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Increased extraction efficiency of dioxins and PCBs in fishmeal using a mixture of toluene:ethanol as extraction solvent compared to hexane:acetone and toluene

Guillaume ten Dam[1,2], Jeroen Markesteijn[1,2], Lotte Bech[3], Chris van Wakeren[1] and Wim Traag[1]
[1] DSP-Systems, Darwinstraat 7a, 6718XR Ede, The Netherlands, [2] QTI services B.V., Keenstraat 46, 3044CD Rotterdam, The Netherlands, [3] FF Skagen, Havnevagtvej 5, 9990 Skagen, Denmark

Goal

To share the latest findings of DPS-systems regarding the extraction of feed samples for the analysis of dioxins using a SER-158.

Introduction

Chemical analyses for the determination of contaminants in food or feed involve several critical steps. The extraction of analytes from a sample is one of those steps. While the extraction process should generally quantitatively extract the analyte from the matrix, other constituents are preferably not extracted as they can disturb the subsequent analytical processes.

For dioxins and PCBs, extraction methods for food and certain feedstuff is focussed on the extraction of the fat content of the sample. It is reasoned that the fat soluble dioxins and PCBs are generally dissolved within the fat content of a sample. So with the extracted fat, also the dioxins and PCBs are considered to be extracted. For fatty samples, hexane, cyclohexane and mixtures of hexane : acetone and cyclohexane : isopropanol are widely used for the quantitative extraction of fat.

Feedstuff however, can also consist of non-fatty materials like mineral feed, trace elements, premixtures and compound feed. It was established that for these kind of samples a mixture of toluene and a polar solvent yields the highest extraction efficiency[1].

Similarly, in 2012 the EURL for POPs reported results of a comparison study for the extraction efficiency of dioxins from liver. This study was performed on request of the European Food Safety Agency when they considered to change the expression of maximum levels from expression on the fat content of the sample to expression on the fresh weight of the sample. Experiments were performed with several extraction solvents and combinations hereof. Extraction with ethanol : toluene 70 : 30 v/v yielded the highest amount of extractable matter, which was considered to be lipids. Also the variation on the determined amount of fat using this solvent was considerably lower. The same was observed for the levels of dioxins. For egg similar results were obtained and these differences were assumed to relate to the phospholipid content in the samples.[2]

As phospholipids are a considerable constituent[3] of fishmeal, extraction efficiency of dioxins from fishmeal would also be lower when ethanol : toluene is not used as extraction solvent. This short communication describes a real-life case and results regarding

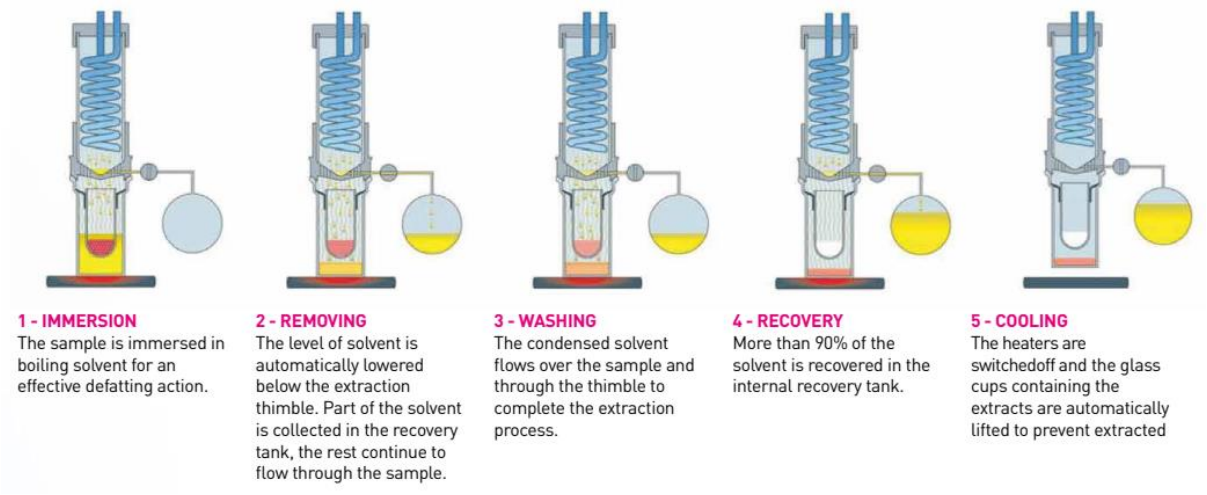

Figure 1 Illustration of the automated extraction sequence using a SER 158 from Velp

the extraction efficiency of dioxins form fishmeal.

In 2021 a sample fish meal was analysed by QTI Services B.V. which was commissioned by FF Skagen, a fishmeal producer. Results delivered by QTI Services B.V deviated from the expected values determined by FF Skagen. The expected values were based on historical results from fish meal supplied by the specific producer.

As for this difference the sample was re-analysed to obtain the same result and the discussion shifted towards the method used by QTI Services B.V. and the in-house method of FF Skagen. Both laboratories used almost the same methods, e.g. automated Soxhlet with a SER-158, followed by automated purification using a Miura GO-eHT and measurement with GC-HRMS. However, at FF Skagen extraction was performed with hexane : acetone while at QTI Services B.V. ethanol : toluene 70 : 30 v/v was used. Based on this observation an experiment was started to replicate the observation. The experiment and results are described below.

Experimental

A fish meal sample was obtained from FF Skagen for analysis. Additionally, the sole additive, an antioxidant, was send and analysed as well. Analysis was performed at QTI Services B.V. which holds an ISO 17025:2017 accreditation for the analysis of dioxins an PCBs under registration no. L678. The accredited method involves extraction of 5 gram fish meal with ethanol : toluene 70 : 30 v/v using a SER-158 (Velp) applying a automated Twisselman and Soxhlet method (figure 1). The residual oil and solvent is evaporated till dryness and reconstituted in 10 ml hexane. The hexane extract is automatically purified on a GO-eHT consisting of four columns containing silver nitrate dispersed in silica, sulfuric acid impregnated silica, carbon and alumina. The automated purification results in two fraction containing 1; Dioxins and non-ortho PCBs and 2; mono-ortho PCBs and non-dioxin-like PCBs each in 1.5 ml toluene. Both fractions are concentrated till dryness and reconstituted in 40 µl injection standard solution containing (1,2,3,4-TCDD in dodecane (Cambridge Isotope Laboratories)). Internal standards (Cambridge Isotope Laboratories) for recovery correction are added before extraction and incubated for 1 hour. Both extracts are analysed with a GC-HRMS from Thermo Scientific.

To determine the extraction efficiencies between 1; ethanol : toluene 30 / 70 v/v, 2; hexane : acetone 88 / 12 v/v and 3; toluene three experiments were performed. For the third experiment another sample fish meal was used as the initial sample was used up for the first two experiments.

Experiment 1; The sample fish meal was extracted according the accredited method to obtain extract 1.1. After extraction internal standards were added again to the residual sample. The sample was extracted once again according the accredited method to obtain extract 1.2.

Experiment 2; The sample fish meal was extracted according the accredited method accept for extraction solvent. The extraction solvent was changed to hexane : acetone 88 : 12 v/v to obtain extract 2.1. After extraction internal standards were added again to the residual sample. The sample was extracted once again, but this time completely according the accredited method to obtain extract 2.2.

Experiment 3; Another sample fish meal was extracted according the accredited method accept for extraction solvent. The extraction solvent was changed to toluene to obtain extract 3.1. The sample was extracted once again, but this time completely according the accredited method to obtain extract 3.2.

In all six residues the mass of the residue was determined gravimetrically and further analysed according the accredited method. The triglyceride content was also determined according NEN-EN-ISO 660:2021[4].

Additionally, FF Skagen analysed 23 samples fish meal, a sample citrus pulp and their control sample.

Results and discussion

Results for the three initial extractions were varying and results of dioxins differed more

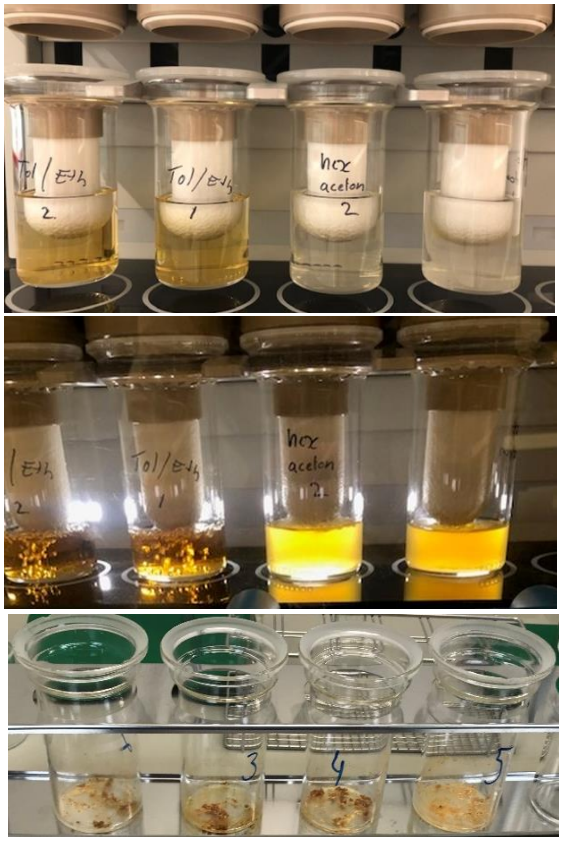

Figure 2 Pictures of the extract during the extraction process; top after adding solvent, middle after Twisselman and Soxhlet extraction and bottom after evaporation of the solvent. From left to right twice hexane : acetone and twice toluene ethanol.

then the results of the gravimetrical determination of the extracted residues (table 1 and 2). The appearance of the extracted matter also differed (fig. 2). After initial extraction with hexane : acetone and toluene the secondary extraction with toluene : ethanol yielded in both cases a substantial amount of additional residues. Additionally, the secondary extraction with ethanol : toluene yielded also a considerable amount of dioxins in experiment 1 and 3.

The results of the analysis triglyceride content was 9.7%. As the total residue content was 24%, 14 % of the extracted matter is unaccounted for. With the hexane : acetone and toluene extractions the total residue contents were 11% and 12%. The total

triglyceride content was not analysed in these samples.

The experiments with toluene : ethanol and hexane : acetone were repeated by FF Skagen for 23 samples fish meal (table 3). Differences in results varied by sample from slightly lower till over 50% higher using toluene : ethanol. A higher yield was also observed for the control sample and the sample citrus pulp.

These results support the suggestion by the EURL for POPs regarding extraction efficiencies of dioxins in materials containing considerable amounts of phospholipids.[2] For such samples method Smedes[5] is generally considered as an exhaustive extraction. The described method in this publication using a combined Twisselman and Soxhlet extraction was not compared to method Smedes.

Although the analysis of Dioxins has a strong historical background, challenges to obtain accurate results are still current. This is shown with the results presented in this publication. More specific, the difficulty of extracting the total lipid content from fish, and thus the total amount Dioxins, was also demonstrated in 2020 studied by Haedrich et al.[6] (fig. 1).

In conclusion, extraction efficiencies vary depending on the applied methods and method parameters. This depends on the sample type as well and for fish meals studied it was demonstrated that the residual content after extraction is not directly proportional to the determined total amount of Dioxins.

Acknowledgements

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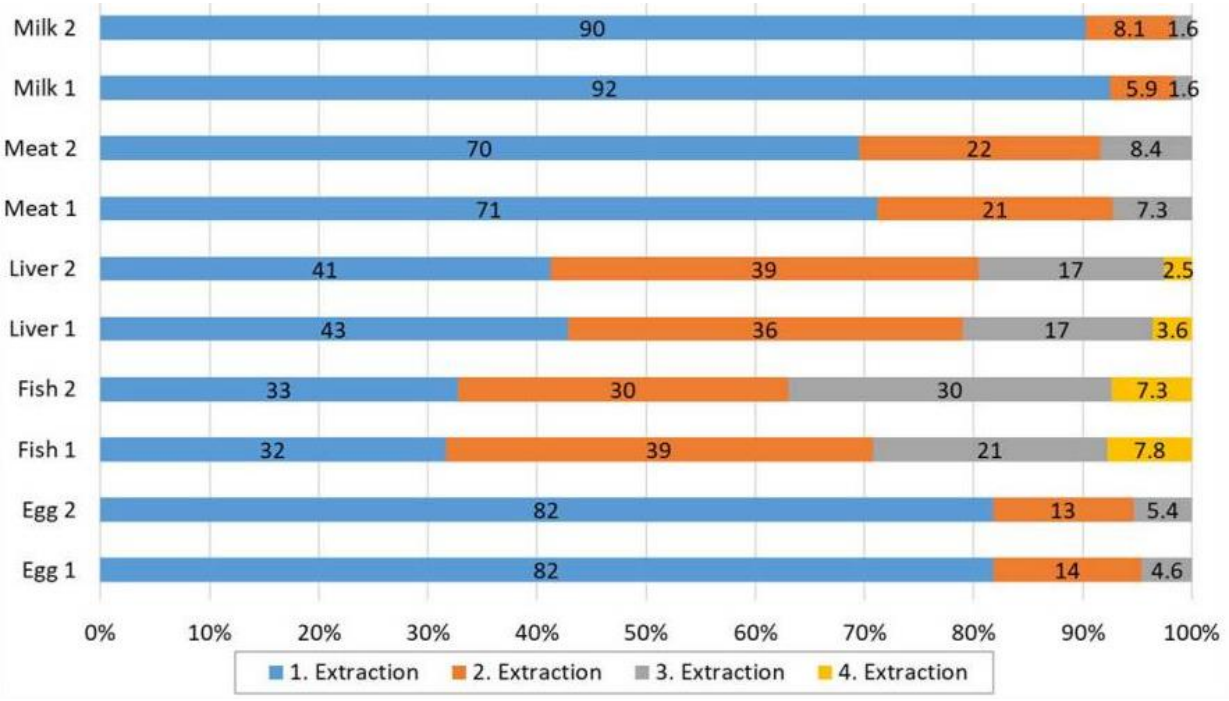

Figure 3 Lipid extraction profles (in duplicate) from applying the modifed Smedes extraction (MSE, this work) under within-lab reproducibility conditions for chicken eggs, fsh meat (brown trout), bovine liver, bovine meat (beef neck) and cow's whole milk. Amounts are given in % of total extracted lipids. (Illustration by Haedrich et al. 2020[4])

References

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4 NEN-EN-ISO 660:2021, Animal and vegetable fats and oils - Determination of acid value and acidity

5 Smedes F, Determination of total lipid using non-chlorinated solvents., 1999, Analyst 124:1711–1718

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Table 1a Results of the analysis of Dioxins, extractable content and triglyceride content

	Subsample 1		Subsample 2		Subsample 3		Sub-sample 4	Anti-oxidant
Extraction nr.	1	2	1	2	1	2		
Solvent	Hexane Acetone	Ethanol Toluene	Ethanol Toluene	Ethanol Toluene	Toluene	Ethanol Toluene	NEN-EN-ISO 660:2021	Ethanol Toluene
residue	0,537 g (11%)	0,51 g (10%)	1,19 g (24%)	0,015 g (0,3%)	0,581 g (12%)	0,285 g (5,7%)	-	-
Triglyceride content	-	-	-	-			9.7%	-
PCDD/F pg TEQ/g WHO2005 (lowerbound)	1,13	0,44	1,50	0,02	1,25	0,12		0,02
Unit	ng/kg	ng/kg	ng/kg	ng/kg	ng/kg	ng/kg	ng/kg	ng/kg
2,3,7,8-TCDD	0,05	0,06	0,11	<0,025	0,08	<0,025	-	<0,025
1,2,3,7,8-PeCDD	0,19	0,05	0,27	<0,025	0,21	<0,025	-	<0,025
1,2,3,4,7,8-HxCDD	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	-	<0,05
1,2,3,6,7,8-HxCDD	0,18	<0,05	0,17	<0,05	0,20	<0,05	-	<0,05
1,2,3,7,8,9-HxCDD	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	-	<0,05
1,2,3,4,6,7,8-HpCDD	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	-	<0,10
OCDD	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	-	0,85
2,3,7,8-TCDF	2,3	2	3,9	0,19	2,6	0,50	-	0,19
1,2,3,7,8-PeCDF	0,3	0,25	0,51	<0,05	0,38	0,08	-	<0,05
2,3,4,7,8-PeCDF	2,0	0,41	2,2	<0,05	2,1	0,22	-	<0,05
1,2,3,4,7,8-HxCDF	0,07	<0,05	0,09	<0,05	0,11	<0,05	-	<0,05
1,2,3,6,7,8-HxCDF	0,12	<0,05	0,12	<0,05	0,12	<0,05	-	<0,05
2,3,4,6,7,8-HxCDF	0,13	<0,05	0,15	<0,05	0,16	<0,05	-	<0,05
1,2,3,7,8,9-HxCDF	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	-	<0,05
1,2,3,4,6,7,8-HpCDF	<0,10	<0,10	0,11	<0,10	0,11	<0,10	-	<0,10
1,2,3,4,7,8,9-HpCDF	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	-	<0,1
OCDF	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	-	<0,20

Table 1b Results of the analysis of PCBs

	Subsample 1		Subsample 2		Subsample 3		Sub-sample 4	Anti-oxidant
Extraction nr.	1	2	1	2	1	2		
Solvent	Hexane Acetone	Ethanol Toluene	Ethanol Toluene	Ethanol Toluene	Toluene	Ethanol Toluene	Ethanol Toluene	Ethanol Toluene
PCB 77	32	4,1	33	<2	35	2,6	-	-
PCB 81	<2	<2	<2	<2	<2	<2	-	-
PCB 126	9,2	0,82	10	<0,5	12	<0,5	-	-
PCB 169	2,4	<2	2,4	<2	2,5	<2	-	-
PCB 105	903	109	1051	17	936	59	-	-
PCB 114	47	<10	44	<10	44	<10	-	-
PCB 118	2217	234	2378	42	2184	109	-	-
PCB 123	43	<10	29	<10	31	<10	-	-
PCB 156	264	26	248	<10	249	14	-	-
PCB 157	67	<10	68	<10	68	<10	-	-
PCB 167	114	11	114	<10	102	<10	-	-
PCB 189	16	<10	17	<10	17	<10	-	-
Unit	µg/kg	ug/kg	ug/kg	µg/kg	µg/kg	µg/kg	-	-
PCB 28	0,24	<0,05	0,23	<0,05	0,26	<0,05	-	-
PCB 52	0,6	0,07	0,65	<0,05	0,63	<0,05	-	-
PCB 101	2,1	0,23	2,2	<0,05	2,0	0,11	-	-
PCB 138	3	0,34	2,9	0,06	2,8	0,16	-	-
PCB 153	3,5	0,36	3,6	0,07	3,4	0,18	-	-
PCB 180	0,82	0,09	0,87	<0,05	0,83	<0,05	-	-

Table 2 Recovery the internal standards of the analysis of Dioxins and PCBs

	Subsample 1		Subsample 2		Subsample 3		Antooxidant
Extraction nr.	1	2	1	2	1	2	1
Solvent	Hexane Acetone	Ethanol Toluene	Ethanol Toluene	Hexane Acetone	Toluene	Ethanol Toluene	Ethanol Toluene
13C Internal standard	%	%	%	%	%	%	%
2,3,7,8-TCDD	98	94	85	92	85	81	-
1,2,3,7,8-PeCDD	92	95	75	87	86	84	-
1,2,3,4,7,8-HxCDD	76	81	70	70	80	80	-
1,2,3,6,7,8-HxCDD	83	80	69	79	80	81	-
1,2,3,7,8,9-HxCDD	78	79	65	76	85	87	-
1,2,3,4,6,7,8-HpCDD	68	77	73	78	94	95	-
OCDD	69	65	62	64	89	95	-
2,3,7,8-TCDF	110	104	96	95	90	83	-
1,2,3,7,8-PeCDF	86	92	79	87	82	78	-
2,3,4,7,8-PeCDF	94	97	85	89	88	80	-
1,2,3,4,7,8-HxCDF	80	80	75	82	76	78	-
1,2,3,6,7,8-HxCDF	79	83	70	82	80	80	-
2,3,4,6,7,8-HxCDF	83	87	74	78	78	77	-
1,2,3,7,8,9-HxCDF	81	88	75	80	83	81	-
1,2,3,4,6,7,8-HpCDF	76	77	70	71	87	89	-
1,2,3,4,7,8,9-HpCDF	74	81	70	98	86	89	-
OCDF	69	65	62	64	89	95	-
PCB 77	76	83	69	78	81	74	-
PCB 81	78	85	69	81	83	74	-
PCB 126	80	85	67	81	77	77	-
PCB 169	77	89	71	85	82	79	-
PCB 105	-	-	-	-	-	-	-
PCB 114	-	-	-	-	-	-	-
PCB 118	-	-	-	-	-	-	-
PCB 123	-	-	-	-	-	-	-
PCB 156	-	-	-	-	-	-	-
PCB 157	-	-	-	-	-	-	-
PCB 167	-	-	-	-	-	-	-
PCB 189	-	-	-	-	-	-	-
PCB 28	-	-	-	-	-	-	-
PCB 52	-	-	-	-	-	-	-
PCB 101	-	-	-	-	-	-	-
PCB 138	-	-	-	-	-	-	-
PCB 153	-	-	-	-	-	-	-
PCB 180	-	-	-	-	-	-	-

Table 3 Results of the analysis of Dioxins by FF Skagen

	WHO-PCDD/F-TEQ upper bound 12 % moisture		
Sample weight	Hexane Acetone	Ethanol Toluene	Diffference (Ethanol:Toluene - Hexane:Acetone / Hexane : Acetone x 100)
Fish meal	0.447	0.416	-7
Fish meal	0.677	0.792	17
Fish meal	0.484	0.451	-7
Fish meal	0.778	0.852	10
Fish meal	0.969	1.05	8
Fish meal	0.816	0.866	6
Fish meal	0.533	0.652	22
Fish meal	0.474	0.597	26
Fish meal	0.512	0.659	29
Fish meal	0.286	0.256	-10
Fish meal	0.574	0.664	16
Fish meal	0.600	0.556	-7
Fish meal	0.499	0.645	29
Fish meal	0.589	0.578	-2
Fish meal	0.559	0.675	21
Fish meal	0.587	0.625	6
Fish meal	0.490	0.539	10
Fish meal	1.14	1.602	41
Fish meal	0.623	0.912	46
Fish meal	0.410	0.487	19
Fish meal	0.645	0.712	10
Fish meal	0.597	0.729	22
Fish meal	0.460	0.556	21
Citrus pulp	0.412	0.478	16
Control sample	0.931	1.43	54