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## **EFFECT OF PRE-TREATMENT ON CONTENT OF PCDD/F + DL-PCB IN MUSCLE TISSUE OF ATLANTIC SALMON (*SALMO SALAR*) AND SEA TROUT (*SALMO TRUTTA*)**

### **S u m m a r y**

The effect of pre-treatment was evaluated on the reduction in the content of dioxins (PCDD/F) and dioxin-like polychlorinated biphenyls (dl-PCBs) in the Atlantic salmon (*Salmo salar*) fillets and sea trout (*Salmo trutta*) caught in the Baltic Sea. The research studies included fish of different sizes.

The levels of contaminants below the acceptable values as set out in the Commission Regulation (EU) No. 1259/2011 were found in the pre-treated products produced from salmon fillets of up to 7900 g in weight and up to 86 cm in length, and from the sea trout, their weight up to 4500 g and length up to 74 cm. In the case of the trimmed fillets (which contained the ventral part), the content of dioxins and dl-PCBs therein did not exceed the acceptable levels in the Baltic salmon of up to 2579 g in weight and up to 65 cm in length nor in the sea trout of up to 3376 g in weight and up to 65 cm in length.

It was concluded that the pre-treatment process involving trimming and removal of the ventral part of fillet (about 25 % of weight) caused the content of contaminants in the final product to decrease. The study results indicate that the monitoring of contaminants in fish with the focus on consumer safety should involve samples of fish directly available to consumers in chain stores. In the case the sample is a whole fish, the procedure of preparing a sample for laboratory tests has a major impact on the final result of assay.

**Key words:** salmon, sea trout, PCDD/Fs, PCBs, pre-treatment

### **Introduction**

Owing to their nutritional value, fish and fishery products are highly valuable food products with a beneficial effect on human health. They contain proteins rich in essential amino acids (lysine, methionine, cysteine, threonine, and tryptophan); micro- and macro-nutrients (calcium, phosphorous, fluorine, and iodine); fats, which are

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a valuable source of energy; fat-soluble vitamins; and n-3 polyunsaturated fatty acids, which exhibit beneficial effect in the prevention against heart diseases [2, 9, 10]. Of the Baltic fishes, salmon, sea trout and sprat are found to be characterized by a fat content ranging from ca. 2 % to 20 % depending on the season; and herring is characterized by a fat content from ca. 2 % to 10 % depending on the season [16, 21, 22].

However, fish and fishery products do not contain only nutrients but, also, undesirable substances that can pose health risk to consumers. The most common toxic substances occurring in fish and fish products are toxic metals (mercury, arsenic, lead, and cadmium), polychlorinated biphenyls (PCBs), organochlorine pesticides, aromatic hydrocarbons, dioxin-like polychlorinated biphenyls (dl-PCBs), polychlorinated dibenzo-p-dioxins, and dibenzofurans (PCDD/Fs) [6, 17].

In the interest of consumer health, the EU has introduced maximum limits for those contaminants in different food groups, among other things, in fish [3, 4]. The amount of contaminants in all the products admitted to the European market should not exceed those levels. Moreover, members of the European community are obliged to regularly monitor the levels of PCDD/Fs and PCBs in food, including fish. In Poland, such monitoring is provided by the National Veterinary Institute in Puławy.

The levels of contaminants in fish vary depending on the species; they are determined by several factors such as quality of ecosystem, in which the fish occur, duration of exposure to environmental factors (age of fish), season of the year, and position in the food web. Dioxins are toxic substances accumulating in fat, which undergo bioaccumulation and bio-magnification in the trophic chain. Therefore, the highest levels of those contaminants should be expected in oily predatory fish. Among them, there are salmon, for which, initially, planktonic crustaceans, insect larvae, smaller fish, and crustaceans constitute a prey.

However, in the light of contamination-related data, the question arises whether or not the consumption of salmon can be recommended to consumers. First of all, it should be noted that, owing to the limited resources of wild salmon and because of the associated severe restrictions on fishing quotas for this species in the Baltic Sea, the market share of wild salmon is small; therefore, the farmed salmon is a species that usually reaches the consumers.

The objective of the present study was to assess the two approaches that ensure that the products, derived from salmon and sea trout caught in the Baltic Sea and directed to the market, meet the EU limits with respect to the content of dioxins (PCDD/F) and dioxin-like polychlorinated biphenyls (dl-PCBs). One of them, used in several countries (e.g., Denmark) involves the determination of the size of individuals that may end up in the market. This approach is justified by the fact that the younger fish is exposed to harmful compounds in its environment for a shorter period of time; so, they should be characterized by a lower level of contaminants. Of course, it is im-

possible to determine the age of fish intended for the market because of practical reasons. Thus, based on the study results, there is a need to determine the size of those fish, in which the amounts of the above indicated contaminants do not exceed the acceptable levels. The second solution is to use a treatment technology, which makes it possible to reduce the levels of contaminants to such a degree that the contaminant levels in the resulting product are in line with the applicable regulations in force. This treatment technology covers trimming and excision of ventral part of the fillet and it was used in the present research study.

## Materials and methods

### *Study material*

The research material included 17 Baltic salmon (*Salmo salar*) and 16 sea trout (*Salmo trutta*) individuals. The fishes of those two species were collected in the fisheries located in the 24th and 25th square of ICES (International Council for the Exploration of the Sea), between 2011 and 2013. They were caught by fishing boats and delivered to a laboratory within 24 h (kept on ice). Tab. 1 and 2 show the detailed parameters (weight and length) of the samples studied.

### *Sample preparation*

Fish delivered to the laboratory were, first, weighed, then headed and filleted. Fillets of the right side of every fish were trimmed and analyzed as a whole. Dorsal parts of the left fish side were weighed, their ventral parts (about 25 % of weight) were removed and, next, the prepared samples were analyzed under this study. In addition, 14 samples of ventral parts of the fillets were analyzed, too.

The samples prepared in this way were ca. 60 s homogenized in a mixer (Multi Processor, Zelmer), at a speed of 1300 rpm. The contents of fat and water were determined in the homogenized samples. To determine the PCDD/F and dl-PCB, the samples were freeze-dried in an Alpha 2-4 LSC freeze dryer (Christ, GmbH, Osterode am Harz, Germany). The samples were collected and prepared for chemical analysis by the National Fisheries Research Institute (NMFRI).

### *Analytical methods*

In Eurofins GfA Lab Service GmbH, Hamburg, an accredited laboratory, the following was determined: polychlorinated dibenzo-paradioxins (PCDDs: sum of seven most toxic congeners), polychlorinated dibenzofurans (PCDFs: sum of ten most toxic congeners), dioxin-like PCBs (dl-PCBs: sum of four non-ortho PCB nos. 77, 81, 126, 169 and sum of eight mono-ortho PCB nos. 105, 114, 118, 123, 156, 157, 167, 189). An isotope dilution analysis was applied according to the EPA Method 1613 [18]. The freeze-dried samples were extracted with n-hexane and the cleanup was per-

formed using adsorption chromatography. Prior to the extraction, the samples were spiked with mixtures of the isotope-labeled standards (Wellington Laboratories, Canada). The final extracts were analyzed using a HRGC/HRMS technique. The quantitation limits varied depending on the congener and the sample size. All the dl-PCBs occurred at measurable concentrations, while the highly chlorinated (hepta and octa) congeners of dioxins and furans occurred at the levels below LOQ. The concentrations below the limits of quantitation (LOQ) were equated with LOQ. The contents of PCDD/Fs and dl-PCBs were expressed as TEQ values, which were calculated for every sample by multiplying the individual congener levels, measured in every sample, by the appropriate toxic equivalency factor (TEF). The TEFs used were set by the World Health Organization (WHO) for humans and were calculated with respect to 2,3,7,8-TCDD [23]. The content of fat and water in the samples were determined in NMFRI based on the methodology as outlined in AOAC [1].

#### *Statistical analysis*

The statistical analysis was conducted using a STAT statistical software package (Statistica, Version 7.0); a significance level of  $p = 0.05$  was applied. The Person's correlation analysis was used to analyze the correlation between the fish length and weight and the concentration of contaminants.

### **Results and discussion**

Based both on the results of a long-standing research project, conducted by NMFRI on very many contaminants in fishes caught in the Polish Marine Areas [13, 14, 15] and on the data published by the National Veterinary Institute in Puławy [12], the following was found: in the salmon and sea trout studied, the limits of the acceptable contents of the sum of dioxin amounts and dl-PCBs (PCDD/F were exceeded, i.e.: 3.5 pg WHO-TEQ/g wet weight; sum of PCDD/F and dl-PCB: 6.5 pg WHO-TEQ). However, owing to the aforementioned effect of fish age on the concentration of contaminants, the amount of exceedance reported strongly depends on the characteristic of the samples analyzed.

The current study confirmed that the concentrations of PCDD/F + dl-PCBs in the muscle tissues of wild salmon samples and sea trout analyzed strongly depended on the fish size and the content of fat in the muscle tissue. Tab. 1 and 2 show detailed data on the fat content and contaminant concentrations in different parts of fillets. The linear regression models as presented in Fig. 1 - 4 that describe the relationship between the fish weight or fish length and the content of PCDD/Fs + dl-PCB demonstrate that there is a high, statistically significant ( $p \leq 0.05$ ) correlation ( $0.5 \leq r < 0.7$ ) between those parameters [11].

Table 1. Content of PCDD/Fs + dl-PCBs [pgTEQ-WHO/g], fat, and water in salmon fillets

Tabela 1. Zawartość PCDD/F + dl PCB [pgTEQ-WHO/g], tłuszczu i wody w filetach z łososia

No	Weight Masa [g]	Length Długość [cm]	Whole fillet Cały filet			Dorsal part Część grzbietowa			Ventral part Część brzuszna		
			Water Woda [%]	Fat Tłuszcz [%]	PCDD/F + dl- PCB	Water Woda [%]	Fat Tłuszcz [%]	PCDD/F + dl- PCB	Water Woda [%]	Fat Tłuszcz [%]	PCDD/F + dl- PCB
1	1688	57	72.42	7.15	5.20	73.43	6.73	2.73	70.65	9.16	6.16
2	2515	66	70.31	8.81	6.35	71.58	7.08	5.06	66.71	13.67	9.69
3	2850	64	72.50	4.38	6.94	74.85	3.95	5.15	na	na	na
4	3216	67	68.64	12.00	5.82	68.09	8.82	3.42	70.90	12.69	6.20
5	3545	72	68.52	12.17	7.43	69.59	10.60	7.09	67.21	13.93	8.16
6	3680	75	65.63	13.81	8.66	67.64	11.01	6.70	63.30	16.90	9.06
7	3875	69	65.36	14.76	7.63	66.50	13.82	4.48	63.98	17.26	8.62
8	4044	75	71.88	7.65	8.21	74.04	5.15	6.50	na	na	na
9	6600	79	72.45	6.98	5.34	76.11	4.32	3.11	na	na	na
10	8050	87	65.51	15.51	12.74	68.75	10.9	5.49	63.78	18.94	13.11
11	8625	92	64.78	14.15	17.79	69.03	8.83	8.02	62.77	16.67	19.82
12	8950	90	67.01	11.20	8.96	69.04	9.05	7.22	na	na	na
13	9090	94	67.32	12.19	7.51	69.58	8.12	4.68	66.20	19.23	10.34
14	9500	90	61.74	19.76	13.62	65.40	15.51	6.60	58.72	23.99	15.59
15	10245	101	67.09	13.49	11.78	70.33	9.10	7.80	61.13	20.84	na
16	10320	96	69.64	10.36	10.45	70.89	8.12	6.90	na	na	na
17	12180	99	61.72	17.27	14.53	63.57	14.81	10.71	55.16	26.59	15.62

Explanatory notes / objaśnienia:

na – not analyzed / nie analizowano

The developed models indicate that:

- as regards the whole trimmed fillet of wild salmon, lower levels of PCDD/F + dl-PCB were predicted only in those characterized by a weight below 2579 g and a length below 65 cm (Fig. 1) compared to the acceptable amount of those compounds as established by the Commission Regulation (EU) No. 1259/2011 [4];
- as regards the whole trimmed fillet of sea trout, lower levels of PCDD/F + dl-PCB were predicted only in those characterized by a weight below 3376 g and a length below 65 cm (Fig. 2) in comparison to the acceptable amount of those compounds as established by the Commission Regulation (EU) No. 1259/2011 [4].

Table 2. Content of PCDD/Fs + dl-PCBs [pgTEQ-WHO/g], fat, and water in trout filets

Tabela 2. Zawartość PCDD/F + dl PCB [pgTEQ-WHO/g], tłuszczu i wody w filetach z troci

No	Weight Masa [g]	Length Długość [cm]	Whole fillet Cały filet			Dorsal part Część grzbietowa			Ventral part Część brzuszna		
			Water Woda [%]	Fat Tłuszcz [%]	PCDD/F + dl- PCB	Water Woda [%]	Fat Tłuszcz [%]	PCDD/F + dl- PCB	Water Woda [%]	Fat Tłuszcz [%]	PCDD/F + dl- PCB
1	1580	51	74.03	5.00	4.35	74.98	4.20	3.57	na	na	na
2	1750	52	75.59	4.31	4.92	76.04	3.64	3.52	73.24	6.48	6.09
3	1753	54	76.93	2.43	4.22	77.82	2.12	3.46	na	na	na
4	1896	50	71.22	7.69	3.86	73.04	5.88	3.17	na	na	na
5	1980	58	78.18	2.81	4.49	78.09	2.62	3.68	na	na	na
6	2003	56	76.64	2.85	5.66	77.96	2.12	4.71	75.68	3.44	7.26
7	2100	57	73.50	4.92	5.03	74.97	3.96	4.13	na	na	na
8	2182	58	74.17	5.10	5.77	75.27	4.23	4.73	na	na	na
9	2380	60	78.43	3.05	4.04	79.78	2.84	3.32	na	na	na
10	2400	58	71.64	6.44	6.20	72.89	5.24	5.08	na	na	na
11	2620	60	67.42	10.68	5.90	69.68	8.34	4.84	na	na	na
12	2750	61	72.50	5.79	6.69	73.89	4.92	6.65	71.88	6.64	7.67
13	3200	64	69.37	8.16	6.79	70.79	6.67	5.43	na	na	na
14	3247	63	75.48	3.14	7.34	75.99	3.06	5.87	na	na	na
15	3638	68	72.19	3.86	3.62	72.88	3.04	2.97	na	na	na
16	4920	75	67.52	11.17	9.33	69.02	9.25	7.46	na	na	na

Explanatory notes / Objasnienia:

na – not analyzed / nie analizowano

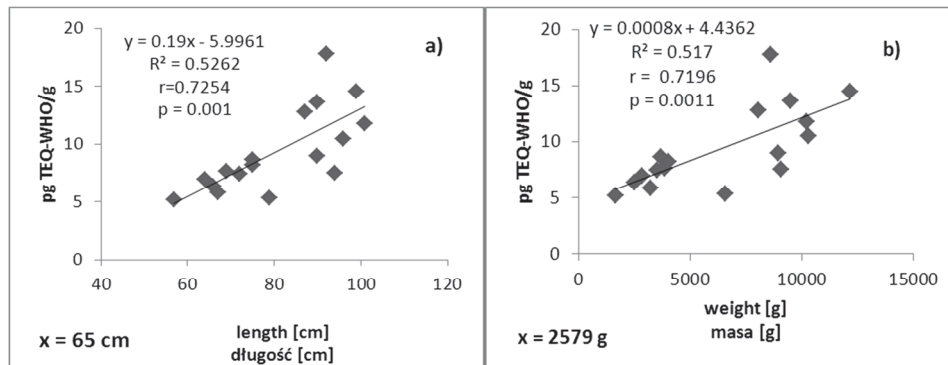


Fig. 1. Correlation between content of PCDD/F + dl-PCB in whole salmon fillet and length (a) and weight (b) of fish individual

Rys. 1. Zależność między zawartością PCDD/F + dl-PCB w całym filecie z łososia a długością osobnika (a) i jego masą (b)

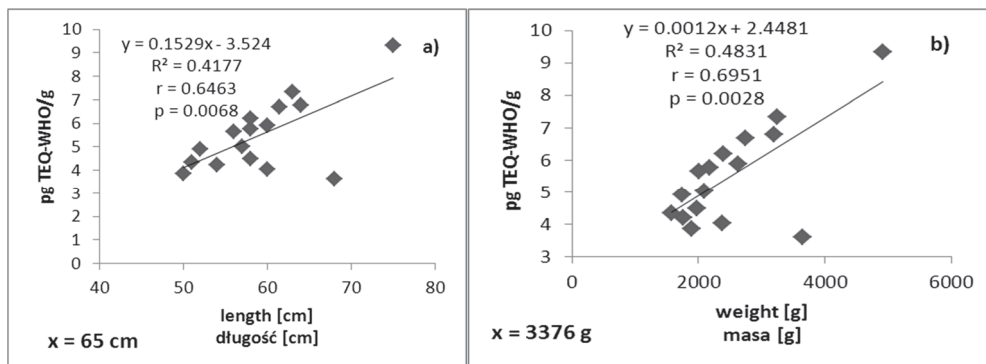


Fig. 2. Correlation between content of PCDD/F + dl-PCB in whole sea trout fillet and length (a) and (b) weight (b) of fish individual

Rys. 2. Zależność między zawartością PCDD/F + dl-PCB w całym filecie z troci a długością osobnika (a) oraz jego masą (b)

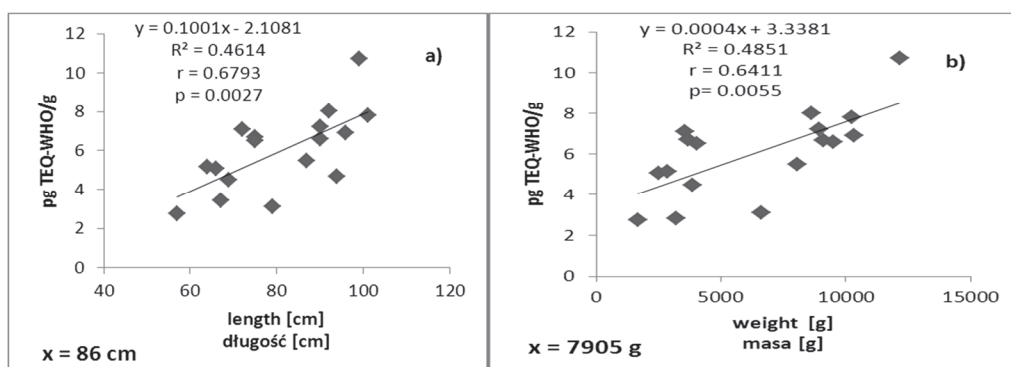


Fig. 3. Correlation between content of PCDD/F + dl-PCB in dorsal part of salmon fillet (after trimming and removal of ventral part) and length (a) and weight (b) of fish

Rys. 3. Zależność między zawartością PCDD/F + dl-PCB w części grzbietowej fileta z łososia (po trymowaniu i usunięciu części brzusznej) a długością osobnika (a) oraz jego masą (b)

Considering that the distribution of fat in the fillet is not homogeneous [7, 8] and the dioxin and other POPs accumulate predominantly in fat, it was assumed that after the removal of the most oily ventral part of the fillet, the remaining dorsal part should contain much lower concentrations of those contaminants than the whole fillet. As expected, the conducted studies proved that the content of PCDD/F and dl-PCBs in the dorsal part of the fillet was lower compared to the whole fillet and that the ventral part of the fish was the most contaminated one (Tab. 1 and 2).

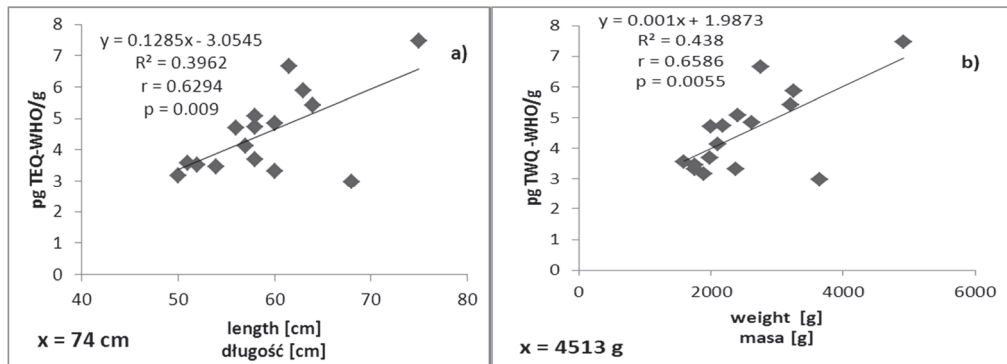


Fig. 4. Correlation between content of PCDD/F + dl-PCB in dorsal part of sea trout fillet (after trimming and removal of ventral part) and length (a) and weight (b) of fish

Rys. 4. Zależność między zawartością PCDD/F + dl-PCB w części grzbietowej fileta z troci a długością osobnika (a) oraz jego masą (b)

In the dorsal part of salmon individuals characterized by a weight of 7900 g and a length up to 86 cm (Fig. 3) as well as in the dorsal part of sea trout fish weighing up to 4500 g and being up to 74 cm long (Fig. 4), lower levels of PCDD/F + dl-PCB should be expected in comparison to the levels as established by the Commission Regulation (EU) No. 1259/2011 [4].

Compared to the whole fillet, the reduction in the content of dioxins and dl PCB in the dorsal part of salmon fillet ranged from 4.6 % to 56.9 % and as regards the sea trout: from 0.6 % to 28.5 %. The lowest reduction in the content of dioxins and dl-PCB in sea trout results probably from smaller differences in the content of fat among different parts of fillet.

The data on contaminants in wild salmon can eventuate in further restrictions for salmon fishing and bring negative economic consequences for coastal fisheries. However, our study indicates that the use of the procedure as suggested in the paper does enable us to get products made of wild salmon and sea trout that meet the requirements of the Commission Regulation (EU) No. 1259/2011 [4]. On the other hand, it was also shown that the majority of samples of ventral parts of fillets contained the amounts of contaminants that exceeded the EU limits. Thus, a question arises how to deal with the removed ventral parts of fillets.

The removed ventral parts of fillets could be treated as animal by-products not intended for human consumption, classified as Category 1 material, which is handled in the manner described in Article 12 of Regulation (EC) No. 1069/2009 of the European Parliament and of Council Regulation of 21<sup>st</sup> October 2009 *laying down health rules as regards animal by-products not intended for human consumption and repealing Regulation (EC) No. 1774/2002 (Animal by-products Regulation)* (OJ EU L 300 of



14.11.2009). Therefore, ventral parts of fillets could be used, for example, as feed for fur bearing animals, in accordance with the articles of the above-mentioned Regulation. In Poland, there are many fur farms. Such approach allows for the partial compensation of economic losses. Another possibility is to obtain oil and apply it as a raw material for the production of animal feed provided that this raw material will meet the requirements concerning acceptable levels of dioxins and dl-PCBs set out in Annex I to Directive 2002/32/EC [5]. However, it should be noted that there is a possibility of reducing the amount of dioxins (PCDD/Fs) in fish oil using activated carbon [20].

In the case of different types of fish oil, with the use of activated carbon, we achieved a reduction in the levels of PCDD/F within the range from more than 60 % to over 90 % [20].

However, it is important to note that the wild salmon fishing in the Polish Marine Areas is very low (about 32.6 tons in 2013 and 18.2 tons in 2014), while the annual production of smoked salmon in Poland is above 50,000 tones [NMFRI data]. The results received under that work refer to wild salmon caught in the Baltic Sea and not to farmed salmon. According to the literature data, the average content of dioxins and dl-PCBs in smoked farmed salmon, present in the Polish market, was approximately 2.5 pgWHO-TEQ<sub>1998</sub>/g of wet weight [19]. The levels of contaminants in farmed salmon, fed with fodder that meets legal requirements regarding contaminant levels, are expected to be low.

## Conclusions

1. Salmon and sea trout are oily fishes and contain a lot of valuable compounds characterized by pro-health properties; however, the content of PCDD/F and dl-PCB therein exceeds the levels as required by law in force.
2. Pre-treatment involving trimming and removal of ventral part of fillet (about 25 % of weight) allow the content of contaminants in the final product to be significantly reduced.
3. The removed ventral part can be rationally used, for example, as a fodder for fur bearing animals.
4. The monitoring of contaminants in fishes, focused on consumer safety, should involve samples derived from the market, which actually reach consumers. When the whole fish constitutes a sample, the procedure of preparing samples in a laboratory has a strong effect on the final result of the assay. It is important, which part of the fillet will constitute a test sample.

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**WPLYW OBRÓBKİ WSTĘPNEJ NA ZAWARTOŚĆ PCDD/F + DL-PCB W TKANCE  
MIĘŚNIOWY ŁOSOSIA ATLANTYCKIEGO (*SALMO SALAR*) I TROCI WĘDROWNEJ  
(*SALMO TRUTTA*)**

S u m m a r y

Oceniono wpływ obróbki wstępnej na zawartość dioksyn (PCDD/F) i dioksynopodobnych polichlorowanych bifenyli (dl-PCB) w filetach z łososia atlantyckiego (*Salmo salar*) i troci wędrownej (*Salmo trutta*), poławianych w Bałtyku. Badaniami objęto ryby o zróżnicowanej wielkości.

Poziomy zanieczyszczeń poniżej wartości dopuszczalnych, określonych w Rozporządzeniu UE nr 1259/2011 stwierdzono w produktach po obróbce wstępnej, pochodzących z osobników łososia o masie do 7900 g i długości do 86 cm oraz troci wędrownej o masie do 4500 g i długości do 74 cm. W przypadku trzymowanych filetów (zawierających część brzuszna) poziomy dioksyn i dl-PCB nie były przekroczone w mięsie łososia bałtyckiego o masie do 2579 g i długości do 65 cm oraz troci wędrownej o masie do 3376 g i długości do 65 cm.

Stwierdzono, że obróbka wstępna polegająca na trzymowaniu i odrzuceniu części brzusznej fileta (ok. 25 % masy) wpłynęła na zmniejszenie zawartości zanieczyszczeń w końcowym produkcie. Wyniki badań wskazują, że monitoring zanieczyszczeń w rybach w zakresie bezpieczeństwa zdrowotnego powinien być prowadzony na próbach ryb bezpośrednio dostępnych dla konsumentów w sieci handlowej. W przypadku

kiedy próbę stanowi cała ryba, sposób przygotowania próbki do badań w laboratorium ma duży wpływ na końcowy wynik oznaczenia.

**Słowa kluczowe:** łosoś, troć, PCDDFa, PCBs, obróbka wstępna ☒

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