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Reduction of Persistent Organic Pollutants in Fishmeal: A Feasibility Study

ÅGE OTERHALS* AND EINAR NYGÅRD

Norwegian Institute of Fisheries and Aquaculture Research Department Bergen, Kjerreidviken 16, N-5141 Fyllingsdalen, Norway

The dioxin, dioxin-like polychlorinated biphenyl (DL-PCB), fat, and dry matter partitioning during fishmeal production have been studied in pilot scale. Most of the dry matter and lipid content in the fishmeal could be ascribed to the press cake intermediate product. Dioxins and DL-PCBs are fat-soluble compounds, and the process partitioning is reflected by the fat partitioning data. Enzyme and heat treatment of the press cake and stickwater concentrate did not improve fat separation. Soybean oil extraction of the press cake reduced the dioxin and DL-PCB content by 97%. Less exchange of fatty acids was observed (56−72%). Combined with fat separation of the stickwater concentrate, the applied process conditions were able to give a fishmeal decontamination rate higher than hexane and isopropanol extraction of the fishmeal. Quantification of fat content based on chloroform/methanol extraction was found to be the best protocol to estimate fat partitioning and decontamination effects. The oil extraction process requires further optimization, but has several advantages compared to organic solvent extraction. These include easy implementation in an existing fishmeal processing line, use of a safe and nonflammable extraction medium, and expected lower investment and operation costs. A new integrated fishmeal and fish oil production and decontamination process line is proposed.

KEYWORDS: Decontamination; extraction; fat separation; dioxins; PCB

INTRODUCTION

Persistent organic pollutants (POPs) are chemical substances that persist in the environment and bioaccumulate in the fatty tissue of living organisms. The content of POPs in feed and food products have been given considerable attention due to recent contamination episodes (*1, 2*) and their potential risk to human health (*3, 4*). High levels of organic pollutants have been found in several fish species and consequently in fishmeal and fish oil produced from industrial fish and byproducts (*1, 5*). The fish stocks of concern for the Northern European industry are sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) in the Baltic Sea and herring, sprat, sand eel (*Ammodytes tobianus* and *Ammodytes marinus*), and blue whiting (*Micromesistius poutassou*) in the North Sea (*5, 6*). The observed contamination levels reflect the general pollution level in the respective fishing areas. Although only a minor part of the produced fish oil and fishmeal has a content of undesirable organic pollutants above the maximum permitted levels, the need for decontamination of the products to comply with the legislations will disfavor producers based on such raw material on a world basis.

The European Commission (EC) implemented in July 2002 new legislation on undesirable substances in animal feed (*7*). The legislation was amended in November 2005 to include maximum levels for the sum of dioxins and dioxin-like polychlorinated biphenyls (DL-PCB) in addition to the present maximum levels for dioxins only (*8*). Consideration will be given by December 31, 2008, to significantly reduce the maximum levels. With regard to fishmeal, fish protein hydrolysates, and fish oil, the level shall be determined on the basis of the technical possibilities of the most effective, economically viable, decontamination procedure (*8*). A large range of other POPs are under evaluation (*9*), and special attention has been given to polybrominated diphenyl ether flame retardants (*10*) and perfluorinated compounds (*11*).

Fishmeal is an important and widely used ingredient in compounded feed to aquatic and domestic animals due to high protein digestibility and content of essential amino acids, vitamin and mineral content, and fat rich in long-chain omega-3 fatty acids (*12*). A high inclusion level of fishmeal is especially used in feed to Atlantic salmon (*Salmo salar*), and this can only to a limited extent be replaced by alternative vegetable or single-cell protein sources without negative impact on feed conversion and growth rate (*13*).

POPs in fish derive predominantly from their diet, and fishmeal and fish oil are the most important sources of these contaminants in fish feed formulations (*4, 14*). To meet the present legislation requirements, feed compounders are using increasing levels of South Pacific fishmeal and fish oil or nonmarine feed ingredients with naturally low contamination

* Corresponding author [telephone (+47) 55 50 12 74; fax (+47) 55 50 12 99; e-mail aage.oterhals@fiskeriforskning.no].

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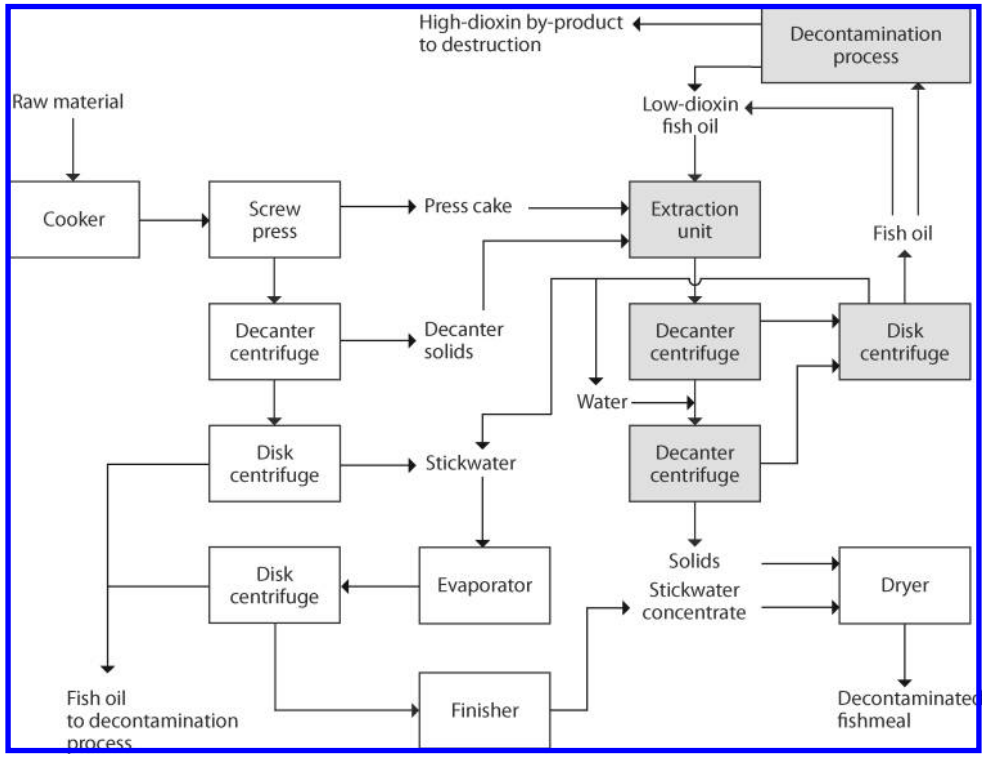

Figure 1. Simplified process flow diagram representing a new integrated fishmeal and fish oil production and decontamination process line based on oil extraction of intermediate products (press cake and decanter solids). New unit operations are marked with shading.

levels. In addition, the fatty acid composition of the farmed fish will reflect the feed composition, and inclusion of a high level of marine oils is necessary to obtain seafood products with significant levels of the essential and health-promoting fatty acids EPA and DHA (*15*).

The annual world fishmeal production is around 6 million metric tons with Peru, Chile, Thailand, China, the United States, Japan, Denmark, Iceland, Norway, and South Africa as the main producing countries in descending 2005 order (*16*). Fishmeal is produced by use of heat coagulation of the raw material followed by a mechanical fat separation and thermal dewatering process (*17, 18*). The processing equipment is fairly standardized worldwide, and product quality is mainly dependent on raw material type and quality (*19*) together with drying conditions (*20*). A general outline of the process unit operations is included in **Figure 1**.

Several process alternatives have been developed to reduce the dioxin and DL-PCB contents in fish oil, for example, activated carbon adsorption (*21*), steam deodorization (*22*), and short path distillation (*23*). Less emphasis has been given to the development of fishmeal decontamination alternatives. POPs are fat-soluble compounds, and reduction of the fat level in the fishmeal will also reduce the content of these undesirable compounds. Høstmark (*24*) has studied the effect of a gentle, low-temperature (60 °C) coagulation procedure on fat separation in the fishmeal process. On average, a 40% reduction of the fishmeal fat level was obtained with herring and mackerel raw material compared to a harsher coagulation procedure (7.2 and 12.0% Soxhlet fat on a dry matter basis, respectively) aimed to simulate large-scale industrial conditions. Attempts in 1995 to scale up the process failed, but part of the same improved fat separation effect has been obtained in large-scale operation by use of hot press or decanter liquid in return to the cooker (Høstmark, Fiskeriforskning, personal communication). However, the obtainable effect in large-scale operation is also dependent on raw material type and quality and the specific equipment used in the processing plant. Increased fat separation has also been reported on the basis of protease treatment of noncoagulated fish byproducts (*25*).

A high and predictable fishmeal decontamination effect can only be obtained on the basis of extraction technology. Hexane (i.e., commercial petroleum fractions consisting of 45−90% *n*-hexane and other branched and cyclic hexane isomers) is the main solvent alternative used for edible oil extraction (*26*) and has also been utilized in Norway in the 1960s and 1970s to produce a special quality low-fat fishmeal (Norsamin) to be used in domestic animal feed (*27*). A new fishmeal decontamination plant based on hexane extraction was put in operation in 2005 at the fishmeal factory TripleNine Fish Protein in Esbjerg, Denmark. A presentation of the plant can be found on the homepage www.999.dk. At present there is no published information available on decontamination efficiency or residual fat content in the fishmeal after the hexane extraction. Hexane is a highly flammable and toxic solvent. Isopropanol has therefore been studied as a more environmentally friendly alternative. The main drawbacks of isopropanol, compared to hexane, have been the lower apparent solvency for oil and higher energy consumption in the recovery process (*28*).

Several alternatives to organic solvent extraction of fishmeal have been described in the literature. The oil extraction process described in this paper has earlier in part been presented to the industry by the principal author (*29*). Baron et al. (*30*) have studied oil extraction of fishmeal and documented a 60−75% decontamination effect. Only limited effect was observed on improved fat separation after protease treatment of the fishmeal (30%) and on polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzofuran (PCDD/F) and DL-PCB degradation by oxidoreductases (10−15%) (*30*). Exposure of fishmeal to UVB light has been shown to reduce the PCDD/F content by 70% (*31*). However, the photodegradation mechanism is probably

Table 1. Recorded Weights, Dry Matter, and Fat Content (Mean ± SD, $n = 6$)[a] in Raw Material and Intermediate Products from Pilot-Scale Production of Herring Fishmeal and Fish Oil

			fat extraction method			
	wt (kg)	dry matter (%)	ethyl acetate (%)	Soxhlet[b] (%)	EC[c] (%)	Bl&D[d] (%)
raw material	311.1	25.2	6.14 (±0.03) a	5.58 (±0.03) b	5.70 (±0.05) c	6.58 (±0.06) d
press cake/jesma solids	117.77	45.9	4.82 (±0.14) a	4.51 (±0.19) b	4.68 (±0.17) a,b	6.39 (±0.21) c
stickwater concentrate	41.74	29.3	2.65 (±0.41) a	3.28 (±0.37) b	4.29 (±0.03) c	4.62 (±0.04) c
separator sludge	6.12	19.65	16.15	NA	NA	NA
fish oil	9.00	NA	NA	NA	NA	NA
fishmeal	74.65[e]	91.0	10.10 (±0.05) a	9.87 (±0.08) b	11.22 (±0.09) c	13.18 (±0.15) d

[a] Different letters in the same row indicate significant differences at $p < 0.05$. [b] Light petroleum Soxhlet extraction. [c] Light petroleum Soxhlet extraction with acid hydrolysis. [d] Chloroform/methanol extraction. [e] Calculated on the basis of average fat-free dry matter mass balances.

coupled to lipid oxidation with non- and mono-*ortho* PCBs as reaction products (*31*). The industrial use of the principle is questionable due to the need for a long exposure time (5 days) and increased lipid oxidation and DL-PCB content in the fishmeal after the UV treatment.

The objectives of this study were (1) to study the fat, dioxin, and DL-PCB partitioning in the fishmeal process, (2) to assess the effect on increased fat separation based on enzyme and heat treatment of the intermediate products (i.e., press cake and stickwater concentrate), and (3) to compare the decontamination efficiency of oil extraction of press cake and organic solvent extraction of fishmeal. The experimental results can be used as a basis for further research and development of an efficient and economically viable industrial fishmeal decontamination solution.

MATERIALS AND METHODS

Materials. Frozen herring (*Clupea harengus*) was purchased from Astrid Fiskexport AB, Rønneng, Sweden. The fish was from the same catch in Skagerrak, April 2003, and stored frozen until pilot-scale production in September 2003. The solvents used in the extraction trials were *n*-hexane for liquid chromatography (Merck KGaA, Darmstadt, Germany), technical grade isopropanol (Arcus Kjemi AS, Vestby, Norway), and food grade soybean oil (Idun AS, Skjetten, Norway). Ethoxyquin FEQ500 was purchased from LL Chemie AB, Helsingborg, Sweden. All solvents and reagents for the analyses were of analytical grade.

Pilot-Scale Fishmeal and Oil Production. The utilized processing steps are equivalent to the unit operations used in the fishmeal industry (**Figure 1**). Frozen blocks of herring were thawed overnight. The fish (311 kg) was divided into 10 batches and heated to 85 °C in 150 L steam-heated kitchen cookers under manual mixing and kept at this temperature for approximately 5 min before mechanical dewatering in a P13-SCR double-screw press (Stord Bartz AS, Bergen, Norway). Ten kilograms of fresh water was added to the first four batches to facilitate heat transfer during the coagulation process. To the remaining batches was added 11−12 L of press water from the screw press instead of fresh water. Ethoxyquin (10 ppm) was added to the press water before heating to 90 °C. Suspended solids were removed by a Jesma VS 20/65 Roto-Fluid sieve (Jesma, Velje, Denmark; equivalent to the decanter centrifuge in **Figure 1**) with a 100 μm sieve net opening before oil separation on an SA-1-03 separator (Westfalia, Oelde, Germany). The jesma solids (equivalent to decanter solids in **Figure 1**) were added to the press cake and mixed by use of an IDE-CON minimixer (IDE-CON AS, Porsgrunn, Norway). Sludge from the oil separator was collected, heated to 90 °C, and separated a second time. The amount of sludge from the second separation was measured, and a sample was collected to estimate the loss of fat and dry matter. The separated press water (stickwater) was concentrated on a four-stage falling film evaporator (APV Anhydro, Søborg, Denmark) at 60−100 °C. On the basis of the recorded amounts of combined press cake and jesma solids (press cake/jesma solids) and stickwater concentrate in the process (**Table 1**), 5.32 kg of stickwater concentrate was added to 15.0 kg of press cake/jesma solids and mixed in the IDE-CON mixer. Ten 500 g batches of press cake/jesma solids plus stickwater concentrate mixture was added ethoxyquin to 100 ppm on a dry matter basis and dried to fishmeal in a laboratory hot air TG1 fluid bed dryer (Retsch GmbH & Co. KG, Germany) at 63 ± 1 °C. The fishmeal samples were pooled and ground on a meat grinder with a 4.5 mm aperture. Care was taken at each step in the process to collect representative samples of the individual intermediate and final products. All samples were stored at −20 °C until analysis or further experimental use.

Heat and Enzyme Treatment. A two-factorial design experiment was performed to test the effect of heat (121 °C) and protease (EC 3.4.21.62) treatment on improved fat separation from press cake/jesma solids and stickwater concentrate. Press cake/jesma solids was ground on a meat grinder with a 4.5 mm aperture and homogenized in a food processor; 50 g samples were conditioned to a 10% dry matter level by the addition of water. Enzyme treatment was performed by the addition of Alcalase 2.4L (Novozymes AS, Bagsvaerd, Denmark) to 1 g/kg dry matter. The mixture was heated to 55 °C and kept at this temperature for 60 min with continuous mixing before further heating to 95 °C for 10 min to stop the enzyme activity. The sample was centrifuged at 4225*g* for 9 min in a Sorvall RC5C centrifuge (Sorvall Instruments, Wilmington, DE). The water phase was decanted off and separated oil quantified by tetrachloromethane extraction (*32*). A control sample was treated accordingly without enzyme addition. Heat treatment, with and without enzyme pretreatment, was performed by heating a conditioned sample for 10 min at 121 °C in an autoclave. Two hundred gram samples of stickwater concentrate were diluted with water to 20% dry matter content and enzyme and/or heat treated according to the same procedure as above. After centrifugation, the samples were frozen, and the upper oil/water layer was scraped off and collected. Separated oil was quantified by extraction of this subsample using tetrachloromethane (*32*).

Organic Solvent Extraction. Two hundred and fifty grams of fishmeal was added to 1250 g of solvent (hexane or isopropanol) and heated to 58 °C. After 30 min of holding time with continuous mixing, the solvent was removed by filtration over a Büchner filter with a Black ribbon grade 589/1 (Schleicher & Schuell GmbH, Dassel, Germany) filter paper. The filter cake was collected and extracted a second time under the same conditions. The final filter cake was washed with 400 g of solvent preheated to 58 °C, transferred to aluminum foil, and air-dried at room temperature for 2 days before analysis.

Soybean Oil Extraction. Press cake/jesma solids were ground on a meat grinder with a 4.5 mm aperture and homogenized in a food processor. A 500 g sample was mixed with 1500 g of soybean oil and heated to 88 °C under continuous mixing. After 60 min of holding time, the sample was centrifuged at 4225*g* for 9 min in a Sorvall RC5C centrifuge. The oil phase was decanted off and the sediment added to 1000 g of distilled water and heated to 87 °C under continuous mixing. After 5 min of holding time, the sample was centrifuged (4225*g*, 9 min) and the top oil layer decanted off. The water phase was decanted off and the sediment washed a second time by use of 300 g of distilled water. The top oil layer was decanted off and the sediment resuspended in the water phase and transferred to a metal tray. The water phase from the first washing step was added, and the pooled sample was freeze-dried and conditioned at room temperature for 2 days before analysis.

Dry Matter and Fat Content. Moisture content was measured gravimetrically after drying in a forced-air oven at 103 ± 1 °C for

4.5 h or overnight if water content was >15% (*33*). Fat content was determined on the basis of ethyl acetate extraction (ethyl acetate) (*34*), light petroleum (boiling range = 40−60 °C) Soxhlet extraction (Soxhlet) (*35*), light petroleum (boiling range = 40−60 °C) Soxhlet extraction with acid hydrolysis (EC) (*36*), and chloroform/methanol extraction (Bl&D) (*37*). All samples were dried at 103 °C for 2 h prior to Soxhlet extraction.

Fatty Acid Composition. Preparation of fatty acid methyl esters (FAMEs) was done according to AOCS method Ce 1b-89 (*38*). C23:0 methyl ester was added as internal standard. The FAMEs were separated by gas chromatography fitted with a methyl-deactivated 2.5 m × 0.32 mm fused silica pre column (Varian, Palo Alto, CA) connected to a 50 m × 0.32 mm i.d., 0.2 μm stationary phase, capillary CP-SIL 88 column (Varian) using a HRGC 5300 Mega Series gas chromatograph equipped with an AS 2000 autosampler, on-column injector, and flame ionization detector (Thermo/Carlo Erba Instruments, Milan, Italy). Helium at 70 kPa injector pressure was used as carrier gas. Injection (0.3 μL) was done at 60 °C. The temperature profile used was 25 °C/min, 165 °C for 12 min, 25 °C/min, 190 °C for 22 min, 25 °C/min, and 205 °C for 17 min. Fatty acid composition was calculated by use of the internal standard method and reported on a sample basis as grams of FAMEs per kilogram. Analyses were run in duplicate.

Lipid Phase Exchange. The percent lipid phase exchange between the press cake/jesma solids and soybean oil after the extraction procedure was defined as the amount of soybean oil that mixed with the press cake/jesma solid lipid phase resulting in the observed fatty acid composition. The lipid phase and fatty acid balances can be described by

$$100 \text{ g of } LP_{SBO\text{-}exPC/JS} = X \text{ g of } LP_{PC/JS} + Y \text{ g of } LP_{SBO} \quad (1)$$

$$100 \times FA\%_{SBO\text{-}exPC/JS} = X \times FA\%_{PC/JS} + Y \times FA\%_{SBO} \quad (2)$$

where $LP_{SBO\text{-}ex\ PC/JS}$ is lipid phase from soybean oil extracted press cake/jesma solids, $LP_{PC/JS}$ is lipid phase from press cake/jesma solids, LP_{SBO} is lipid phase from soybean oil, and FA% is the weight percent of a specific fatty acid in the respective lipid phases. Combining eqs 1 and 2 gives

$$Y = \frac{FA\%_{SBO\text{-}exPC/JS} - FA\%_{PC/JS}}{FA\%_{SBO} - FA\%_{PC/JS}} \times 100 \quad (3)$$

Dioxin and DL-PCB Analysis. Wet samples were lyophilized and homogenized before pressure−solvent extraction. PCDD/F and DL-PCB congeners were analyzed as previously described (*39*). The congeners analyzed included the 17 PCDD/Fs and 12 DL-PCBs for which the World Health Organization (WHO) has established toxic equivalency factors (TEFs) for human risk assessment (*40*).

In the present study no experimental or analytical replicates were included to estimate uncertainty. The analytical laboratory (NIFES, Bergen, Norway) is accredited according to ISO 17025. During validation of the method an expanded measurement uncertainty (U) was calculated as a combined uncertainty from the internal reproducibility (u_{ir}) and the uncertainty of the true-value estimate of a ring-test reference material (u_{rrm}), which was fish muscle. If $\bar{X}$ is the mean measured value, the 95% probability expanded measurement uncertainty is

$$U(\%) = \frac{2 \times 100 \times \sqrt{u_{ir}^2 + u_{rrm}^2}}{\bar{X}} \quad (4)$$

The relevant U values have been validated in fish meat, for low (0.4 ng/kg), medium (1.9, 1.2 and 1.3 ng/kg), and high (21 and 380 ng/kg) PCDD/F concentration levels. The corresponding non-*ortho* PCB concentration levels were 2 and 12 ng/kg (low) and 39 and 120 ng/kg (medium), and those for mono-*ortho* PCB were 790 ng/kg (low) and 2500 and 6200 ng/kg (medium). For simplicity the highest of these calculated U values, 40%, can be applied to all PCDD/F and non/mono-*ortho* PCB congener data in the accredited measurement range for fish meat and closely related matrices. It can also be applied to the ΣTEQ values.

The TEQ level was expressed as nanograms of WHO-TEQ per kilogram on the basis of the sum concentration of each congener times the TEF value (*40*). The contribution of nondetected congeners was set to zero.

Statistical Analysis. Statistical analyses were performed with assistance of Statistica 7 from Statsoft (Tulsa, OK). The obtained data were analyzed by use of ANOVA and significant differences ($p < 0.05$) between the means evaluated by use of Tukey HSD post hoc comparison test.

RESULTS AND DISCUSSION

Mass Balance and Dry Matter and Fat Partitioning. Fat separation in a conventional fishmeal process is accomplished by use of a combination of a twin screw press, decanter centrifuge, and disk centrifuge technology (*17, 18*) (**Figure 1**). The process is designed to bring the final fat content in the fishmeal below 10−12% with a typical level in commercial parcels in the 8−10% range. PCDD/Fs and DL-PCBs are lipophilic compounds with high octanol−water partitioning coefficients (K_{ow}) (*41, 42*) and will accumulate in the available fat phase in the fish, increasing the levels on lipid basis with decreasing fat level (*6*). POPs in the raw material will follow the fat partitioning, and any improvement in the fat separation unit operations utilized in the manufacturing process will have a proportional effect on the level of these compounds in the fishmeal product. Optimization of the fat separation will also be the most cost-effective way to reduce the dioxin and PCB content in fishmeal.

To assess the effect of alternative fat separation strategies, detailed knowledge is needed on fat partitioning and how well alternative analytical fat extraction methods indirectly reflect the persistent organic pollutant partitioning in the process. As a basis for such process studies, we have conducted a pilot plant fishmeal and fish oil production based on herring raw material. The fat content in the raw material, press cake/jesma solids (jesma solids is equivalent to decanter solids in **Figure 1**), stickwater concentrate, and fishmeal was followed on the basis of four frequently applied analytical extraction protocols (**Table 1**). Ethyl acetate extraction (*34*) is used by the Scandinavian fishmeal industry to measure the fat content in the raw material and to estimate the oil yield. Combined with total dry matter, it is also used for final price setting between the fishermen and purchasers. Ethyl acetate is reported to give comparable results to *n*-hexane extraction (*43*). Soxhlet extraction (*35*) is the industry standard for analysis of the fat content in fishmeal. However, the method extracts only 40−60% of the phospholipids present in the fishmeal (*44*). The EC method (*36*) is used by feed compounders to quantify the total lipid content in feed products. Bl&D extraction (*37*) is the preferred technique used in research activity due to the use of low-temperature conditions and quantitative measurement of both polar and neutral lipids in biological samples.

Significant differences in the lipid content between the tested analytical extraction protocols were observed for the raw material and fishmeal samples (**Table 1**). The intermediate products showed a more complex pattern with no significant difference between the EC and ethyl acetate and the EC and Soxhlet method in the case of press cake/jesma solids. Similarly, the EC and Bl&D method did not give significantly different stickwater concentrate levels. The ranking Bl&D > Soxhlet > EC was systematic for all samples and in agreement with earlier studies on fishmeal extraction (*44*). Ethyl acetate extraction gave a less systematic picture with levels higher than the EC method in raw material and press cake/jesma solids, lower than the Soxhlet method in the stickwater concentrate, and between the

Table 2. Fat-free Dry Matter Partitioning (Percent) Relative to Raw Material Content in Pilot-Scale Herring Fishmeal and Fish Oil Production Based on Different Fat Extraction Methods

	fat extraction method			
	ethyl acetate	Soxhlet[b]	EC[c]	Bl&D[d]
press cake/jesma solids	81.7	80.0	80.1	80.4
stickwater concentrate[a]	19.0	18.0	17.5	18.1
sum	100.7	98.0	97.6	98.5

[a] Including contribution from separator sludge. [b] See **Table 1**. [c] See **Table 1**. [d] See **Table 1**.

Table 3. Fat Partitioning (Percent) Relative to Raw Material Content in Pilot-Scale Herring Fishmeal and Fish Oil Production Based on Different Fat Extraction Methods

	fat extraction method			
	ethyl acetate	Soxhlet[b]	EC[c]	Bl&D[d]
press cake/jesma solids	29.7	30.6	31.1	36.8
stickwater concentrate	6.2	8.2	10.3	9.6
fish oil[a]	55.8	61.4	60.1	52.1
sum	91.7	100.3	101.5	98.4

[a] Including contribution from separator sludge and corrected for average Soxhlet, EC, and Bl&D oil loss in the process. [b] See **Table 1**. [c] See **Table 1**. [d] See **Table 1**.

Soxhlet and EC method in the fishmeal sample (**Table 1**). The unsystematic behavior of the ethyl acetate method is difficult to explain but renders this extraction technique less suitable to follow the fat partitioning in the fishmeal process.

By combining the recorded mass balance and composite data (**Table 1**) it is possible to obtain a fat-free dry matter and fat partition overview of the process (**Tables 2** and **3**). In large-scale continuous operations, the separator sludge from the oil separators is normally recycled back to the decanter centrifuge to enable separation of particulate matter and oil. This is not practical in pilot plant experiments, and the separator sludge fat-free dry matter content (**Table 1**) is therefore included in the stickwater concentrate partition (**Table 2**). Correspondingly, the fat content (**Table 1**) in the separator sludge is included in the fish oil partition (**Table 3**).

Almost all fat-free dry matter in the raw material could be accounted for in the intermediate products (**Table 2**). Only small variations were observed between the tested fat extraction methods, giving a normalized average fat-free dry matter partitioning between the press cake/jesma solids and stickwater concentrate of 82 and 18%, respectively. This is in good agreement with earlier observations at our institute based on fresh herring and the process equipment and conditions utilized in this study (*24*).

The fat balances showed a much higher process loss (ethyl acetate, 17.0%; Soxhlet, 9.4%; EC, 7.9%; Bl&D, 9.7%). Because almost all fat-free dry matter could be accounted for on the basis of press cake/jesma solids and stickwater concentrate (**Table 2**), this discrepancy must mainly be caused by loss of oil in the process equipment. In **Table 3** this is corrected for on the basis of the average Soxhlet, EC, and Bl&D oil loss (0.54 kg of oil/100 kg of raw material). The observed large discrepancy based on ethyl acetate extraction might be caused by the comparatively high fat content in the raw material based on this procedure. The oil yield ranking Soxhlet > EC > Bl&D can be explained by the respective method's ability to extract polar lipids that are not extractable by the wet rendering technology used in the fishmeal industry.

Table 4. PCDD/F and DL-PCB Concentrations and TEQ Levels (Nanograms per Kilogram of Dry Matter) in Raw Material, Intermediate and Final Products from Pilot-Scale Herring Fishmeal and Fish Oil Production

	raw material	press cake/jesma solids	stickwater concentrate	fishmeal	fish oil
PCDD	3.49	1.37	0.65	2.02	12.65
PCDF	18.30	6.03	3.90	9.71	78.71
sum PCDD/F	21.79	7.40	4.55	11.73	91.36
non-*ortho* PCB	136.7	48.9	29.0	67.1	578.9
mono-*ortho* PCB	8452.7	3254.5	1698.9	3805.0	34910.8
sum DL-PCB	8589.3	3303.3	1727.9	3872.1	35489.7
PCDD/F-TEQ	5.53	1.72	1.13	2.86	22.74
DL-PCB-TEQ	4.41	1.66	0.88	1.96	18.04
TEQ ratio	1.25	1.04	1.28	1.45	1.26

POPs Partitioning. To obtain a fishmeal with high PCDD/F and DL-PCB content, herring from the Skagerrak region was chosen as raw material in this study. Purchase of herring with a low fat content (i.e., caught in April) further increased this effect (*45*). The obtained fish oil and fishmeal had a WHO-PCDD/F-TEQ content (**Table 4**) significantly above the present EC legislation (*8*) for the maximum permitted levels for use in animal feed (6.0 and 1.25 ng of WHO-TEQ/kg, respectively; 12% water basis). Only the fish oil was above the combined maximum level of PCDD/F+DL-PCB (24 and 4.5 ng of WHO-TEQ/kg, respectively; 12% water basis). The corresponding content in the herring raw material on a wet weight basis (**Table 4**) was below the present maximum permitted levels in fish for human consumption (*46*) (2.25 and 6.0 ng of WHO-TEQ/kg, respectively). This is in good agreement with levels reported in fillets based on herring caught in the same region (*45*).

The experimental fishmeal was produced by mixing the intermediate products, press cake/jesma solids and stickwater concentrate, on the basis of the obtained process mass balance (**Table 1**). Consequently, the PCDD/F and DL-PCB levels in the intermediate products (**Table 4**; **Tables 1** and **2** of the Supporting Information) should reflect the level found in the fishmeal. However, the observed press cake/jesma solids and stickwater concentrate levels are both below the expected values. Analysis of the two intermediate products at another laboratory gave a higher PCDD/F+DL-PCB TEQ level in press cake/jesma solids (24%) and a lower level in stickwater concentrate (14%) (data not reported). The observed discrepancy can therefore not be explained by the uncertainty of the analytical methods and might also be caused by a systematic analytical problem linked to this type of sample matrix.

On the basis of the lipophilic nature of the studied POPs (*41, 42*) it can be assumed that the process partitioning is directly reflected by the fat partitioning data (**Table 3**). The level found in fishmeal (**Table 4**) will be controlled by the dry matter partitioning between press cake/jesma solids and stickwater concentrate and the fat content on dry matter basis obtained in the respective intermediate products (**Table 6**). Corrected for oil loss in the process, the combined mass balance (**Table 1**) and dioxin and PCB data (**Table 4**) can explain 98% of the PCDD/F+DL-PCB content in the raw material with a normalized partitioning between fishmeal and fish oil of 43 and 57%, respectively. Depending on the used analytical protocol, 75−85% of the fishmeal fat content originates from the press cake/jesma solids fraction (**Table 3**). This is in the upper part of the typical 65−80% range observed in commercial scale fishmeal production based on ethyl acetate extraction (Oterhals and Høstmark, Fiskeriforskning, Bergen, Norway, unpublished

Table 5. Effect of Protease (Alcalase) and Heat Treatment (121 °C) on Bl&D Fat Reduction in Press Cake/Jesma Solids and Stickwater Concentrate

	process variable (coded value)		fat reduction (%)	
expt no.	protease	heat	press cake/jesma solids	stickwater concentrate
1	−1	−1	2.9	64.0
2	1	−1	0.4	61.0
3	−1	1	0.7	65.0
4	1	1	0.7	65.0
mean ± SD			1.2 ± 1.2	63.8 ± 1.9

results). The obtainable level is dependent on type and quality of the raw material, process equipment, and conditions. In general, any actions directed on press cake and decanter solids (equivalent to jesma solids in this study) will have a higher fishmeal decontamination potential compared to the stickwater concentrate fraction.

Improved Mechanical Fat Separation. Neither enzyme nor heat treatment gave a significant increase in the fat separation compared to the reference conditions used in this study (**Table 5**). The overall effect on press cake/jesma solids was negligible and of no practical interest in fishmeal production. The highest effect observed in experiment 1 (**Table 5**) was caused by formation of an emulsion phase containing particulate material contributing to the total fat in the water phase. The use of high-temperature−short-time (HTST) treatment to split emulsions and coagulate protein before oil separation is described in the patent literature (*47*). The treatment did not give any additive effect in this study, but might find applications in the processing of decanter or stickwater with high content of fine particulate dry matter and emulsion problems.

The applied centrifugation conditions gave a substantial overall fat reduction in the stickwater concentrate (**Table 5**), corresponding to a fishmeal decontamination effect of 13% based on Bl&D fat. Fat separation of the stickwater concentrate is limited by the viscosity increase after concentration (*24*). To improve the efficiency, the separation step is often performed before the final concentration of the stickwater concentrate (**Figure 1**). Alcalase treatment is utilized by the fishmeal industry to reduce the viscosity and improve the dry matter obtainable in falling film evaporators. Although not documented in this study, this effect might also improve the second fat separation step if performed at a higher dry matter content compared to the level used in this study. Baron et al. (*30*) have studied the use of Alcalase treatment to reduce the fat content in fishmeal. The utilized hot water extraction conditions will also extract water-soluble inorganic and organic compounds, including protein. In industrial application, the water phase has to be added back to the fishmeal and dried together with the solid phase to restore the original fishmeal fat-free dry matter composition. Although a 20−30% fat reduction in the separated fishmeal solid phase was documented (*30*), no experimental data were given to document the possible separation of oil transferred to the water phase. Further studies are needed to elucidate this process limitation. In general, the use of enzyme treatment will also increase the water-soluble protein level and induce changes in the nutritional and physical properties of the fishmeal (*25*).

Organic Solvent Extraction. Hexane and isopropanol extraction reduced the fat level in the fishmeal to a very low level (**Table 6**). Isopropanol was the most effective organic solvent quantified by the EC and Bl&D methods. Due to very low residual fat levels quantified by the ethyl acetate and Soxhlet methods, these analytical methods were not able to differentiate between the two tested extraction conditions. The observed

Table 6. Fat Level (Grams per Kilogram of Dry Matter) before and after Organic Solvent Extraction of Fishmeal and Soybean Oil Extraction of Press Cake/Jesma Solids

	fat extraction method			
	ethyl acetate	Soxhlet[b]	EC[c]	Bl&D[d]
press cake/jesma solids	105	98	102	139
stickwater concentrate	91	112	147	158
fishmeal	111	108	123	145
hexane-extracted fishmeal	3	2	18	31
isopropanol-extracted fishmeal	2	2	10	17
soybean oil extracted press cake/jesma solids	107	104	124	144
soybean oil extracted fishmeal[a]	104	105	128	146

[a] Calculated on the basis of overall mass balance and combined content in soybean oil extracted press cake/jesma solids and stickwater concentrate. [b] See **Table 1**. [c] See **Table 1**. [d] See **Table 1**.

differences might be explained by the polarity of the tested organic solvents and their ability to extract phospholipids from the fishmeal (*26, 30, 44*). Excess hot solvent was used in the applied extraction procedure, and the residual fat levels (**Table 6**) can be expected to be lower than what is normally obtained in commercial scale countercurrent percolation extractors. Opstvedt and Hansen (*27*) reported residual lipid levels in four commercially produced hexane-extracted fishmeal samples to be in the 0.3−0.7 and 3.4−6.0% range based on Soxhlet (i.e., ethyl ether) and Bl&D extraction, respectively. Baron et al. (*30*) have conducted comparable extraction trials based on ethanol, isohexane, and isopropanol extraction of fishmeal at room temperature. Although a shorter contact time (30 min) was used in these trials, they obtained reductions of 85, 66, and 71% in total fat content, respectively, and a residual Bl&D extractable fat content of 2% after ethanol extraction. The results are in good agreement with the residual levels obtained in this study (**Table 6**). The lower Bl&D fat level obtained after isopropanol extraction (1.7%) might be explained by the use of hot solvent, higher solvent to matrix ratio, and longer extraction time.

Oil Extraction. The obtained soybean oil extracted press cake/jesma solids had a fat content close to the starting material (**Tables 1** and **6**). The results demonstrate the technical feasibility of removing the excess oil content after the extraction step based on the combined use of mechanical separation and water washing. The estimated fat content of soybean oil extracted fishmeal, based on the process mass balance and combined composition of soybean oil extracted press cake/jesma solids and stickwater concentrate, is close to the respective fat content in ordinary fishmeal from the process (**Table 6**). This is a critical success factor if the process should be used in industrial application. A high fat content in the fishmeal gives reduced flowability and is known to create problems during milling operations and in conveying and storage facilities. In contrast, solvent extraction gives a low fat fishmeal with sandy and dusty characteristics, properties that might introduce technical problems when used in feed pellet and extrusion operations. This can, however, be corrected by the addition of oil to the extracted fishmeal.

Decontamination Effect. Both organic solvent and soybean oil extraction resulted in fishmeal products with a TEQ level below the present maximum permitted levels (**Table 7**; **Table 2** of the Supporting Information). The lowest levels were observed in isopropanol-extracted fishmeal and soybean oil extracted press cake/jesma solids corresponding to a sum PCDD/F+DL-PCB-TEQ reduction of 88 and 97%, respectively. The less efficient decontamination rate based on hexane extraction (75%) might be explained by the higher Bl&D extractable

Table 7. Levels of PCDD/F and DL-PCB (Nanograms per Kilogram of Dry Matter) after Organic Solvent Extraction of Fishmeal and Soybean Oil Extraction of Press Cake/Jesma Solids

	hexane-extracted fishmeal	isopropanol-extracted fishmeal	soybean oil extracted press cake/jesma solids	soybean oil extracted fishmeal[a]
PCDDs	0.60	0.93	0.70	0.97
PCDFs	3.29	1.50	0.38	2.25
non-*ortho* PCBs	15.2	14.9	7.5	19.5
mono-*ortho* PCBs	374.4	221.6	133.6	866.8
PCDD/F reduction (%)[b]	67	79	91	73
DL-PCB reduction (%)[b]	90	94	96	77
PCDD/F-TEQ	0.94	0.44	0.09	0.64
DL-PCB-TEQ	0.24	0.12	0.07	0.44
TEQ ratio	3.91	3.51	1.42	
PCDD/F-TEQ reduction (%)[b]	67	85	97	77
DL-PCB-TEQ reduction (%)[b]	88	93	97	77
PCDD/F+DL-PCB-TEQ reduction (%)[b]	75	88	97	77

[a] Calculated on the basis of overall mass balance and combined soybean oil extracted press cake/jesma solids and estimated stickwater concentrate content. [b] Relative to the fishmeal level (**Table 4**).

fat content after this treatment (**Table 6**). The addition of stickwater concentrate to the soybean oil extracted press cake/jesma solids considerably increased the final POP level, corresponding to a fishmeal decontamination rate of 77% (**Table 7**). Due to the low analyzed level in stickwater concentrate (**Table 4**) the calculations are based on an estimated stickwater concentrate level equal to the fishmeal level on a Bl&D fat basis. The effect is caused by the high PCDD/F and DL-PCB concentration in the lipid phase of untreated stickwater concentrate. As demonstrated in this study, a second separation step reduced the fat level by 64% (**Table 5**). Assuming the same effect on the PCDD/F and DL-PCB levels, this combined treatment would give a fishmeal decontamination rate of 90%. Although not tested in this study, it is also possible to contact the stickwater concentrate with an oil with low dioxin level on the basis of the same principle as demonstrated for press cake/jesma solids. This would have the potential to bring the soybean oil extracted fishmeal level further down and render this decontamination alternative more efficient than organic solvent extraction. The obtained decontamination rates are higher than reported by Baron et al. (*30*) on the basis of olive oil extraction of fishmeal (60 and 75% of the PCDD/F- and DL-PCB-TEQ, respectively, transferred to the olive oil phase). The difference might be explained by the combined use of a higher extraction temperature (88 °C vs room temperature) and oil/matrix ratio (1:3 vs 1:1) in this study. A direct comparison is not possible due to the use of different solid matrices.

Decontamination rates based on congener concentrations show similar results (**Table 7**; **Table 1** of the Supporting Information). However, in the case of soybean oil extracted press cake/jesma solids less reduction of PCDD was observed compared to PCDF (**Table 7**). This was caused by high levels of the 1234678-HpCDD (1.3 ng/kg) and OCDD (18.4 ng/kg) congeners (**Table 1** of the Supporting Information) in the applied soybean oil, giving rise to less reduction and increased level, respectively, in the soybean oil extracted press cake/jesma solids. An increased level of the OCDD congener was also observed in isopropanol-extracted fishmeal, reducing the total PCDD decontamination effect. No information is, however, available on the level of PCDD/F in the used isopropanol. Due to the low TEF values of the two congeners (*40*) the effect on the TEQ decontamination rate was insignificant (**Table 7**).

PCDD/F to DL-PCB Ratio. An increased TEQ ratio between PCDD/F and DL-PCB in the fishmeal compared to the raw material was observed in this study (**Table 4**). The ratio was further increased after hexane and isopropanol extraction (**Table 7**). However, soybean oil extraction gave a TEQ ratio similar to that of the fishmeal (**Table 7**). The results indicate that PCDD/Fs have a stronger affinity to the extracted matrix compared to PCBs. The lipophilic nature of the compounds expressed as the octanol−water partition coefficient (K_{ow}) is in the same range (*41, 42*) and cannot explain the observed selectivity. A possible mechanism might be based on the partitioning of the two groups of compounds in membrane structures (i.e., phospholipids) and adipose tissue (i.e., triglycerides) in the fish. PCDD/Fs have a coplanar molecular conformation that fit into membrane structures. DL-PCBs have a twisted conformation due to steric effects (*48*), and this might shift the partition coefficient between membrane structures and adipose tissue. The wet rendering process used in the fishmeal industry mainly extracts the triglyceride fraction, and the fish oil product contains a very low level of phospholipids (*49*). Consequently, almost all membrane-bound lipids in the fish are retrieved in the fishmeal product. Such a mechanism might favor a higher retention of PCDD/Fs compared to DL-PCBs in the fishmeal and after solvent extraction. The nonselective response after oil extraction (**Table 7**) does not fit into such a model. More studies are needed to confirm this hypothesis.

Table 8. Ratio between WHO-TEQ Decontamination Effect and Fat Reduction after Organic Solvent Extraction of Fishmeal

		fat extraction method			
extraction solvent	WHO-TEQ	ethyl acetate	Soxhlet[a]	EC[b]	Bl&D[c]
hexane	PCDD/F	0.69	0.68	0.79	0.85
hexane	DL-PCB	0.90	0.89	1.03	1.12
hexane	av	0.80	0.79	0.91	0.98
isopropanol	PCDD/F	0.86	0.86	0.92	0.96
isopropanol	DL-PCB	0.96	0.96	1.02	1.06
isopropanol	av	0.91	0.91	0.97	1.01

[a] See **Table 1**. [b] See **Table 1**. [c] See **Table 1**.

Decontamination to Fat Reduction Ratio. Measurement of fat reduction based on the Bl&D method shows the best agreement to the observed decontamination rate after hexane and isopropanol extraction of fishmeal (**Table 8**). Ethyl acetate and Soxhlet extraction gave less satisfactory results. A systematic lower ratio is observed for PCDD/Fs, possibly reflecting the increased PCDD/F to DL-PCB ratio after solvent extraction discussed above (**Table 7**). The results indicate that a Bl&D or equivalent fat extraction protocol could be used to estimate the combined PCDD/F+DL-PCB-TEQ fishmeal de-

Table 9. Levels of Selected Fatty Acids (Grams per Kilogram of Lipid) in Soybean Oil and Press Cake/Jesma Solids before and after Soybean Oil Extraction

fatty acid	soybean oil	press cake/jesma solids[a]	soybean oil extracted press cake/jesma solids[a]	lipid phase exchange (%)
18:2 n-6	558	10	337	60
18:3 n-3	59	5	37	59
20:5 n-3	ND	56	16	72
22:6 n-3	ND	115	50	56

[a] Chloroform/methanol extract.

contamination effect of improved fat separation in the fishmeal process or after organic solvent extraction. More studies are needed to confirm this covariance.

Fatty Acid Exchange. The use of soybean oil in the extracting process was chosen to enable the assessment of fatty acid exchange between the press cake/jesma solids matrix and the continuous oil phase. The observed fatty acid composition after oil extraction (**Table 9**) reflects a high mobility of the lipid phase as reported by others (*30*). On the basis of selected fatty acids typical for fish oil (EPA and DHA) and soybean oil (linoleic and α-linolenic acid), an exchange of 56−72% of the lipids in press cake/jesma solids with soybean oil could be estimated (**Table 9**). Twenty to forty percent of the total lipids in fishmeal from herring are phospholipids (*50*), and the observed levels might be explained by a lower mobility of membrane-bound phospholipids in the press cake/jesma solids matrix. If desirable, the change in fatty acid composition can be avoided by use of fish oil instead of a vegetable oil in the oil extraction process.

Process Flow Diagram. The oil extraction process described in this paper can easily be integrated in an existing fishmeal and fish oil processing plant. A simplified process flow diagram is given in **Figure 1**. The intermediate products press cake and decanter solids are transferred to an extraction unit and contacted with low-dioxin fish oil. Excess fat in the solid matrix after the oil extraction step is removed by use of a decanter centrifuge. The decanter solids are mixed with hot water and separated a second time over a decanter centrifuge to reduce the fat level to the initial level. The oil/water phase from the decanter centrifuges is separated by use of a disk centrifuge. Separated water can be removed in the evaporator to recover dissolved dry matter or reused in the water washing step. Oil used in the extraction process might be recycled several times before it needs to be decontaminated. The number of applicable cycles will depend on the raw material dioxin level and the solid/oil ratio used in the extraction step. Decontamination of the used fish oil can be based on the same process equipment as utilized for ordinary fish oil produced at the plant. The described oil extraction process has several advantages compared to organic solvent extraction, for example, easy integration in an existing fishmeal processing line, use of a safe and nonflammable extraction medium, and expected lower investment and operation costs. In addition, the decontaminated fishmeal will have chemical composition and technical properties comparable to those of commercial fishmeal products.

The process can also be utilized for decontamination of stickwater concentrate, fish protein hydrolysates, and fishmeal. Use of the technology demands detailed knowledge on POP levels on a fat basis in different fish species relative to fishing areas, seasonal variation, and age. Such knowledge will enable the industry to predict the resulting fishmeal level and to make decisions on when the use of the oil extraction process is needed. More studies are needed to optimize the extraction process including the impact on fishmeal and fish oil quality, fat separation, scaling up, and operation costs.

Supporting Information Available: PCDD/F and DL-PCB congener concentration levels and congener WHO-TEQ levels in samples given in **Table 7**; fatty acid composition of samples given in **Table 9**. This material is available free of charge via the Internet at http://pubs.acs.org.

NOTE ADDED AFTER ASAP PUBLICATION

The original posting of February 20, 2008, contained coding for several symbols. These have been converted and are now correct in the posting of February 28, 2008.

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