Removal of Dioxins and PCB from Fish Oil by Activated Carbon and Its Influence on the Nutritional Quality of the Oil

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ABSTRACT: Fish oils are well-known sources of nutritionally valuable components such as the n-3 FA EPA and DHA as well as the fat-soluble vitamins A, D, and E. However, some fish oils can be contaminated with considerable amounts of dioxins and dioxin-like PCB. The most important challenge during fish oil refining is to remove these contaminants without altering the levels of nutritionally valuable compounds and the oxidative status and stability of the oil. Treatment with an apolar adsorbent, e.g., activated carbon (AC), seems to be the most efficient process today. Very little information about the adsorption of different dioxin and PCB congeners is available. Four grades of AC were evaluated for their efficiency in removing these compounds. In addition, the effects of the treatment on the nutritional and oxidative quality of the oil were evaluated. After treatment of contaminated cod liver oil [5.4 ppt toxic equivalents (TEQ) polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F), 18.1 ppt TEQ dioxin-like PCB] with 0.5% AC, almost all PCDD/F and up to 80% of the dioxin-like PCB could be removed. AC showed low affinity for mono-*ortho* PCB (<30% removal), which could be explained by their noncoplanar structure. Removal efficiencies were dependent on the grade and percentage of AC used. The treatment of contaminated cod liver oil caused no important effects on oil quality or FA composition in the conditions tested.

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KEY WORDS: Activated carbon, adsorption, dioxin-like PCB, dioxins, fish oil, GC-HRMS, oil quality, omega-3 fatty acids.

Over the last decades, the growing consumer awareness of healthy nutrition and a healthy lifestyle has caused an increased interest in the nutritional aspects of food oils and fats. High-quality food oils should have a balanced FA composition and should contain high levels of valuable minor compounds such as vitamins and natural antioxidants. Levels of fat isomerization and degradation products such as *trans*-FA and fat oxidation components should be low. In addition, the level of contaminants, such as PAH, pesticides, dioxins, and PCB, should be as low as possible. According to the European Scientific Committee on Food, benzo(a)pyrene can be used as a marker for the occurrence and effect of carcinogenic PAH in food. Recently, a maximum level of 2.0 ppb benzo(a)pyrene was established in oils and fats for human consumption (1). Current European Union (EU) and Member State legislation on pesticides, which does not yet include Maximum Residue Levels for fish oils, is being replaced throughout 2005 (2). The EU regulation on dioxins and PCB is discussed further in this article.

Fish oils are an excellent source of n-3 FA, mainly EPA and DHA. Several epidemiological studies have shown a correlation between the intake of these compounds and a reduced risk of arterial thrombosis, cardiovascular diseases, multiple sclerosis, and autoimmune and inflammatory disorders such as psoriasis, asthma, and type 1 diabetes (3–8). The n-3 FA are claimed to be essential for normal growth and development and to play an important role in the prevention and treatment of hypertension, cancer, and several inflammatory and autoimmune disorders (9). Today, about 2% of the total fats and oils produced in the world are fish oils. They are mainly incorporated into n-3-enriched margarines (70%) and animal feeds (15%), but some are also used for the production of nutraceuticals (e.g., EPA- and DHA-enriched products) (10). Because of their health-protective and health-promoting effects, the market share of foods enriched in n-3 FA is becoming more important. Therefore, the marine oil market is experiencing a revival, and commercial interest in using fish oils for food and feed supplementation is growing.

Two main issues should be taken into account when considering marine oils as a dietary source of n-3 FA: (i) their low oxidative stability and (ii) their contamination with persistent organic pollutants (POP) such as dioxins and PCB. The term "dioxins" is a general name for polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) (hereafter, PCDD/F). Intake of dioxins can cause a broad range of toxic effects in the human body, including teratogenicity and carcinogenicity (11). In the PCB fraction, the non-*ortho* (coplanar) and mono-*ortho* chlorine-substituted congeners are considered to be the most important compounds from a toxicological point of view. They can cause the same adverse effects as

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*To whom the correspondence should be addressed at De Smet Technologies and Services S.A., Corporate Village, Da Vincilaan 2, B-1935 Zaventem, Belgium, e-mail: MAJ@desmetgroup.com (J. Maes); and Laboratory of Food Chemistry–Department of Food Safety and Food Quality, Ghent University, Coupure Links 653, B-9000 Gent, Belgium, e-mail: Bruno.DeMeulenaer@UGent.be (B. De Meulenaer) dioxins and are therefore generally referred to as "dioxin-like" PCB. Levels of dioxins and dioxin-like PCB are expressed in terms of "toxic equivalents" (TEQ), a value that represents the total "2,3,7,8-tetrachlorodibenzo(*p*)dioxin (2,3,7,8-TCDD)-equivalent toxic potency" of a mixture of dioxin-like compounds.

At present, a maximum PCDD/F level for food and feed purposes of, respectively, 2 and 6 ppt TEQ in fish oil has been set by the European Commission (12). Legislation regarding maximally accepted dioxin-like PCB levels in food oils is expected in the near future. The POP expert team of the European Commission recently reported high dioxin-like PCB levels in European fish oil samples, ranging between 10 and 74 ppt TEQ (13). Therefore, fish and the derived products, including fish oils, are the main targets in which to reduce the contamination by dioxin-like compounds.

One of the main challenges in fish oil refining is to remove these dioxins and dioxin-like PCB without losing the valuable minor components and without altering the oxidative status and stability of the oil. Contaminants can generally be removed by high-temperature deodorization or adsorption on apolar adsorbents such as activated carbon (AC). The latter technique is usually preferred because of the mild processing conditions applied during the process. AC has been shown to be very effective in removing dioxins and other dioxin-like compounds from oils (14). However, very little information about the adsorption of the different dioxin and PCB congeners is available.

This paper focuses on the use of AC powders to remove dioxins and dioxin-like PCB from cod liver oil. Various AC grades are evaluated for their efficiency in removing dioxins and dioxin-like PCB and to determine the resulting effect on the overall quality of the decontaminated oil.

EXPERIMENTAL PROCEDURES

Raw material. Crude cod liver oil of Scandinavian origin was used as a feedstock for the adsorption trials. The oil was stored in the dark under a nitrogen atmosphere and at refrigeration temperature (4°C) to minimize quality deterioration. The AC grades used were obtained from NORIT (Amersfoort, The Netherlands) and were coded as follows: Norit SA4 PAH HF "Classic": AC-A; Norit Exp 21B "New" (since April 2004): AC-B; Norit Exp 21A: AC-C; Norit Exp 21F: AC-D.

AC treatment. The crude oil (300 g) was preheated to 70°C and added under reduced pressure (50 mbar) to a rotary evaporator containing an appropriate amount of AC powder (0.1 or 0.5% w/w). The oil was mixed at 85 rpm for 30 min at 70°C, followed by filtration over a Büchner filter with Whatman No. 1 filter paper. Preliminary experiments (results not shown) indicated complete equilibrium after a 30-min reaction time. The temperature of 70°C was chosen as the best compromise between increased viscosity (hence better adsorption) and lower oil stability at higher temperatures. Treated oil samples were stored in the dark at 4°C under a nitrogen atmosphere.

Analytical methods. (i) Physical and chemical properties. The crude and AC-treated cod liver oils were characterized using following AOCS Official Methods and Recommended Practices (15): FFA content (Ca 5a-40), PV (Cd 8-53), *p*-anisidine value (Cd 18-90), oxidative stability index (Cd 12b-92), and Lovibond color, measured in a Tintometer Type E instrument (The Tintometer Ltd., Salisbury, United Kingdom) (Cc 13b-45).

(ii) FA profile. Preparation of FAME was done according to AOCS Official Method Ce 1b-89 (15). The FAME were separated on a Varian 3380 gas chromatograph equipped with an FID and a BPX 70 capillary column (60 m × 0.22 mm i.d.; Supelco, Bellefonte, PA). The column temperature was held for 1 min at 140°C, elevated at 2°C/min to 230°C, and held for 4 min at 230°C. The injector and detector temperatures were set at 250°C. Helium was used as a carrier gas, flowing at 1 mL/min. The flow rates of hydrogen and air were, respectively, 30 and 300 mL/min. The injection volume was 1 μL. Peak identification was based on the Nu-Check-Prep commercial reference standard GLC68D (Nu-Check-Prep Inc., Elysian, MN). Quantitative information was obtained by internal normalization, in which the sum of the areas of the peaks in the chromatogram, except that of the solvent, was set at 100%. The content of a constituent was calculated by determining the area of the corresponding peak as a percentage of the sum of the area of all the peaks. For each calculation, a correction factor, obtained from the standard solution, was included.

(iii) Elemental analysis. Elements (P, Ca, Fe, and Mg) were determined by inductive coupled plasma (ICP) spectrometry according to AOCS Official Method Ca 20-99 (15) using an ICP Atomscan 25 (Thermo Jarell Ash, Franklin, MA).

(iv) Retinol content. Saponification and extraction were carried out basically according to the procedure described in IUPAC Method 2.411 (16). The extract was evaporated to dryness in a rotary evaporator, redissolved in 5 mL methanol, and diluted 10 times. For separation and detection, an HPLC system with a single supply pump (1.2 mL/min), 10 μL loop injection system, and Varian LiChrosorb 10 RP 18 HPLC column (Varian, Palo Alto, CA) was used in combination with a UV detector at 325 nm, recorder, and integrator. Quantification was done by means of an external standard curve.

(v) Contaminant analysis. GC in combination with high-resolution MS (GC-HRMS) was used for the detection of dioxins and dioxin-like PCB in the oil samples. The analyses were carried out at the University of Liège. A detailed description of the GC-HRMS analysis is given by Focant *et al.* (17). Calculations were done using the World Health Organization toxic equivalency factor (TEF) values proposed by Van den Berg *et al.* (18).

RESULTS AND DISCUSSION

Efficiency of the AC treatment. A cod liver oil from the Baltic Seas was selected as the feedstock for the adsorption experiments using the four types of AC powder at two concentration levels, 0.1 and 0.5%.

All adsorption tests were single trials. The results shown in the tables are averages of duplicate analyses. Results for the

TABLE 1
Levels of PCDD/F (in ppt TEQ) of Feedstock and Activated Carbon-Treated Samples[a]

	Feedstock	Treated samples							
AC-grade	—	AC-A		AC-B		AC-C		AC-D	
AC-conc. (%)	—	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5
2,3,7,8-TCDD	0.56	<LOQ[b]	ND[c]	<LOQ	ND	ND	ND	<LOQ	ND
1,2,3,7,8-PeCDD	0.74	ND	ND	<LOQ	ND	ND	ND	<LOQ	ND
1,2,3,4,7,8-HxCDD	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,6,7,8-HxCDD	0.14	ND	<LOQ	0.03	<LOQ	<LOQ	ND	<LOQ	ND
1,2,3,7,8,9-HxCDD	0.03	ND	ND	ND	ND	ND	ND	ND	ND
1,2,3,4,6,7,8-HpCDD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	ND	<LOQ	<LOQ
OCDD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2,3,7,8-TCDF	2.00	0.47	0.07	0.47	0.09	0.22	<LOQ	0.19	<LOQ
1,2,3,7,8-PeCDF	0.18	ND	<LOQ	0.02	<LOQ	0.01	<LOQ	0.01	ND
2,3,4,7,8-PeCDF	1.47	0.30	0.10	0.37	ND	0.20	ND	0.19	<LOQ
1,2,3,4,7,8-HxCDF	0.08	ND	<LOQ	ND	ND	<LOQ	ND	<LOQ	<LOQ
1,2,3,6,7,8-HxCDF	0.09	ND	<LOQ	ND	ND	<LOQ	ND	<LOQ	ND
1,2,3,7,8,9-HxCDF	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4,6,7,8-HxCDF	0.11	0.04	<LOQ	ND	<LOQ	<LOQ	ND	<LOQ	ND
1,2,3,4,6,7,8-HpCDF	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
1,2,3,4,7,8,9-HpCDF	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND
OCDF	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	ND	<LOQ	<LOQ
Total PCDD/F	5.41	0.80	0.17	0.89	0.09	0.43	0.00	0.39	0.00
Total PCDD/F removal (%)	—	85	97	83	98	92	100	93	100

[a] Activated carbon (AC) samples were as follows: AC-A, Norit SA4 PAH HF "Classic"; AC-B, Norit Exp 21B "New" (since April 2004); AC-C, Norit Exp 21A; AC-D, Norit Exp 21F (all obtained from NORIT, Amersfoort, The Netherlands). PCDD/F, polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF); TEQ, toxic equivalent(s).
[b] LOQ, level of quantification.
[c] ND, not detectable.

contaminant analyses are shown in Tables 1 and 2. The feedstock was contaminated with 5.41 ppt TEQ PCDD/F, 14.32 ppt TEQ non-*ortho* PCB, and 3.74 ppt TEQ mono-*ortho* PCB. In the non-*ortho* PCB fraction, PCB 126 was responsible for 98% of the toxicity. PCB 118 contributed about half of the mono-*ortho* PCB TEQ. The maximum level set by the European Commission (12) for food purposes—2 ppt TEQ PCDD/F in fish oil—was clearly exceeded.

Decontamination efficiencies were strongly dependent on the AC load and grade. Almost full removal of dioxins could be achieved when 0.5% of AC was used. Even with 0.1% of AC, more than 80% of the total PCDD/F fraction could be removed from the oil. Most of the residual dioxin content was caused by 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF, which were also present in the feedstock at the most elevated concentrations.

Generally, the removal of dioxins was more efficient than the removal of dioxin-like PCB. For the total dioxin-like PCB, removal remained restricted to 20–33% with 0.1% AC and to 58–80% with 0.5% AC. The lower PCB removal was mainly caused by a low adsorption of the mono-*ortho* PCB fraction. Maximally, a 28% decrease in mono-*ortho* PCB-TEQ could be achieved (0.5% AC-C). The low adsorption rate of the mono-*ortho* PCB can be explained by their geometrical structure. AC has the highest affinity for compounds with a perfect coplanar structure, such as dioxins. In the PCB fraction, the compounds substituted on both *para*- and at least two *meta*-positions, but not on the *ortho*-positions, are approximately isostereomers of 2,3,7,8-TCDD (11). This similar structure not only causes the strong dioxin-like toxicity effects of these compounds but also causes them to be easily adsorbed on AC. The geometrical structure of the mono-*ortho*-substituted PCB molecules differs significantly more from that of dioxins. Because of steric hindrance by the chlorine atom at the *ortho*-position, no perfect coplanar structure is obtained. This partially explains the lower TEF values of these congeners (18). At the same time, noncoplanar compounds will adsorb less easily on the AC surface, explaining the difficult removal of the mono-*ortho* PCB fraction in this study.

Influence of contaminant removal on oil quality. The results for selected oil quality parameters are summarized in Table 3. Levels of the n-3 FA EPA and DHA remained unaffected. No significant changes in FFA content and oxidative quality were noted. The oxidative stability, however, seemed to decrease for some of the AC types. Also, a concentration-dependent decrease in color parameters could be noticed. For three samples—one feedstock and two samples treated with, respectively, 0.1 and 0.5% AC-D—a linear relation ($R^2 = 0.9414$) between the retinol content and the total Lovibond color (Y + 10R) could be established. In this way, discoloration was linked to adsorption of vitamin A on the AC.

TABLE 2
Levels of Non-*ortho* and Mono-*ortho* PCB (in ppt TEQ/g) of Feedstock and Activated Carbon-Treated Samples

	Feedstock	Treated samples							
AC grade	—	AC-A		AC-B		AC-C		AC-D	
AC conc. (%)	—	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5
PCB 77	0.03	0.02	0.01	0.03	0.01	0.02	<LOQ[a]	0.02	0.00
PCB 81	0.00	0.00	0.00	0.00	0.00	0.00	<LOQ	0.00	0.00
PCB 126	14.05	10.65	3.34	10.64	3.98	8.39	0.91	8.77	1.28
PCB 169	0.23	0.17	0.07	0.16	0.07	0.13	0.02	0.16	0.03
Total non-*ortho* PCB	14.32	10.84	3.42	10.83	4.06	8.54	0.93	8.94	1.31
Non-*ortho* PCB removal (%)	—	24	76	24	72	40	93	38	91
PCB 105	0.63	0.59	0.53	0.58	0.57	0.64	0.48	0.59	0.47
PCB 114	0.20	0.11	0.16	0.13	0.13	0.20	0.20	0.21	0.10
PCB 118	1.90	2.04	1.79	1.91	1.75	1.78	1.28	1.62	1.41
PCB 123	0.03	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.01
PCB 156	0.70	0.68	0.51	0.59	0.50	0.60	0.55	0.75	0.58
PCB 157	0.25	0.17	0.12	0.10	ND[b]	0.25	0.15	0.20	0.21
PCB 167	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
PCB 189	0.02	0.01	0.01	0.01	0.01	ND	ND	0.02	0.01
Total mono-*ortho* PCB	3.74	3.62	3.14	3.33	2.99	3.50	2.69	3.42	2.80
Mono-*ortho* PCB removal (%)	—	3	16	11	20	6	28	9	25
Total dioxin-like PCB	18.06	14.46	6.56	14.16	7.05	12.04	3.62	12.36	4.11
Total dioxin-like PCB removal	—	20	64	22	61	33	80	32	77

[a] LOQ, level of quantification. For a detailed description of the PCB identified by the GC-high-resolution MS analysis, see Focant *et al.* (17).
[b] ND, not detectable. For other abbreviations see Table 1.

From the experiments, we concluded that AC is an efficient adsorbent for the removal of dioxins from contaminated fish oil. AC treatment of fish oil is applied industrially. Depending on the initial dioxin content, the activated carbon dose can be adjusted to reach the desired reduction in TEQ. The main limitation of AC is the very poor adsorption of (dioxin-like) PCB. The first indicative trials (data not shown) have demonstrated that these components are better removed by stripping at optimized process conditions. For the complete decontamination of fish oil, a combined AC treatment/stripping therefore seems to be the best process option.

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TABLE 3
Selected Quality Parameters of Feedstock and Activated Carbon-Treated Samples

	Feedstock	Treated samples							
AC-grade	—	AC-A		AC-B		AC-C		AC-D	
AC-conc. (%)	—	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5
EPA (%)	9.8	9.4	9.4	9.6	9.8	9.7	9.7	9.7	9.8
DHA (%)	11.4	11.5	11.6	11.6	11.3	11.1	11.3	11.1	11.7
FFA (% oleic)	0.17	0.19	0.18	0.19	0.19	0.17	0.16	0.17	0.16
PV (meq O_2/kg)	13.6	13.2	14.4	13.2	15.2	13.0	13.3	14.2	14.5
AV	18.0	16.6	15.2	17.4	17.8	17.4	17.3	16.5	17.0
OSI (h) at 80°C	5.8	5.3	4.6	4.7	4.7	5.9	5.5	5.9	5.7
Elemental analysis by ICP									
Ca (ppm)	0.55	0.36	0.34	0.21	0.15	0.41	0.50	0.26	0.19
P (ppm)	0.18	0.37	0.18	ND[a]	ND	ND	0.22	ND	ND
Fe (ppm)	0.06	0.01	0.01	ND	ND	ND	ND	0.05	0.06
Mg (ppm)	1.06	0.56	0.74	0.27	0.34	0.88	0.62	0.34	0.30
Retinol (UI/g)	1430	NA[b]	NA	NA	NA	NA	NA	1301	1188
Lovibond color at 1″									
Yellow	50	50	30	50	30	50	15	40	20
Red	5.5	5.0	4.1	4.5	3.6	4.0	1.7	4.1	2.0

[a] ND, not detectable.
[b] NA, not analyzed; AV, anisidine value; OSI, oxidative stability index; ICP, inductive coupled plasma spectrometry; for other abbreviations see Table 1.

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