuna from Pacific, respectively (table 5).

Table 5 – Per Fluorinated compounds (PFCs) content (µg/kg ww) obtained in raw and cooked seafood samples. Bold letters represent cooked samples where the highest increase/decrease percentage in contaminant levels above 50% was registered compared to raw samples.

Sample ID	Species	Sampling site	PFBA		PFPeA		PFHxA		PFHpA		PFOA		PFNA		PFDcA		PFUnA	
			raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked
ILVO0005a	Plaice, small	Channel	<0.3	<0.2	<0.3	<0.2	<0.3	<0.2	<0.04	<0.03	<0.3	<0.2	<0.04	<0.03	<0.04	<0.03	<0.3	<0.2
ILVO0005b	Plaice, large	Channel	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.03	<0.03	<0.2	<0.2	<0.03	<0.03	<0.03	<0.03	<0.2	<0.2
ILVO0006	Mussels	France	<0.1	0.2	<0.1	<0.2	<0.1	<0.2	<0.02	<0.03	<0.1	<0.2	<0.02	<0.03	0.03	<0.03	<0.1	<0.2
IPMA0011a	Pacific hake, small	South America	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.02	<0.02	<0.1	<0.1	<0.02	<0.02	<0.02	<0.02	<0.1	<0.1
IPMA0011b	Pacific hake, large	South America	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.03	<0.03	<0.2	<0.2	<0.03	<0.03	<0.03	<0.03	<0.2	<0.2
IPMA0012a	Atlantic hake, small	South Africa	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.02	<0.02	<0.1	<0.1	<0.02	<0.02	<0.02	<0.02	<0.1	<0.1
IPMA0012b	Atlantic hake, large	South Africa	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.03	<0.02	<0.2	<0.2	<0.03	<0.02	<0.03	<0.02	<0.2	<0.2
IPMA0013a	Imported Tuna, small	Pacific	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.03	<0.03	<0.2	<0.2	<0.03	<0.03	<0.03	**0.5**	1.8	7.4
IPMA0013b	Imported Tuna, large	Pacific	<0.1	<0.3	<0.1	<0.3	<0.1	<0.3	<0.02	<0.04	<0.1	<0.3	<0.02	<0.04	<0.02	0.1	1.3	4.2

Sample ID	Species	Sampling site	PFDoA		PFTrA		PFTeA		PFBS		PFHxS		PFHpS		PFOS		PFDS	
			raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked
ILVO0005a	Plaice, small	Channel	<0.3	<0.2	<0.3	<0.2	<0.04	<0.03	<0.2	<0.1	<0.2	<0.1	<0.2	<0.1	0.5	0.6	<0.3	<0.1
ILVO0005b	Plaice, large	Channel	<0.2	<0.2	<0.2	<0.2	<0.03	<0.03	<0.2	<0.1	<0.2	<0.2	<0.2	<0.2	0.3	0.3	<0.2	<0.2
ILVO0006	Mussels	France	<0.1	<0.2	<0.1	<0.2	<0.02	<0.03	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
IPMA0011a	Pacific hake, small	South America	<0.1	<0.1	<0.1	<0.1	<0.02	<0.02	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
IPMA0011b	Pacific hake, large	South America	<0.2	<0.2	<0.2	<0.2	<0.03	<0.03	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
IPMA0012a	Atlantic hake, small	South Africa	<0.1	<0.1	<0.1	<0.1	<0.02	<0.02	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
IPMA0012b	Atlantic hake, large	South Africa	<0.2	<0.2	<0.2	<0.2	<0.03	<0.02	<0.2	<0.1	<0.2	<0.1	<0.2	<0.1	<0.2	<0.1	<0.2	<0.1
IPMA0013a	Imported Tuna, small	Pacific	0.2	**2.3**	1.1	5.6	0.3	0.8	<0.1	<0.2	<0.1	<0.2	<0.1	<0.2	<0.1	<0.2	<0.2	<0.2
IPMA0013b	Imported Tuna, large	Pacific	3.5	3.2	7.7	16	1.4	1.3	<0.1	<0.2	<0.1	<0.2	<0.1	<0.3	0.6	**0.3**	<0.1	<0.3

While PFBA, PFDcA, PFUnA, PFDoA, PFTrA and PFTeA presented increased contamination levels above 50% in cooked samples of mussels and tuna (large and small), PFOS only revealed an increase of 20-50% in small plaice and a decrease of more than 50% in large tuna. Both large and small tuna from Pacific were more affected by cooking. Although the highest increase was observed for PFDcA levels (1,566.67%) in cooked small tuna from the Pacific, followed by PFDoA (1,050.00%) (table 5).

5.3 Endocrine Disruptors (EDCs)

Tris(2-butoxyethyl)phosphate (TBEP) was the only contaminant not detected in the 14 samples analyzed after cooking. In contrast, bisphenol A (BPA), triclosan and methylparaben were detected with increased levels above 50% in 8 (seabream, mussels from Goro, mussels from Netherlands, brown crab, large octopus, canned mackerel and large Atlantic hake), 5 (raw and canned mackerel, mussels from Goro, large octopus and large Atlantic hake) and 5 (seabream, mussels from Goro, brown crab, large octopus and large Atlantic hake) samples after cooking, respectively. Additionally, small cooked imported tuna revealed 12.79% increase in triclosan levels after cooking. The highest level of BPA (17.81 µg/kg) and triclosan (14.45 µg/kg) were found in canned mackerel, whereas the highest methylparaben level was found in cooked large Atlantic hake (7.14 µg/kg) after cooking (table 6).

Table 6 – Endocrine disruptors (EDCs) content (µg/kg ww) obtained in raw and cooked seafood samples. Bold letters represent cooked samples where the highest increase percentage in contaminant levels above 50% was registered compared to raw samples.

Sample ID	Species	Sampling site	Bisphenol A		Triclosan		Methylparaben		TBEP	
			raw	cooked	raw	cooked	raw	cooked	raw	cooked
AEIF0007b	Sole, large	Goro. IT	<0,001	<0,002	<0,000	<0,001	<0,000	<0,001	<0,000	<0,000
AEIF0008	Mackerel, fresh	Goro. IT	<0,02	<0,01	<0,001	1.086	<0,000	<0,001	<0,003	<0,000
AEIF0009	Seabream	Other origin	4.62	13.89	<0,000	<0,000	0.581	2.81	<0,000	<0,000
AEIF00010	Mussels	Goro. IT	1.67	12.10	<0,001	**5.48**	0.408	5.97	<0,005	<0,02
IMAR0010	Mackerel, fresh	North Sea	<0,002	<0,002	<0,000	<0,000	<0,000	<0,000	<0,001	<0,000
IMAR0012	Mussels	Netherlands	<0,01	**2.28**	<0,008	<0,009	<0,001	<0,002	<0,006	<0,007
IMAR0014	Brown crab	Ireland	<0,007	**1.42**	<0,02	<0,007	<0,000	**0.42**	<0,004	<0,005
IRTA008b	Octopus, large	Spain	1.21	10.57	<0,006	**8.16**	<0,001	**3.71**	<0,01	<0,02
ICETA0008	Canned mackerel	Spain	7.45	17.81	0.27	14.45	<0,000	<0,000	<0,001	<0,001
ILVO0005a	Plaice, small	Channel	<0,002	<0,002	<0,000	<0,000	<0,000	<0,001	<0,000	<0,000
ILVO0005b	Plaice, large	Channel	<0,002	<0,002	<0,000	<0,000	<0,000	<0,001	<0,000	<0,000
IPMA0012b	Atlantic hake, large	South America	2.04	15.52	<0,03	5.80	<0,002	**7.14**	<0,02	<0,03
IPMA0013a	Imported Tuna, small	Pacific	<0,006	<0,007	0.86	0.970	<0,000	<0,000	<0,001	<0,001
IPMA0013b	Imported Tuna, large	Pacific	<0,006	<0,009	<0,006	<0,004	<0,003	<0,000	<0,003	<0,001

The effect of cooking triggered the highest contaminant increase in: a) mussels from Netherlands and brown crab from Ireland (22,700.00% and 20,185.71%, respectively) for BPA; b) mussels from Goro and large octopus from Spain (547,900.00% and 135,900.00%, respectively) for triclosan; and c) brown crab from Ireland (419,900.00%) and octopus from Spain (370,900.00%) for methylparaben.

5.4 Brominated Flame Retardants (BFRs)

Among brominated flame retardants (BFRs), the effect of cooking was assessed in polybrominated diphenyl ethers (PBDEs: BDE28, BDE47, BDE100, BDE99, BDE154, BDE153, BDE209, BDE183), hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), decabromodiphenyl ethane (DBDPE) and methoxylated polybrominated diphenyl ethers (MeO-PBDEs: 2-MBDE-68, 6-MBDE-47, 4-MBDE-99, 5-MBDE-100, 4-MBDE-100, 5-MBDE-99, 4-MBDE-101) (table 7).

Table 7 – Brominated flame retardants (BFRs) content (µg/kg ww) obtained in raw and cooked seafood samples. Bold letters represent cooked samples where the highest increase percentage in contaminant levels above 50% was registered compared to raw samples.

Sample ID	Species	Sampling site	BDE28		BDE47		BDE100		BDE99		BDE154		BDE153		BDE209		BDE183		∑PBDEs		HBB		PBEB	
			raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked
AEIF0008	Mackerel, fresh	Goro. IT	<0.04	5.01	<0.04	<0.05	<0.004	<0.005	5.68	5.25	9.97	<0.04	1.66	<0.05	<0.01	<0.02	<0.03	<0.04	17.32	10.26	6.62	8.31	<0.03	18.81
AEIF0009	Seabream	Other origin	2.81	5.45	6.77	7.85	<0.002	<0.003	<0.01	3.28	<0.02	<0.03	0.87	<0.01	<0.01	<0.01	<0.06	<0.03	10.46	16.58	3.86	8.89	<0.02	10.00
AEIF00010	Mussels	Goro. IT	<0.01	5.16	<0.02	<0.03	<0.002	<0.002	2.07	<0.002	<0.02	<0.02	<0.01	0.75	<0.01	<0.01	<0.05	<0.02	2.07	5.91	3.21	4.21	<0.02	<0.02
IMAR0009a	Plaice, small	North Sea	0.04	0.14	0.35	0.36	<0.002	<0.002	0.04	0.05	<0.02	<0.02	<0.02	<0.02	<0.01	<0.01	<0.02	0.59	0.44	1.14	0.07	0.08	<0.02	<0.02
IMAR0012	Mussels	Netherlands	0.07	0.08	0.20	0.22	<0.002	<0.002	0.05	0.05	<0.02	<0.02	<0.02	<0.03	<0.01	**0.93**	<0.02	<0.02	0.32	1.28	0.07	0.08	<0.02	<0.02
IMAR0013	Mussels	Ireland	0.04	0.09	0.11	0.35	<0.001	<0.002	0.03	0.04	<0.01	<0.02	<0.01	<0.02	<0.005	<0.01	<0.01	<0.02	0.18	0.47	0.04	0.06	<0.01	<0.02
IRTA0007	Mussels	Spain	0.06	0.08	0.38	**0.14**	<0.001	<0.002	0.07	0.11	<0.01	<0.02	<0.02	<0.02	<0.01	**1.88**	0.21	<0.02	0.71	2.20	0.05	0.07	<0.01	<0.02
IRTA0009	Mackerel fresh	Spain	<0.03	<0.04	<0.03	<0.04	<0.003	<0.004	3.59	4.29	<0.08	8.90	2.01	1.83	<0.03	<0.01	<0.03	<0.09	5.60	15.02	5.56	6.28	<0.03	<0.03
DTU0006	Norwegian salmon	DanSalmon. Denmark	0.08	0.22	1.63	2.95	<0.004	<0.004	0.08	0.07	<0.04	<0.03	<0.04	<0.04	<0.02	<0.01	<0.04	<0.03	1.79	3.25	<0.04	0.15	<0.04	<0.03
DTU0007	Atlantic Cod	North Sea. Denmark	0.04	0.05	0.11	6.32	<0.002	<0.002	0.04	0.05	<0.02	<0.02	<0.02	0.10	<0.01	<0.01	0.57	<0.02	0.76	**6.51**	0.08	0.10	<0.02	0.07
DTU0008	Mackerel	North Sea. Denmark	0.09	0.11	9.01	<0.06	<0.004	<0.01	0.09	0.11	<0.04	<0.05	<0.05	<0.02	<0.02	<0.02	<0.04	<0.05	9.18	**0.21**	<0.04	0.21	<0.04	0.37
ILVO0006	Mussels	France	0.07	0.12	0.15	0.27	<0.002	**2.39**	0.05	0.06	<0.02	<0.03	<0.03	<0.03	<0.01	<0.01	2.71	2.09	2.98	4.92	0.10	0.12	<0.02	<0.03
IPMA0013a	Imported Tuna, small	Pacific	0.10	0.19	0.52	0.79	<0.003	<0.004	0.07	0.08	<0.03	<0.03	<0.03	<0.04	<0.01	<0.01	<0.03	<0.03	0.70	1.06	0.11	<0.03	<0.03	<0.03
IPMA0013b	Imported Tuna, large	Pacific	0.17	0.22	0.68	1.24	<0.003	<0.004	0.07	0.10	<0.03	<0.04	<0.04	<0.05	<0.01	0.44	<0.03	<0.04	0.92	2.00	0.12	0.16	<0.03	<0.04

Sample ID	Species	Sampling site	DBDPE		2-MBDE-68		6-MBDE-47		5-MBDE-47		4-MBDE-99		5-MBDE-100		4-MBDE-100		5-MBDE-99		4-MBDE-101		∑MeO-PBDEs	
			raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked
AEIF0008	Mackerel, fresh	Goro. IT	<0.01	<0.01	<0.01	<0.02	<0.49	<0.20	<0.03	<0.04	<0.01	<0.02	3.3	4.14	<0.004	<0.005	<0.08	<0.10	<0.04	<0.02	3.30	4.14
AEIF0009	Seabream	Other origin	<0.01	<0.01	<0.02	<0.03	2.27	<0.13	<0.02	<0.03	<0.01	<0.01	1.96	<0.08	<0.002	<0.003	<0.05	<0.06	<0.01	<0.01	4.23	**0.00**
AEIF00010	Mussels	Goro. IT	<0.01	<0.01	<<0.01	<0.01	<0.08	<0.10	<0.02	<0.02	<0.01	<0.01	1.60	**2.10**	<0.002	<0.002	<0.04	<0.05	<0.01	<0.01	1.60	2.10
IMAR0009a	Plaice, small	North Sea	<0.01	<0.01	<0.01	<0.01	<0.08	<0.10	<0.02	<0.02	<0.01	<0.01	<0.05	<0.05	<0.002	<0.002	<0.04	<0.05	<0.01	<0.01	0.00	0.00
IMAR0012	Mussels	Netherlands	<0.01	<0.01	1	2.15	<0.09	<0.10	<0.02	<0.06	<0.01	<0.01	<0.06	<0.05	<0.002	<0.002	<0.05	<0.05	<0.01	<0.01	0.85	2.15
IMAR0013	Mussels	Ireland	<0.004	<0.01	<0.01	**1.23**	<0.05	1.13	<0.01	<0.01	<0.005	<0.01	<0.03	<0.04	<0.001	<0.002	<0.03	<0.04	<0.005	<0.01	0.00	2.36
IRTA0007	Mussels	Spain	<0.004	<0.01	<0.02	<0.02	<0.20	<0.26	<0.04	<0.05	<0.01	<0.02	0.03	<0.05	<0.001	<0.002	<0.03	<0.04	<0.01	<0.01	0.03	**0.00**
IRTA0009	Mackerel fresh	Spain	<0.01	<0.01	<0.03	<0.04	<0.13	<0.15	<0.03	<0.003	<0.01	<0.01	2.7	3.34	<0.003	<0.004	<0.07	<0.08	<0.01	<0.01	2.67	3.34
DTU0006	Norwegian salmon	DanSalmon. Denmark	12.21	<0.01	<0.04	0.22	<0.54	<0.16	<0.04	<0.03	<0.02	<0.01	<0.04	<0.09	<0.004	<0.004	<0.09	<0.08	<0.02	<0.01	0.00	0.22
DTU0007	Atlantic Cod	North Sea. Denmark	<0.01	<0.01	<0.01	0.12	<0.08	<0.11	<0.02	<0.02	<0.01	<0.01	<0.05	<0.06	<0.002	<0.002	<0.04	<0.05	<0.01	<0.03	0.00	0.12
DTU0008	Mackerel	North Sea. Denmark	<0.01	<0.02	<0.02	**3.16**	<0.19	<0.23	<0.04	<0.05	<0.02	<0.02	<0.04	<0.14	<0.004	<0.01	<0.09	<0.12	<0.02	<0.02	0.00	**3.16**
ILVO0006	Mussels	France	<0.01	<0.01	<0.03	0.03	<0.33	0.40	<0.02	0.02	<0.01	0.01	<0.07	0.07	<0.002	0.003	<0.05	0.07	<0.01	0.01	0.00	0.61
IPMA0013a	Imported Tuna, small	Pacific	<0.01	<0.01	<0.03	<0.04	0.8	<0.16	<0.03	<0.03	<0.01	<0.01	<0.08	<0.07	<0.003	<0.004	<0.07	<0.08	<0.01	<0.01	0.85	**0.00**
IPMA0013b	Imported Tuna, large	Pacific	<0.01	<0.01	2	<0.05	<0.14	<0.19	<0.03	<0.04	<0.01	<0.02	<0.09	<0.09	<0.003	<0.004	<0.07	<0.10	<0.01	<0.02	2.38	**0.00**

From the 14 samples analysed, no detectable contamination levels were registered for DBDPE, 5-MBDE-47, 4-MBDE-99, 4-MBDE-100, 5-MBDE-99 and 4-MBDE-101. In contrast, detectable levels were found in BDE28, BDE47, BDE100, BDE99, BDE154, BDE153, BDE209, BDE183, HBB, PBEB, 2-MBDE-68, 6-MBDE-47 and 5-MBDE-100. The highest contamination levels of PBDEs (16.58 µg/kg) and HBB (8.89 µg/kg) was observed in seabream, while mackerel from Goro showed the highest levels for PBEB (18.81 µg/kg) and MeO-PBDEs (4.14 µg/kg). Overall, the contamination levels of PBDEs increased after cooking, with some exceptions (61.58% decrease in mussels from Spain for BDE47 levels and 23% decrease of BDE183 in mussels from France). Indeed, the highest increase of contamination levels among PBDEs was observed for BDE100 in cooked samples of mussels from France (119,260%), whereas the highest

increase was observed in Atlantic cod from the North Sea for ∑PBDEs (756.89%) and the highest decrease of ∑PBDEs was registered in mackerel from the North Sea (97.70%). On the other hand, the effect of cooking only increased the contamination levels of HBB and PBEB. From the 14 samples analysed for HBB, 2 cooked samples revealed increased levels up to 20%, 10 cooked samples with increased levels up to 50% and 3 samples above 50% increase. In contrast, the only 4 cooked samples that presented detectable contamination levels of PBEB, revealed increases above 50% in the concentration levels. Mackerel samples from France and Italy, showed the highest increases for HBB (427.00%) and PBEB (62,613.16%), respectively. Regarding ΣMeO-PBDEs, the effect of cooking revealed either lower or higher levels in cooked samples depending on species. Increase in levels above 50% were observed in cooked mussels from Netherlands, Ireland and France, as well as in cooked Norwegian salmon, cooked Atlantic cod and cooked mackerel from the North Sea. In contrast, a strong reduction in levels of ΣMeO-PBDEs were registered in cooked samples of seabream, mussels from Spain and tuna from the Pacific. The highest increase in ΣMeO-PBDEs and 2-MBDE-68 (15,710%) contamination levels was observed in mackerel from the North Sea.

Tetrabrombisphenol A (TBBPA), 2,4,6 tribromophenol (2,4,6-TBP) and α-, β-, γ-Hexabromocyclododecane (α-, β-, γ-HBCD) were also analysed in 16 samples after cooking (table 8).

Table 8 – Brominated flame retardants (BFRs: TBBPA, 2,4,6-TBP, α-HBCD, β-HBCD, γ-HBCD) content (µg/kg ww) obtained in raw and cooked seafood samples

Sample ID	Species	Sampling site	TBBPA		2.4.6-Tribromophenol		α-HBCD		β-HBCD		γ-HBCD	
			raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked
AEIF0007b	sole, large	Goro. IT	na	<0.05	na	<0.5	na	<0.05	na	<0.05	na	<0.05
AEIF0008	mackerel, fresh	Goro. IT	<0.05	<0.05	<0.5	<0.5	1.55	2.03	<0.05	<0.05	<0.1	<0.05
AEIF0009	seabream	Other origin	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05	<0.05	<0.05	<0.1	<0.05
AEIF00010	Mussels	Goro. IT	<0.05	<0.05	2.15	**0.94**	0.11	0.20	<0.05	<0.05	0.06	0.05
IMAR0009a	Plaice, small	North Sea	<0.05	<0.05	0.25	**0.85**	0.02	<0.05	<0.05	<0.05	<0.05	<0.05
IMAR0012	Mussels	Netherlands	<0.05	<0.05	0.73	0.85	0.06	<0.05	<0.05	<0.05	0.07	**0.03**
IMAR0013	Mussels	Ireland	<0.05	<0.05	1.97	5.71	0.25	0.54	0.02	0.04	0.06	0.07
IRTA0007	Mussels	Spain	<0.05	<0.05	1.82	2.22	0.05	0.08	<0.05	<0.05	<0.05	<0.05
IRTA0009	mackerel fresh	Spain	0.57	<0.05	<0.5	<0.5	0.60	0.63	<0.05	<0.05	<0.05	<0.05
DTU0006	Norwegian salmon	DanSalmon. Denmark	<0.05	<0.05	<0.5	<0.5	0.18	0.12	<0.05	<0.05	0.11	<0.05
DTU0007	Atlantic Cod	North Sea. Denmark	<0.05	<0.05	<0.5	<0.5	0.02	<0.05	<0.05	<0.05	<0.05	<0.05
DTU0008	mackerel	North Sea. Denmark	<0.05	<0.05	<0.5	<0.5	0.18	0.36	<0.05	0.06	<0.05	**0.21**
ICETA0006a	Monkfish, small	Portugal	0.09	0.18	<0.5	<0.5	<0.05	<0.05	<0.05	<0.05	<0.05	0.05
ICETA0006b	Monkfish, largel	Portugal	<0.05	<0.1	<0.5	<0.5	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
ILVO0006	Mussels	France	<0.05	<0.05	2.89	<0.5	0.04	<0.05	<0.05	<0.05	<0.05	<0.05
IPMA0013a	Imported Tuna, small	Pacific	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05	<0.05	<0.05	0.03	<0.05
IPMA0013b	Imported Tuna, large	Pacific	<0.05	<0.05	<0.5	<0.5	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

While TBBPA and β-HBCD contamination levels increased after cooking in one sample, 2,4,6-TBP, α-HBCD and γ-HBCD contamination levels increased or decreased after cooking depending on the analysed samples. Regarding TBBPA, only small monkfish from Portugal presented detectable contamination levels, with an increase of 47.93% after cooking. As for β-HBCD, mussels from Ireland was the only sample contaminated, with an increase of 51.85% in the contamination levels after cooking. Mussels from Ireland revealed the highest increase in 2,4,6-TBP and α-HBCD contamination levels after cooking (65.45% and 53.46%, respectively), whereas mackerel from the North Sea registered the highest increase in γ-HBCD levels after cooking (51.85%). In contrast, cooked mussels from Italy presented a decrease of 56.35% in 2,4,6-TBP contamination levels, while cooked mussels from The Netherlands decreased γ-HBCD levels by 61.06% and cooked Norwegian salmon decreased the α-HBCD by 33.33%. The effect of cooking was more relevant in γ-HBCD, where the highest increase in contamination level was registered (76.30% in cooked mackerel from the North Sea), as well as the highest decrease (61.06% in cooked mussels from Netherlands). Nevertheless, 2,4,6-TBP showed the highest contamination levels after cooking (5.71 µg/kg in mussels from Ireland).

5.5 Pharmaceuticals (PhACs)

The results of pharmaceutical compounds were inconclusive as the levels of diclofenac, azithromycin, sulfamethoxazole, sotalol, diazepam, carbamazepine, venlafaxine and citalopram found in the 6 samples were below the detection limits in all raw and cooked samples (table 9).

Table 9 – Pharmaceuticals compounds (PhACs) content (µg/kg ww) obtained in raw and cooked seafood samples.

Sample ID	Species	Sampling site	Diclofenac		Azithromycin		Sulfamethoxazole		Sotalol		Diazepam		Carbamazepine		Venlafaxine		Citalopram	
			raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked
AEIF0008	Mackerel, fresh	Goro. IT	<0,004	<0,005	<6,14	<2,31	<0,07	<0,03	<0,004	<0,005	<0,004	<0,005	<0,01	<0,01	<0,01	<0,02	<0,27	<0,1
AEIF00010	Mussels	Goro. IT	nm	nm	<0,002	<0,002	<0,004	<0,002	<0,04	<0,05	<0,1	<0,13	<0,005	<0,007	<0,002	<0,002	<0,01	<0,01
IRTA0007	Mussels	Spain	nm	nm	<0,001	<0,002	<0,001	<0,002	<0,02	<0,04	<0,08	<0,1	<0,004	<0,006	<0,001	<0,002	<0,009	<0,01
ILVO0006	Mussels	France	nm	nm	<0,002	<0,003	<0,002	<0,003	<0,05	<0,06	<0,1	<0,2	<0,007	<0,009	<0,002	<0,003	<0,01	<0,02
IPMA0013a	Imported Tuna, small	Pacific	<0,003	<0,004	<1,57	<1,77	<0,02	<0,02	<0,003	<0,004	<0,003	<0,004	<0,009	<0,04	<0,01	<0,01	<0,07	<0,08
IPMA0013b	Imported Tuna, large	Pacific	<0,003	<0,004	<5,41	<2,19	<0,02	<0,03	<0,003	<0,004	<0,003	<0,004	<0,04	<0,05	<0,01	<0,02	<0,07	<0,1

5.6 Polycyclic aromatic hydrocarbons (PAHs)

Seventeen polycyclic aromatic hydrocarbons (PAH) were analysed in 5 raw and cooked samples of mussels and one raw and cooked sample of brown crab. The results included in table 10 indicate that the effect of cooking can vary according to the sample and compound. Indeed, the contamination levels of acenapthylene, acenapthene, fluorene, phenanthrene, anthracene, pyrene and benzo(a)pyrene increased or decreased depending on the mussel sample, whereas fluoranthene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(j)fluoranthene, benzo(e)pyrene, indeno(123cd)pyrene, dibenzo(ah)anthracene and benzo(ghi)perylene levels generally increased after cooking. The highest increase was observed in pyrene levels (541.51%), followed by fluorene (376.90%) in cooked mussels from Italy. In contrast, the highest decrease was observed in fluorene levels (45.54%) in mussels from Spain. Also, fluoranthene and pyrene presented the highest contamination levels (13.83 µg/kg and 12.66 µg/kg, respectively) in cooked samples of mussels from Spain (table 10). Interestingly, cooked brown crab has not revealed any increase in PAHs compared to raw samples, even revealing a decrease of phenanthrene levels in cooked samples.

Table 10 – Polycyclic aromatic hydrocarbons (PAH) content (µg/kg ww) obtained in raw and cooked seafood samples. Bold letters represent cooked samples where the highest increase percentage in contaminant levels above 50% was registered compared to raw samples.

Sample ID	Species	Sampling site	acenapthylene		acenapthene		fluorene		phenanthrene		anthracene		fluoranthene		pyrene		benzo(a)anthracene		chrysene		benzo(b)fluoranthene	
			raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked
AEIF00010	Mussels	Goro. IT	<0,18	0.16	<1,59	<1,24	0.60	**2.86**	<1,67	5.10	<0,18	0.14	<1,57	4.36	<0,47	**3.02**	<0,18	0.60	0.49	1.67	0.30	0.70
IMAR0012	Mussels	Netherlands	0.24	0.21	2.24	1.51	5.44	5.87	12.92	12.89	0.41	0.46	11.04	11.66	8.28	7.23	1.79	1.83	2.75	2.92	2.28	2.47
IMAR0013	Mussels	Ireland	0.14	0.18	<1,20	<1,17	2.03	3.39	3.73	7.68	0.35	0.71	6.57	13.83	6.25	12.66	2.30	4.56	1.93	3.87	2.85	6.06
IMAR0014	Brown crab	Ireland	ND	ND	ND	ND	ND	ND	1.03	0.92	<0,10	<0,11	0.21	<0,22	<0,20	<0,22	<0,10	<0,11	0.19	0.21	0.08	0.08
IRTA0007	Mussels	Spain	0.15	<0,15	<1,16	<1,40	1.68	**0.91**	3.18	4.10	<0,13	<0,15	4.61	8.50	4.20	5.82	2.33	4.07	3.79	5.83	3.93	6.40
ILVO0006	Mussels	France	0.16	<0,13	<1,17	<1,14	1.73	1.58	1.90	2.03	<0,13	<0,13	1.27	1.99	1.24	0.87	0.25	0.46	0.43	0.64	0.54	0.90

Sample ID	Species	Sampling site	benzo(k)fluoranthene		benzo(j)fluoranthene		benzo(e)pyrene		benzo(a)pyrene		indeno(123cd)pyrene		dibenzo(ah)anthracene		benzo(ghi)perylene	
			raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked
AEIF00010	Mussels	Goro. IT	<0,18	0.25	0.21	0.39	0.32	0.82	<0,18	0.15	<0,18	0.17	<0,18	0.21	<0,18	0.17
IMAR0012	Mussels	Netherlands	1.17	1.28	1.16	1.27	2.35	2.44	1.21	1.18	1.08	1.13	0.31	0.38	1.34	1.21
IMAR0013	Mussels	Ireland	1.43	2.96	1.35	2.93	2.87	6.15	1.69	2.52	1.27	2.09	0.30	0.55	1.60	2.77
IMAR0014	Brown crab	Ireland	<0,10	<0,11	0.16	0.17	<0,10	<0,11	<0,10	<0,11	<0,10	<0,11	<0,10	<0,11	<0,10	<0,11
IRTA0007	Mussels	Spain	1.97	3.31	1.81	2.95	3.21	4.95	1.03	1.83	1.07	1.60	0.33	0.66	1.14	1.57
ILVO0006	Mussels	France	0.23	0.37	0.30	0.54	0.42	0.77	<0,13	0.15	0.24	0.28	<0,13	0.20	0.28	0.36

5.7 Musks

From the 10 musks fragances quantified, only DPMI, HHCB, AHTN and HHCB-Lactone were detected in the analyzed species. The effect of cooking generally increased the contamination levels of DPMI (in mussels from Italy and large plaice) and HHCB-Lactone (large sole, fresh mackerel, large plaice and small plaice). On the other hand, the effect of cooking increased the HHCB levels in large sole, mussels from Italy, large plaice, brown crab and small plaice, as well as AHTN levels increased in large sole, fresh mackerel, mussels from Italy and large plaice. In contrast, HHCB levels decreased in fresh mackerel (29.04%), whereas AHTN levels decreased in brown crab (20.95%). Cooked plaice showed the highest increase in DPMI levels (679.37%), cooked mussels from Italy showed the highest increase in HHCB (1,733.74%) and AHTN1 (538.00%) levels, and cooked sole registered the highest increase in HHCB-Lactone levels 4,882.19% (table 11). Cooked mussels from Italy presented the highest concentration levels of DPMI (6.32 µg/kg) and HHCB (80.50 µg/kg), while cooked mackerel from Italy presented the highest concentration levels of AHTN (5.12 µg/kg) and HHCB-Lactone (106.79 µg/kg) (table 11).

Table 11 – Musks content (µg/kg ww) obtained in raw and cooked seafood samples. Bold letters represent cooked samples where the highest increase percentage in contaminant levels above 50% was registered compared to raw samples.

Sample ID	Species	Sampling site	DPMI		ADBI		AHMI		ATII		HHCB		AHTN		MX		MM		MK		HHCB-Lactone	
			raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked
AEIF0007b	sole, large	Goro. IT	1.31	<1.6	<0.3	<0.6	<0.3	<0.6	<0.3	<0.6	4.82	13.66	1.03	3.19	<0.7	<1.6	<0.7	<1.6	<0.7	<1.6	<0.3	**14.95**
AEIF0008	mackerel, fresh	Goro. IT	<1.5	<1.8	<0.7	<0.9	<0.7	<0.9	<0.7	<0.9	33.46	23.75	3.43	5.12	<7.8	<2.3	<1.8	<2.3	<1.8	<2.3	70.07	106.79
AEIF00010	Mussels	Goro. IT	<1.8	6.32	<0.7	<0.9	<0.7	<0.9	<0.4	<0.5	4.30	**80.50**	<0.2	**3.28**	<1.8	<2.3	<1.8	<2.3	<1.8	<2.3	<0.9	<1.2
IMAR0009b	Plaice, large	North Sea	<0.4	**3.12**	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	3.87	6.88	1.24	2.05	<1.0	<1.1	<1.0	<1.1	<1.0	<1.1	9.42	19.28
IMAR0012	Mussels	Netherlands	1.67	<2.3	<0.8	<0.9	<0.8	<0.9	<0.4	<0.5	<1.0	<0.2	<0.2	<0.2	<2.1	<2.3	<2.1	<2.3	<2.1	<2.3	<1.0	<1.2
IMAR0014	Brown crab	Ireland	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	11.17	17.49	5.02	3.96	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<0.8	<0.8
ILVO0005a	Plaice/Sole, small	Channel	<0.5	<0.6	<0.5	<0.6	<0.5	<0.6	<0.5	<0.6	5.05	5.85	1.63	1.71	<1.2	<1.4	<1.2	<1.4	<1.2	<1.4	8.96	14.24
ILVO0005b	Plaice/Sole, large	Channel	<1.1	<0.6	<0.4	<0.6	<0.4	<0.6	<0.4	<0.6	5.04	5.13	1.28	1.87	<1.1	<1.4	<1.1	<1.4	<1.1	<1.4	<0.4	<0.6

Legend: DPMI- 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone; ADBI- 4-acetyl-1,1-dimethyl-6-tert-butylindane; AHMI-6-acetyl-1,1,2,3,3,5-hexamethylindane; ATII- 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane; HHCB- 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyran); AHTN- 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene; MX -2,4,6-trinitro-1,3-dimethyl-5-tert-butylbenzene; MM-1,1,3,3,5-pentamethyl-4,6-dinitroindane: HHCB-lactone-1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyran-1-one;

5.8 UV-filters

In the 14 cooked samples of seafood analysed, only 7 UV-filter analytes were quantified (EHS, HS, IMC, 4-MBC, BP1DHMB and DBENZO) in 3 species (mackerel, mussels and monkfish) (table 12).

Table 12 – UV-filters content (µg/kg ww) obtained in raw and cooked seafood samples. Bold letters represent cooked samples where the highest increase percentage in contaminant levels above 50% was registered compared to raw samples.

Sample ID	Species	Sampling site	EHS		HS		IMC		4-MBC		EPABA		EHMC		OC		BP3		BP1		DHMB		DBENZO	
			raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked	raw	cooked
AEIF0008	Mackerel, fresh	Goro. IT	<1.10	11.08	<0.74	7.84	<3.68	4.62	<2.94	<3.70	<0.55	<2.31	<1.84	<2.31	<1.84	<2.31	<3.68	<4.62	3.68	**65.29**	<4.42	<5.54	<7.36	<9.24
AEIF0009	Seabream	Other origin	9.21	<2.93	7.17	<1.47	<2.15	<2.93	<0.53	<0.72	<1.08	<1.47	<1.08	<1.47	22.21	<1.47	<2.15	<2.93	21.25	<2.93	85.75	<3.52	<4.30	<5.86
AEIF00010	Mussels	Goro. IT	<1.79	<2.34	<0.57	<1.17	<1.79	<2.34	<1.43	<0.57	<0.90	<1.17	<0.90	<1.17	<0.90	<1.17	<1.79	<2.34	8.88	<2.34	<2.15	<2.81	<3.58	<4.68
IMAR0012	Mussels	Netherlands	3.54	<0.35	2.50	<1.15	<2.08	<2.30	<1.67	<1.84	<1.04	<1.15	<1.04	<1.15	11.67	<1.15	<2.08	<2.30	<2.08	<2.30	<2.50	<2.76	<4.17	<4.60
IMAR0013	Mussels	Ireland	<1.22	<1.73	<0.61	<0.87	<1.22	<1.73	<0.97	<1.38	<0.61	<0.87	<0.61	<0.87	<0.61	<0.87	<1.22	<1.73	<1.22	<1.73	<1.46	<2.08	<2.44	<3.46
IMAR0014	Brown crab	Netherlands	<3.95	<4.08	<1.97	<2.04	<3.95	<4.08	<3.16	<3.26	<1.97	<2.04	<1.97	<2.04	<1.97	<2.04	<3.95	<4.08	<3.95	<4.08	<4.74	<4.89	<7.89	<8.15
IRTA0007	Mussels	Spain	<0.44	<1.93	2.81	**1.08**	<1.47	<1.93	<0.36	<0.47	<0.22	<0.29	<0.73	<0.97	<0.73	<0.97	<1.47	<1.93	10.16	<1.93	<1.76	<2.32	<2.94	<3.86
IRTA0009	Mackerel fresh	Spain	<2.98	<3.51	<1.49	1.24	<2.98	<3.51	<2.38	<2.80	<0.45	<0.53	<1.49	<1.75	<1.49	<1.75	<2.98	<3.51	<2.98	<3.51	<3.57	<4.21	<2.98	7.01
DTU0006	Norwegian salmon (farmed)	DanSalmon. Denmark	9.50	<3.69	6.32	<1.85	<4.14	<3.69	<3.31	<2.95	<0.62	<1.85	<2.07	<1.85	<2.07	<1.85	<4.14	<3.69	<4.14	<3.69	<4.97	<4.43	<4.14	<7.38
DTU0007	Atlantic Cod	North Sea. Denmark	5.16	<2.43	<0.97	<1.21	<1.93	<2.43	<1.54	<1.94	<0.29	<1.21	<0.97	<1.21	7.55	<1.21	<1.93	<2.43	<1.93	<2.43	<2.32	<2.91	<1.93	<4.86
DTU0008	Mackerel	North Sea. Denmark	7.17	<5.27	2.76	<2.64	<4.33	<5.27	<1.06	<4.22	<2.17	<2.64	<2.17	<2.64	18.70	<2.64	<4.33	<5.27	<4.33	<5.27	<5.20	<6.33	<4.33	<10.55
ICETA0006a	Monkfish, small	Portugal	2.92	<2.42	<0.24	<1.21	<1.91	<2.42	<1.53	<1.94	<0.95	<1.21	<0.62	<1.21	2.25	<1.21	<1.91	<2.42	<1.91	<2.42	3.39	<2.91	<3.82	<4.84
ICETA0006b	Monkfish, large	Portugal	<0.61	**16.25**	<0.77	1.29	<2.04	<2.36	<1.63	<1.89	<1.02	<1.18	<1.02	<1.18	3.92	<1.18	<2.04	<2.36	<2.04	<2.36	18.46	11.96	<4.07	<4.72
ILVO0006	Mussels	France	<2.98	<2.98	<1.49	<1.49	<2.98	<2.98	<2.38	2.39	<2.17	<1.49	<2.17	<1.49	<2.07	<1.49	<2.98	<2.98	<2.98	<2.98	<3.57	<3.58	<2.98	<5.97

Legend: EHS - 2-Ethylhexyl salicylate; HS - 3,3,5-Trimethylcyclohexylsalicylate; IMC - Isoamyl-4 methoxycinnamate; 4-MBC - 3-(4-Methylbenzylidene)camphor; EPABA - 2-Ethylhexyl 4-(dimethylamino)benzoate; EHMC - 2-Ethylhexyl 4-methoxycinnamate; OC – Octocrylene; BP3 - benzophenone 3; BP1 - benzophenone 1; DHMB - 2,2-Dihydroxy-4,4-dimethoxybenzophenone; DBENZO - Hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate.

Variations in contamination level were observed after cooking depending on the UV-filter and seafood species. Cooked mackerel from Italy showed an increase above 50% in EHS (907.58%), HS (960.10%) and BP1 (1,674.31%) levels, and 25.54% increase in IMC levels. In contrast, cooked mackerel from Spain presented 135.23% increase in DBENZO levels, but 16.49% decrease in HS levels. Large monkfish from Portugal showed an increase in EHS (2,563.25%) and HS (68.10%) levels, and 35.19% decrease in DHMB. On the other hand, mussels from Spain revealed a decrease in HS levels (61.63%) after cooking. Although the highest contamination level was observed in cooked mackerel from Italy for BP1 (65.29 µg/kg), the cooking effect was more pronounced in EHS, where the highest increase in the contamination level was observed (2,563.25%).

5.9 Microplastics

Because of the extremely low levels of microplastics and fibres quantified in both hotspot, as well as in commercial samples from round I, and for budgetary reasons, it was decided not to perform further analysis of microplastics in round II commercial samples, and not to assess the effect of cooking in microplastic levels found in seafood.

5.10 Biotoxins

Marine phycotoxins are worldwide problem for food safety and public health, and consequently for aquaculture and for the activities of food processing companies.

More than 40% of the produced mussels are transformed by food processing industries, mostly canned mussels or other minor derivatives. During the required processing, the toxins or mussels can undergo changes that could lead to modifications of their toxin content or their potential toxicity (which depends on toxin concentration and on the potency of the involved toxins).

Steaming is one of the simplest processing/cooking methods. This method is an important component of thermal treatment. It is known that cooking or, in general, thermal processing of molluscs dehydrates the meat and consequently leads to a weight decrease, while the degradation of marine toxins is null or very limited. An increase of toxin concentration proportional to the meat weight loss should, therefore, be expected after steaming. Steaming of puffer fish gonads containing high concentration of tetrodotoxin and mussels containing concentrations of Azaspiracids (AZA) were evaluated (table 13).

Table 13 – Azaspiracids (AZA) and Tetrodotoxins (TTX) content (µg equiv/g, ww) obtained in raw and cooked seafood samples. Bold letters represent cooked samples where the highest increase percentage in contaminant levels above 50% was registered compared to raw samples.

	AZA equiv. (1-3)		TTX equiv	
Matrix	**raw**	**cooked**	**raw**	**cooked**
Puffer fish (gonads)			14.34	18.35
Mussels W	0.10	0.18		
Mussels SW	0.24	0.30		
Mussels SW	0.15	0.20		
Mussels NW	0.07	0.17		
Mussels W	0.33	0.48		
Mussels W	0.18	0.24		
Mussels SW	0.16	0.18		
Mussels W	0.07	**0.25**		
Mussels SW	0.12	0.16		
Mussels W	0.06	0.18		

[a]Equivalents of total regulated AZAs(1-3) calculated following application of the toxic equivalence factors for 2 (1.8) and 3 (1.4) relative to 1. The EU regulatory limit is 0.16 µg/g. The harvesting location was the Irish Atlantic coast (W: West, SW: Southwest, NW: Northwest).

Experiments with mussels were conducted in a way that prevented water loss that one normally sees upon cooking mussels. Thus, the concentration of AZAs would approximately have doubled under normal cooking conditions.

Analysis of raw and heat-treated mussels (*Mytilus edulis*) naturally contaminated with AZAs revealed significant increase (up to 4.6-fold) in AZA1−3 (1−3) and 6 (6) values due to heat-induced chemical conversions. NVI data with AZAs showed that 22-carboxyAZAs were converted in 22-desmethylAZAs by heating e.g. AZA17→-AZA3 in mussels. All of the other toxins were stable towards short periods of heating (which corresponds to normal cooking of seafood). This includes other analogues of AZAs that are carboxylated at other positions in the molecule (i.e. extra carboxylic group present, but not at position-22, as these do not decarboxylate upon heating). Many of the details are described in the publication of Kilcoyne et al. " Effect of heating on proportions of Azaspiracids 1-10 in Mussels (*Mytilus edulis*) and Identification of Carboxylated Precursors for Azaspiracids 5, 10, 13 and 15". In summary, analysis of heat-treated mussels from Ireland that were naturally contaminated with AZAs revealed high levels of AZA3 and AZA6. These compounds were not present at significant levels in the uncooked shellfish, highlighting the fact that AZA equivalent values for raw mussels can grossly underestimate the toxicity of the AZAs present (up to at least a 4.6-fold difference for 1-3 and 6). This effect is further compounded by the increase in concentration of these compounds due to water loss during cooking. Due to the huge variation in levels of the decarboxylated analogues, which is dependent on the time since the shellfish were exposed to the algal bloom, it is difficult to build in a safety factor that deals with these bioconversions effectively based on the currently regulated toxins. The EU harmonized LC-MS method has been amended to deal with a concentration effect due to the loss of water during cooking processed samples.

In addition, a puffer fish steaming study comparing the analyses of raw and cooked fish have shown no big differences in concentration. Levels of TTXs were found slightly higher in tissues that were cooked due to loss of water from the matrix. Changes in toxin concentration could be induced by both changes in the weight of tissues or changes in toxin content. In this study, steaming produced an important decrease of the gonad weight and, therefore, a parallel increase in toxin concentration.

6. Conclusions

The results clearly indicate that the culinary processing can indeed affect the levels of most contaminants analysed in this deliverable. In most cases an increase in contaminant levels was found after cooking unlikely due to water loss during steaming since all samples were freeze dried before analysis. For public health, from a theoretical point of view, it is clear that the risk can indeed increase taking into account that the concentration of contaminants in cooked samples can significantly increase. Therefore, it is strongly recommended that in the analysis of raw samples a heating step should be included before analysis. This measure is recommended to enhance human health protection and prevent loss of valuable processed product due to rejection by importing countries.

7. References

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