

Experimental Study on the Removal of Dioxins and Coplanar Polychlorinated Biphenyls (PCBs) from Fish Oil

AYATO KAWASHIMA,* RYOUJI IWAKIRI, AND KATSUHISA HONDA

Environmental Science for Industry, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama-shi, Ehime 790-8566, Japan

Recently, it has been found that fish oils contain a high proportion of contaminants, namely, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (cPCBs). In this study, the removal of contaminants from fish oil by supercritical CO₂ extraction (SCE) and by using adsorbents (0.13 wt % of oil) was investigated. Dioxins and cPCBs were extracted from fish oil by SCE at a temperature of 60 °C and a pressure of 28 MPa, and the removal efficiencies for PCDDs and PCDFs were in the range of 15–90% and those for cPCBs were in the range of 70–90%. However, 40% of the oil was extracted simultaneously with contaminants. On the adsorbent treatment, activated carbon showed high efficiency, and the removal efficiencies were >90% for PCDDs and PCDFs, but below 30% for cPCBs. A combination of both of these methods is more effective, and almost 100% of the total toxicity equivalence quantity value could be reduced.

KEYWORDS: Fish oil; dioxins; supercritical CO₂; extraction; activated carbon

INTRODUCTION

A global increase in contamination due to polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (cPCBs), which are lipophilic, persistent, bioaccumulative, and toxic, has caused grave concerns due to their adverse effects on the environment (1–6). For humans, dietary intakes are the primary intake sources of these contaminants: the main sources are fish, seafood, oil, and fat (7–10). In particular, it was recently revealed that the concentrations of these contaminants are significantly greater in farmed fish as compared to wild fish (11–15). This is due to the contamination of feedstuffs for farmed fish, which comprise fish oil and meal. Because fish oil is a unique source of highly unsaturated fatty acids, it is widely used not only as foodstuffs but also as food products and dietary supplements. Therefore, the research and development regarding the technology for the removal of contaminants from fish oil assume considerable importance for reducing health risks to humans.

Fish oils are usually refined by neutralization, absorption, and distillation processes. However, in many cases, the levels of contaminants following these refining processes are not adequately low for human consumption; furthermore, it is known that the removal of cPCBs from fish oil, particularly mono-*ortho* cPCBs, is difficult (16–18). Supercritical CO₂ extraction (SCE) is applied to a wide range of processes such as selective extraction of valuable resources from natural products (19–21), separation of contaminants (22–25), and so on (26–28)

due to its extraction selectivity, low critical point, nonflammability, nontoxicity, and noncorrosive characteristics. However, the removal of PCDDs, PCDFs, and cPCBs from fish oil by SCE has been attempted a few times (29, 30). In our previous research, we demonstrated the optimization of SCE conditions for the removal of PCDDs, PCDFs, and cPCBs from fish oil, and the determined optimal conditions were a temperature of 60 °C and a pressure of 28 MPa (30). We also described the relationship between molecular weights and extraction efficiencies. However, the extraction property on SCE was not well established and the removal ratios of contaminant were not sufficient.

In this study, we examine the extraction property of SCE in the removal of PCDDs, PCDFs, and cPCBs from fish oil. As the model fish oil, menhaden-oil-added standard solutions of PCDDs, PCDFs, and cPCBs were used. Next, adsorptive activities of four adsorbents such as activated carbon, carboxen-1000, carboxen-1012, and carbon black were investigated, and the property of contaminant removal of this method was reported. Then, a combination of both of these methods, which is expected to be a more effective removal process, is also reported.

MATERIALS AND METHODS

Samples. In these experiments, fish oil spiked with standard solutions of PCDD/DFs and cPCBs was used as the fish oil sample to improve the measurement accuracy. This is because commercially available fish oils have variations in the concentrations of PCDD/DFs and cPCBs. The sample fish oil was prepared by adding 100 μL of hexane standard solution containing 10 ng of tetra- to penta-CDD/DFs, 20 ng of hexa-

* Corresponding author (telephone +81-089-946-9970; fax +81-089-946-9980; e-mail a-kawa@agr.ehime-u.ac.jp).

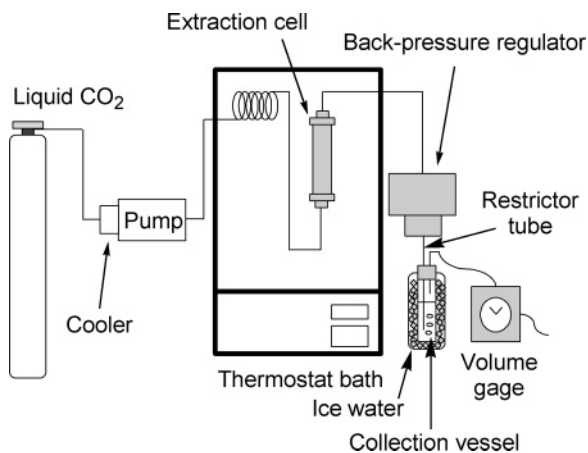


Figure 1. Schematic diagram of supercritical fluid extraction apparatus.

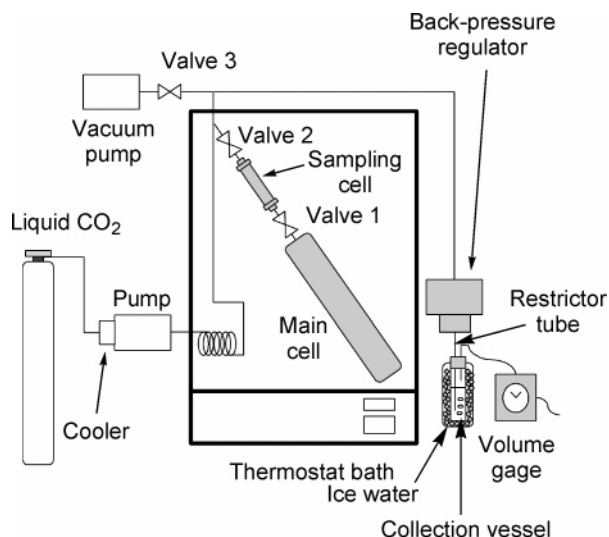


Figure 2. Schematic diagram of the apparatus for partition coefficient measurement.

to hepta-CDD/DFs, 50 ng of octa-CDD/DFs, and 20 ng of each congener of cPCBs to 10 g of menhaden oil (Sigma, St. Louis, MO).

Supercritical Fluid Extraction. A schematic diagram of the supercritical fluid extraction apparatus is shown in Figure 1. Approximately 2.5 g of the fish oil sample was added to the extraction cell (200 mm × 0.5 in. o.d., SUS316). The extraction cell was sealed and attached to the CO₂ delivery pump (SCF-Get; JASCO International Co., Ltd.) and back-pressure regulator (SCF-Bpg; JASCO International Co., Ltd.) with a restrictor tube (1/16 SUS). Supercritical CO₂ dissolving dioxins are exhausted from the back-pressure regulator, and the pressure was reduced. The flow from the restrictor tube was trapped in a collection vessel with 100 mL of hexane, which was cooled in an ice-water jacket. The CO₂ volume was measured by using a dry-gas test meter (DC-1C; Shinagawa Co., Ltd.).

The details of the extraction process are as follows. The extraction cell with the fish oil sample was heated in the thermostated bath up to 60 °C; then, CO₂ was pumped into the cell. At 28 MPa, supercritical CO₂ was flowed at 2 mL/min to the predetermined total CO₂ flow volume at standard temperature and pressure (STP). The extracted sample was collected in a recovery flask with hexane and then analyzed.

The schematic diagram of the apparatus for partition coefficient measurement is shown in Figure 2. The volumes of the main cell (SUS 316) and sampling cell (SUS 316, 0.25 in. o.d.) were 300 and 6.5 mL, respectively.

Approximately 30 g of fish oil and 230 g of CO₂ was introduced into the main cell and valve 1 was closed. The cells were heated at 60 °C in the thermostated bath and the pressure increased to 28 MPa. The cell was allowed to stand for 24 h. Then, valves 2 and 3 were opened, and the sampling cell was first evacuated with a vacuum pump. Valves

2 and 3 were closed, and then valve 1 between the sampling cell and the main cell was opened to sample the equilibrating supercritical CO₂ fluid. Valve 1 was closed and valve 2 opened, and supercritical CO₂ in the sampling cell flowed through the back-pressure regulator with the restrictor tube and was transferred to 100 mL of hexane in the collection vessel. PCDD/DFs and cPCBs present in this hexane solution were analyzed. Fish oil was extracted concurrently with the removal of PCDD/DFs and cPCBs; however, the amount of extracted fish oil was 0.4 g/92 g of CO₂. Therefore, the calculation of partition coefficient was conducted on the basis of the assumption that fish oil did not dissolve in supercritical CO₂.

Absorption Test. Four different carbon adsorbents—activated carbon (Kanto Chemical Co., Inc.), carboxen-1000 (Supelco, Bellefonte, PA), carboxen-1012 (Supelco), and carbon black (Tokai Carbon Co., Ltd.)—were examined. The surface areas of the adsorbents were 1200 m²/g for activated carbon, 1200 m²/g for carboxen-1000, 1500 m²/g for carboxen-1012, and 84 m²/g for carbon black. Fifteen grams of fish oil and 20 mg of adsorbent were stirred with a magnetic stirrer in a beaker for 24 h. One portion of the sample was treated in a centrifuge (13000 rpm, 90 min) to separate the adsorbent, and the oil sample was analyzed.

Fish Oil Analysis. The chemical analysis of PCDD/DFs and cPCBs (molecular structures are shown in Figure 1 of the Supporting Information) followed the *Quantitative Method Guideline for Dioxins in Feed* (31) and JIS K0312 (32) with some modifications. Approximately 5 g of the fish oil sample was spiked with a decane solution of ¹³C-labeled surrogate PCDD/DFs (for PCDDs, 2,3,7,8-TeCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD; for PCDFs, 2,3,7,8-TeCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, and OCDF) and ¹³C-labeled cPCBs (PCB 77, PCB 81, PCB 105, PCB 114, PCB 118, PCB 123, PCB 126, PCB 156, PCB 157, PCB 167, PCB 169, and PCB 189) as internal standards (Wellington Laboratories Inc.) and digested with 1.5 M KOH solution (water/ethanol = 1:2) for 2 h at room temperature. Then, the alkaline hydrolysis sample was extracted twice with *n*-hexane. The extract was washed with 2% NaCl aqueous solution and then concentrated. The resulting extract was applied on a multilayer silica gel column packed with anhydrous Na₂SO₄ (0.5 g), 10 wt % AgNO₃/silica gel (4 g), 44 wt % H₂SO₄/silica gel (6 g), silica gel (0.5 g), 2 wt % KOH/silica gel (1 g), and anhydrous Na₂SO₄ (0.5 g). The column was eluted with hexane (120 mL), and the eluent was concentrated. The resulting eluent was applied on 700 mg of reversible activated carbon-dispersed silica gel column and then introduced into a 40 mL hexane solution. Mono-*ortho* cPCBs were eluted with 60 mL of hexane containing dichloromethane (25 vol %). PCDD/DFs and non-*ortho* cPCBs were eluted with 100 mL of toluene in a reverse flow of the mobile phase. The fraction of mono-*ortho* cPCBs was spiked with ¹³C-labeled PCB 138. The fraction of PCDD/DFs and non-*ortho* cPCBs was spiked with ¹³C-labeled 1,2,7,8-TeCDF, 1,2,3,4,6,9-HxCDF, 1,2,3,4,6,8,9-HpCDF, and PCB 138 for their recovery calculation; then, all of the fractions were concentrated to a specific volume (30–100 μL).

The identification and quantification of PCDD/DFs and cPCBs were performed by using high-resolution gas chromatography coupled with a high-resolution mass spectrometric detector (HRGC/HRMSD) (Agilent 6890/JEOL JMS-700D, Agilent 6890/JEOL JMS-800; Agilent Technologies, Avondale, PA; JEOL Ltd., Tokyo, Japan) at a resolution of >10000. An SP2331 capillary column (film thickness = 0.20 μm, 0.25 mm i.d., length = 60 m, Supelco) was used for the quantification of tetra- to hexa-CDD/DFs, and an RH-12ms (0.25 mm i.d., length = 60 m, Inventex) column was used for the quantification of 1,2,3,7,8,9-HxCDF, hepta- to octa-CDD/DFs, and cPCBs. PCDD/DFs and cPCBs were monitored in the selective ion monitoring mode at two intensive ions of the molecular ion cluster and quantified using the corresponding ¹³C-labeled congeners (for GC-MS condition, see Table 1 in the Supporting Information).

Supercritical Fluid Extract Analysis. ¹³C-Labeled surrogate PCDD/DFs and coplanar PCBs as the internal standards were added to the samples trapped in the hexane solution and then concentrated. The

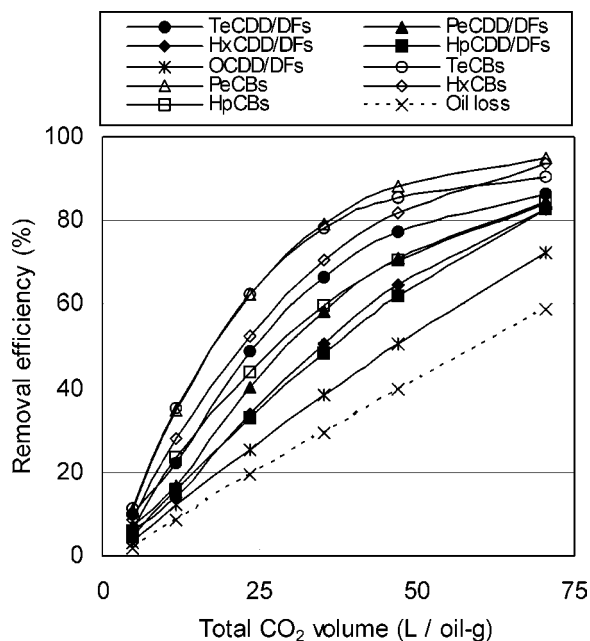


Figure 3. Removal efficiency of the PCDD/DFs and cPCBs of the treated fish oil as a function of the ratio of the total CO₂ volume at STP to 1 g of fish oil. The extraction conditions were 60 °C and 28 MPa. The removal efficiency was the ratio of the removal amount on the basis of the original sample content of PCDD/DFs and cPCBs.

samples were applied to a multilayer column chromatography for purification. The analytical methods were performed as described above.

Quality Assurance and Quality Control (QA/QC). All QA/QC were conducted in conformity with the reference manual and JIS K0312 (32). The recoveries of the ¹³C-labeled PCDD/DFs and cPCBs, which were added prior to the ethanolic KOH digestion, were within 50–120%. Variations in the concentrations between the duplicate analyses were within 30%.

Fatty Acid Content. Fatty acids were esterified according to the literature (33) with minor modification. An aliquot of fish oil with heptadecanoic acid as internal standard were esterified using a methanolic NaOH and then boron trifluoride in methanol solution. Fatty acid methyl esters were extracted with hexane and quantified by gas chromatography using a 6890N (Agilent Technologies) with a flame ionization detector, equipped with an OmegaWax fused silica capillary column (film thickness = 0.25 μm, 0.25 mm i.d., length = 30 m, Supelco). Helium was used as carrier gas. Oven temperature was programmed from 50 to 220 °C at 4 °C/min and held for 25 min. Injector and detector were set at 250 °C. Fatty acids were identified by comparison of retention times to those of authentic standards (Supelco 37 Component FAME Mix).

RESULTS AND DISCUSSION

Effect of the SCE Process. The extraction efficiency is significantly influenced by the fluid density, which can be regulated by adjusting the fluid temperature and pressure. Our previous research indicates that the optimized SCE conditions for PCDD/DFs and cPCBs in fish oil are 60 °C and 28 MPa (30).

Figure 3 shows the removal efficiency of the PCDD/DFs and cPCBs in treated fish oil according to the total CO₂ flow volume at STP. The value indicates the mean value of the removal efficiency of each isomer of PCDD/DFs and cPCBs. For PCDDs and PCDFs, the removal efficiency is collectively shown because the extraction behavior of each isomer with the same chlorine number congener is similar. The removal efficiency of each congener of PCDDs, PCDFs, and cPCBs

increased with an increase in the total CO₂ flow volume. At a total CO₂ flow volume of 50 L/g of oil, it was possible to extract 70% TeCDD/DFs and PeCDD/DFs, 60% HxCDD/DFs and HpCDD/DFs, 50% OCDD/DFs, 80–90% TeCBs through HxCBs, and 70% HpCBs. When the total CO₂ flow volume exceeded 50 L/g of oil, the increase of removal efficiency slowed. The removal efficiency decreases with an increase in the number of chlorine atoms in the molecule, that is, as the molecular weight increases. Moreover, the extraction efficiencies of PCBs are greater than those of PCDDs and PCDFs. In particular, the extraction efficiency of high-chlorinated PCDD/DFs was low. As shown in **Figure 3**, oil was extracted simultaneously, and the difference of slope of removal efficiency lines between dioxins (PCDD/DFs and cPCBs) and oil was large in the CO₂ flow volume range of 0–50 L/g of oil. Therefore, effective removal of dioxins is possible in this range. In the real process, the amount of total CO₂ flow volume should be determined on the basis of the reduction level of dioxins to meet the safety standard of dioxins.

Correlation between Partition Coefficient ($K_{CO_2/Oil}$) and Molecular Weight. To clarify the extraction behavior of PCDD/DFs and cPCBs by SCE in further detail, we measured the partition coefficient of PCDD/DFs and cPCBs between supercritical CO₂ and fish oil ($K_{CO_2/oil}$). **Figure 4a** shows the correlation between partition coefficient ($K_{CO_2/oil}$) and molecular weight of each congener (data are listed in Table 2 of the Supporting Information).

It is clear that the $K_{CO_2/oil}$ values decrease with an increase in the number of chlorine atoms; that is, extraction with supercritical CO₂ becomes difficult due to the increase in the molecular weight. In cPCBs, the total $K_{CO_2/oil}$ values are generally greater than those of PCDD/DFs values. Furthermore, non-ortho cPCBs have lower partition coefficients than mono-ortho cPCBs of the same molecular weight, and their extraction with supercritical CO₂ becomes more difficult. This is considered to be an influence of the difference in the conformation of cPCBs derived from the numbers of ortho-substituted chlorine atoms. On the basis of these observations, it is possible to extract cPCBs, particularly mono-ortho cPCBs, more effectively than PCDD/DFs in the SCE process for fish oil.

Correlation between Partition Coefficient ($K_{CO_2/Oil}$) and Octanol/Water Partition Coefficients (K_{ow}). We compare the $K_{CO_2/oil}$ values and octanol/water partition coefficients (K_{ow}) of PCDD/DFs and cPCBs (34–36). The observed $-\log K_{CO_2/oil}$ values versus the $\log K_{ow}$ values are plotted in **Figure 4b** (data are listed in Table 2 of the Supporting Information). A high correlation can be observed between the $\log K_{CO_2/oil}$ and $\log K_{ow}$ values. In the congeners with small values of $\log K_{ow}$, the $K_{CO_2/oil}$ values become large and they can be conveniently extracted by SCE. Furthermore, mono-ortho cPCBs can be more easily removed from fish oil than the others. These results show that the $\log K_{CO_2/oil}$ values of PCDD/DFs and cPCBs can be roughly estimated from the $\log K_{ow}$ values. This implies the possibility of using K_{ow} values as one of the indexes influencing the decision of operation conditions that predict the removal efficiency by SCE corresponding to the content of each oil sample.

Effect of the Adsorption Removal. For the effective removal of high-chlorinated PCDD/DFs, we considered an adsorptive removal by using carbon adsorbents. Four different carbon adsorbents were examined to reveal the adsorption character of PCDD/DFs and cPCBs in fish oil. The removal efficiencies (the

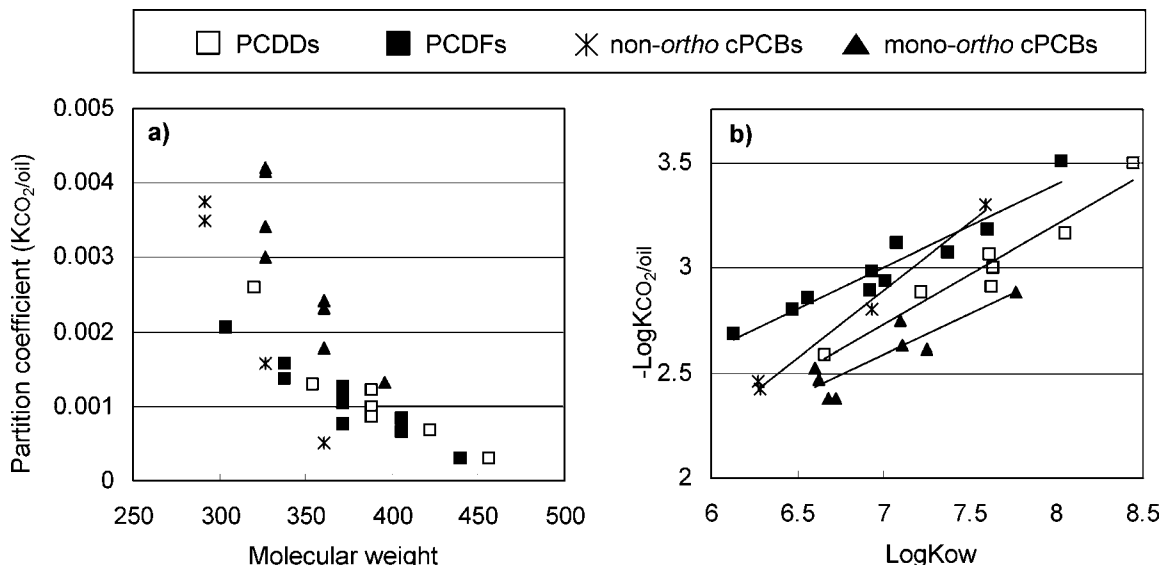


Figure 4. (a) Plot of the partition coefficient ($K_{CO_2/oil}$) versus molecular weight of PCDD/DFs and cPCBs. (b) Plot of the observed $-\log K_{CO_2/oil}$ values versus the $\log K_{ow}$ values. Linear regression analysis on PCDDs yields the equation $-\log K_{CO_2/oil} = 0.47 \log K_{ow} - 0.58$ with $r^2 = 0.93$; on PCDFs, $-\log K_{CO_2/oil} = 0.40 \log K_{ow} + 0.22$ with $r^2 = 0.92$; on non-ortho cPCBs, $-\log K_{CO_2/oil} = 0.78 \log K_{ow} - 2.57$ with $r^2 = 0.99$; and on mono-ortho cPCBs, $-\log K_{CO_2/oil} = 0.45 \log K_{ow} - 0.60$ with $r^2 = 0.82$.

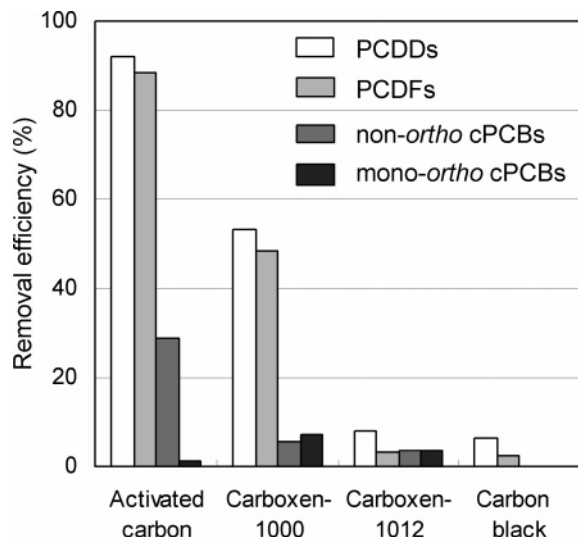


Figure 5. Removal efficiency of PCDD/DFs and cPCBs treated with adsorbents. The removal efficiency was calculated on the basis of the original sample concentrations of PCDD/DFs and cPCBs without adsorption treatment.

mean value of the removal efficiency of each PCDD, PCDFs, non-ortho cPCB, and mono-ortho cPCBs) are shown in Figure 5.

In activated carbon, which has a large surface area and fine pore volume, the removal ratios are $>90\%$ for all of the isomers of PCDD/DFs. On the contrary, the removal ratios for non-ortho cPCBs were about 30 and 1% for mono-ortho cPCBs. In carboxen-1000, the removal ratios are half those of activated carbon, irrespective of the same surface area and same absorption behavior. In carboxen-1012, a fairly low adsorption activity was observed, although the surface area was comparatively greater. These might be due to the difference in the pore distribution of the adsorbent: activated carbon and carboxen-1000 have micro-, meso-, and macropores; however, carboxen-1012 has only mesopores. Carbon black had a small surface area and did not exhibit any absorption activity. In this experiment, it is evident that activated carbon is more suitable

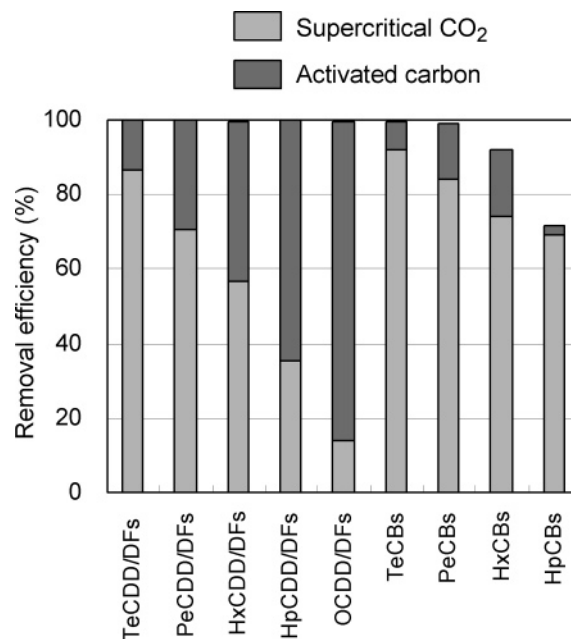


Figure 6. Removal efficiency of PCDD/DFs and cPCBs from fish oil after each SCE process and adsorption treatment. The removal efficiency was calculated on the basis of the original sample TEQ values of PCDD/DFs and cPCBs without SCE and adsorption treatment.

for the adsorption removal. This can be attributed to its large surface area and effective pore distribution for absorption removal. However, activated carbon effectively adsorbs PCDD/DFs, but it has no effect on the removal of mono-ortho cPCBs. The possibility of intramolecular rotation can be attributed to the influence of the removal efficiency of cPCBs.

Effect of the Combined Process: SCE and Adsorption Removal. A removal process combined with these two methods was then examined. First, fish oil was processed with SCE for a CO₂ flow volume of 50 L/g at 60 °C and 28 MPa; then, it was treated with activated carbon adsorption. Figure 6 shows the removal efficiency of PCDD/DFs and cPCBs in fish oil based on toxicity equivalence quantity (WHO-TEQ) level after

each process (data are listed in Table 3 of the Supporting Information).

The value represents the mean value of the removal efficiency of each isomer of the same chlorine number of each PCDD/DF and cPCB. First, the cPCBs and low-chlorinated PCDD/DFs were effectively extracted by the SCE process; then, high-chlorinated PCDD/DFs were removed by the activated carbon adsorption treatment. Finally, almost 100% PCDD/DFs, $\geq 99\%$ TeCBs and PeCBs, 92% HxCBs, and 72% HpCBs were removed from fish oil. The total TEQ level changes for these processes were calculated. PCDD/DFs and cPCBs were totally removed up to a TEQ level of 69% by the SCE process, and the remaining 31% was removed by a subsequent activated carbon adsorption treatment. Finally, almost 100% of the total TEQ value could be reduced using these processes.

In conclusion, the SCE process for fish oil is fairly effective in the removal of cPCBs and low-chlorinated PCDD/DFs; however, it has no effect for high-chlorinated PCDD/DFs. Adsorption removal with activated carbon was fairly effective for the removal of PCDD/DFs.

As mentioned above, it is possible to reduce the levels of almost all of the dioxins and cPCBs by completely using the characteristics of each removal method: SCE process for the removal of PCBs and adsorption treatment with activated carbon for the removal of PCDD/DFs. In particular, the SCE process is efficient in the removal of mono-*ortho* cPCBs. Furthermore, no significant changes were observed in the fatty acid contents such as n-3 fatty acids (EPA and DHA). However, in the SCE process, the oil amount in the extract increased with the total CO₂ flow volume, and 40% of the oil was transferred to the extract at a CO₂ flow volume of 50 L/g of oil. One of the reasons of this oil transfer can be assumed to be as follows: in this experiment, an empty column without packing was used in the SCE process; therefore, a droplet spray of oil was transferred on the CO₂ stream, and the contact efficiency between the oil and CO₂ was not sufficient. The extraction efficiency can be improved by using a column with packing such as Dixon packing or a countercurrent flow extraction. This SCE is a biphasic process: oil (liquid phase) and the CO₂ (supercritical phase), and contact efficiency is one of the important factors for the extraction efficiency. The PCDD/DFs and cPCBs are included in oil at very low concentration in comparison with oil itself. Therefore, CO₂ (extracting agent) contacts with contaminants are much more difficult than with oil. By using a countercurrent extractor, the sample is exposed to a greater contact area between the contaminants and the CO₂. Thus, the countercurrent process is more efficient than the batch extraction process. Furthermore, continuous process is possible.

It would be possible to develop an efficient process in terms of oil yield and reduction level of PCDD/DFs and cPCBs by adjusting the SCE and adsorption processes.

Supporting Information Available: Structures of PCDD/DFs and cPCBs, GC-MS conditions, and tables of data for **Figures 4 and 6**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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