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Food safety and added value of Icelandic fishmeal. Determination of toxic and non-toxic arsenic species in fish meal

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Report summary

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Titill / Title	**Food safety and added value of Icelandic fishmeal – Determination of toxic and non-toxic arsenic species in fish meal** / Verðmæti og öryggi íslensks fiskimjöls – Greining eitraðra og hættulausra efnaforma arsens í fiskimjöli		
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Ágrip á íslensku:	Í lífríkinu er mikið til af arseni í lífrænum efnasamböndum sem og á ólífrænu formi og hafa fundist meira en 50 náttúruleg efnaform af arseni. Sjávarfang inniheldur frá náttúrunnar hendi háan styrk heildararsens miðað við t.d. landbúnaðarafurðir. Stærsti hluti arsens í sjávarfangi er hins vegar bundið á lífrænu formi sem kallast arsenóbetaníð, sem er talið hættulaust. Önnur form arsens í sjávarafurðum eru að jafnaði til staðar í lægri styrk, m.a. ólífrænt arsen (arsenít og arsenat) sem er eitrað og fer sjaldan yfir 3% af heildarstyrk arsens í fiski og krabbadýrum. Formgreining arsens í sjávarfangi er mikilvæg vegna þess að upptaka (bioavailability) og eiturvirkni arsens er háð því á hvaða efnaformi það er. Nýlega kallaði EFSA (European Food Safety Authority) eftir upplýsingum um ólífræn og lífræn efnaform arsens í fæðu og eftir efnagreiningaraðferðum til að greina ólífrænt arsen. Í þessari ritgerð koma fram niðurstöður og mat á mælingum á heildarstyrk í yfir 100 sýnum af íslensku fiskimjöli. Meðal annars var skoðað hvort árstíðamunur á heildarstyrk arsens væri til staðar. Sýnin voru fyrst brotin niður með örbylgjun og því næst mæld á ICP massagreini, ICP-MS (Inductively coupled plasma mass spectrometry). Til að meta hvaða efnaform arsens eru til staðar í mjölinu var fyrst þróuð þrískipt úrhlutunaraðferð. Síðan var áhersla lögð á greiningu eitraðs ólífræns arsens. Áður birt alkalí-alkóhól úrhlutunaraðferð, til að greina ólífrænt arsen, var aðlöguð og sýnin mæld með HPLC búnaði tengdum við ICP-MS. Í ljós kom að arsenóbetaníð var í öllum tilfellum ríkjandi efnaform arsens. Ólífrænt arsen reyndist vera undir fjórum prósentum af heildarstyrk í tólf mældum fiskimjölssýnum. Aftur á móti kom í ljós, þegar annarri efnagreiningartækni (HPLC-HGAFS) var beitt á sýni af stöðluðu viðmiðunarefni (certified reference material), að styrkur ólífræns arsens mældist þrisvar sinnum lægri. Reyndist alkalí-alkóhól úrhlutunaraðferðin gefa sannfærandi efri mörk á styrk ólífræns arsens. Niðurstöðurnar sýna ennfremur að ekki er nóg að reiða sig á eina aðferð þegar efnaform arsens eru greind og magngreind. Aukinheldur sýna þær nauðsyn á vottuðum styrk ólífræns arsens í stöðluðu viðmunarefni til að kanna áreiðanleika efnagreiningaraðferða. Þörfin fyrir frekari þróun efnagreiningaaðferða á þessu sviði er brýn.		
Lykilorð á íslensku:	*Fæðuöryggi, arsen form, ólífrænt arsen, fiskimjöl*		

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ummary in English:	Arsenic is found in the biosphere both in organic and inorganic forms, and there have been recognized more than 50 naturally occurring arsenic species. Seafood products have naturally high concentration of total arsenic compared to e.g. agricultural produce. Arsenic is toxic to humans and animals and is known to be carcinogenic. The toxicity of the arsenic species varies severely and a large portion of the arsenic in seafood is present in the form of the organic compound arsenobetaine, which is considered non-toxic. Other arsenic species are generally present in lower concentrations, including the most toxic inorganic arsenic species, arsenite, As(III) and arsenate, As(V), which usually do not exceed 3% of the total arsenic in fish and crustaceans. Existent European regulations on limits of arsenic in foodstuff and feed only take into account total arsenic concentration, not the toxic arsenic species. Recently the EFSA (European Food Safety Authority) stressed the need for more data on levels of organic and inorganic arsenic in different foodstuffs and the need for robust validated analytical methods for the determination of inorganic arsenic. In this thesis results from total arsenic concentration from over 100 samples of Icelandic fish meal are presented and evaluated. The samples were microwave digested and measured with inductively coupled plasma mass spectrometry (ICP-MS). The samples were screened for a seasonal difference in the total arsenic concentration. To evaluate the arsenic species present in the meal a sequential method of extraction was developed. In addition, a special focus was on the determination of inorganic arsenic and a previously published method for an alkaline-alcoholic extraction of the inorganic arsenic was modified and applied. For determination of arsenic species high pressure liquid chromatography (HPLC) was coupled to the ICP-MS. The predominant arsenic species found in all samples was the non-toxic arsenobetaine. Inorganic arsenic was found not to exceed 4% of total arsenic concentration in 12 samples of fish meal. However, a suspicion of co-elution arose, and when another analytical instrument technique (Hydride generation atomic fluorescence spectroscopy (HPLC-HG-AFS)) was applied, concentration of inorganic arsenic was approximately three times lower in a certified reference material, TORT-2. The alkaline-alcoholic extraction method was found to give convincing upper limits of the inorganic arsenic concentration in fish meal samples. These results show the necessity of further method development and separate methods when identifying and quantifying species. This furthermore stresses the need for a certified value of inorganic arsenic in a certified material to check the robustness of developed methods.
English keywords:	*Food safety, arsenic species, inorganic arsenic, fish meal*

Table of Contents

1 INTRODUCTION AND AIM

Arsenic is found in the biosphere both in organic and inorganic forms, and there have been recognized more than 50 naturally occurring arsenic species, main species are shown in Table 1 on page 3. The toxicity of arsenic is highly dependent on the chemical form[2]. Organoarsenic species such as arsenobetaine are considered non-toxic. The inorganic arsenic (As(III), As(V)) is the most toxic followed by the simple methylated compounds [3]. Human exposure to arsenic is mainly through intake of food and beverages [4]. Normally, arsenic is found in low levels in natural water except for specific regions of the world, e.g. West Bengal [5]. Consumers who are most exposed to arsenic are those with a high consumption of seafood or people from areas where the drinking water is high in arsenic. To assess the health risk associated with ingestion of arsenic in food the variation in toxicity of the arsenic species must be taken into account rather than only the total concentration. Special notice should be taken of species that are toxicologically important, especially the inorganic arsenic. Soluble inorganic arsenic is rapidly and almost completely absorbed after ingestion in humans. The absorption of different organic arsenic species is generally greater than 70%. After the absorption the arsenic is widely distributed to almost all organs [6].

Recently the European Food Safety Authority (EFSA) published a scientific opinion [6] where the risk related to the presence of arsenic in food to human health was assessed. From over 100,000 occurrence data on arsenic in food approximately 98% were reported as total arsenic where only a few investigations took various arsenic species into account. Since representative speciation data is limited the EFSA Panel on Contaminants in the food chain (CONTAM Panel) could not assess typical ratios between inorganic and organic arsenic in foodstuffs. For exposure assessment based on the limited data on inorganic arsenic a number of assumptions were made for the estimation of the contribution of inorganic arsenic to total arsenic. The proportion of inorganic arsenic was assumed to range from 50 to 100% of the total arsenic in food other than fish and seafood. In fish and seafood the proportion of inorganic arsenic is small and tends to decrease as the total arsenic concentration increases, where the ratio depends on the seafood type. A considered realistic value for calculating human dietary exposure was set as a fixed value for inorganic arsenic of 0.03 mg/kg in fish and 0.1 mg/kg in seafood [6]. The provisional tolerable weekly intake (PTWI) of 15 µg/kg body weight (b.w.) was established by the World Health Organisation (WHO) in 1989 [7]. Since then new data that establishes that inorganic arsenic causes cancer in the lungs, the urinary tract, the skin, as well as other adverse effects, has been reported at lower exposure levels than previously considered. The PTWI value of 15 µg/kg is thus no longer appropriate according to the EFSA opinion. The CONTAM Panel has recommended that dietary exposure to inorganic arsenic should be reduced and in order to refine the risk assessment of inorganic arsenic a need for more extensive speciation data for different food commodities is needed [6].

The EU commission has not established maximum levels for total or inorganic arsenic in foodstuffs but maximum levels for total arsenic have been established in animal feeding stuffs [8]. If inorganic/total arsenic ratio varied within a narrow range for different food- or feedingstuffs so that reliable extrapolations were possible, maximum levels for total arsenic might be justifiable. However, generally this does not appear to be the case [9]. Year 2003 the EU commission recognised that more than 95% of the arsenic present in feed materials of marine origin is in the less toxic organic forms and revised the maximum contents permitted for undesirable substances in animal feed [10] and this change enter into

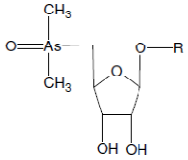
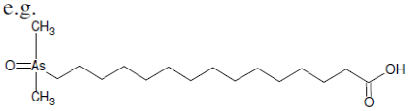
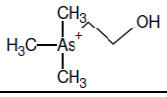
force with the European Parliament Directive 2002/32/EC [10]. When the present work started, year 2008, the maximum level of arsenic in feedingstuffs obtained from the processing of fish or other marine animals was 15 mg/kg, while for seaweed meal and feed materials derived from seaweed it was 40 mg/kg and for complete feedingstuffs for fish, 6 mg/kg [11]. Even though the high percentage of organoarsenicals in material of marine origin was taken into consideration when these maximum levels entered into force with Directive 2002/32/EC, feedingstuffs with low levels of inorganic arsenic but high levels of total arsenic were still at a risk of unnecessary exclusion from the market. However, shortly after the EFSA opinion was published (October 2009) [6] the EU commission amended Directive 2002/32/EC and raised the maximum levels of total arsenic further (November 2009) [8]:

> *As regards feedingstuffs obtained from the processing of fish or other marine animals, recent information provided by competent authorities of the Member States on the presence of total arsenic (sum of organic and inorganic arsenic) indicates that it is necessary to increase certain maximum levels for total arsenic. By- products of the fish filleting industry are valuable raw materials for the production of fish meal and fish oil for use in compound feed, in particular fish feed [8].*

The amendment further states that the increase of the maximum levels for total arsenic does not entail a change in the maximum levels for inorganic arsenic, and thus the increased levels for total arsenic does not affect the protection of animal and human health [8]. Hence the current maximum level of arsenic in feedingstuffs obtained from the processing of fish or other marine animals is 25 mg/kg, while for seaweed meal and feed materials derived from seaweed it is 40 mg/kg and for complete feedingstuffs for fish it is 10 mg/kg [8]. Nevertheless, the responsible operator must perform an analysis to demonstrate that the content of inorganic arsenic is lower than 2 mg/kg upon a request of the competent authorities [8].

Arsenic species and their abbreviation and chemical structure are illustrated in Table 1.

Table 1: Names, abbreviations and chemical structures for selected arsenic species.

Name	Abbreviation	Chemical structure
Arsenite	As(III)	$As(O^-)_3$
Arsenate	As(V)	$AsO(O^-)_3$
Arsenobetaine	AB	$(CH_3)_3As^+CH_2COO^-$
Arsenosugars		
Arsenolipids		e.g. 
Trimethylarsonio propionate	TMAP	$(CH_3)_3As^+CH_2CH_2COO^-$
Methylarsonate	MA	$CH_3AsO(O^-)_2$
Methylarsonite	MA(III)	$CH_3As(O^-)_2$
Dimethylarsinate	DMA	$(CH_3)_2AsO(O^-)$
Dimethylarsinite	DMA(III)	$(CH_3)_2AsO^-$
Trimethylarsine oxide	TMAO	$(CH_3)_3AsO$
Tetramethylarsonium ion	TETRA	$(CH_3)_4As^+$
Arsenocholine	AC	

The main aims of this project were:

- Determine the total arsenic concentration in Icelandic fish meal and screen for seasonal differences.
- Develop analytical techniques for determination of toxic and nontoxic arsenic species in fish meal.
- Evaluate the number and quantity of toxic arsenic species in Icelandic fish meal.
- Include analysis of seafood based certified reference material/s to provide arsenic speciation data for comparative purposes.
- Discuss the obtained results in relation to the maximum contents of total arsenic permitted according to EU regulations.

2 MATERIAL AND METHODS

This was a two year project and an overview of the project plan is shown in Figure 1. Altogether the project was separated into six different work packages (**WP1 – WP6**). Cooperation in method development and identification of arsenic species was with Prof. Jörg Feldmann, University of Aberdeen, Scotland. Details on the analytical method and material used are described in the M.Sc. thesis of Ásta H. E. Pétursdóttir, entitled “Determination of toxic and non-toxic arsenic species in Icelandic fish meal”.

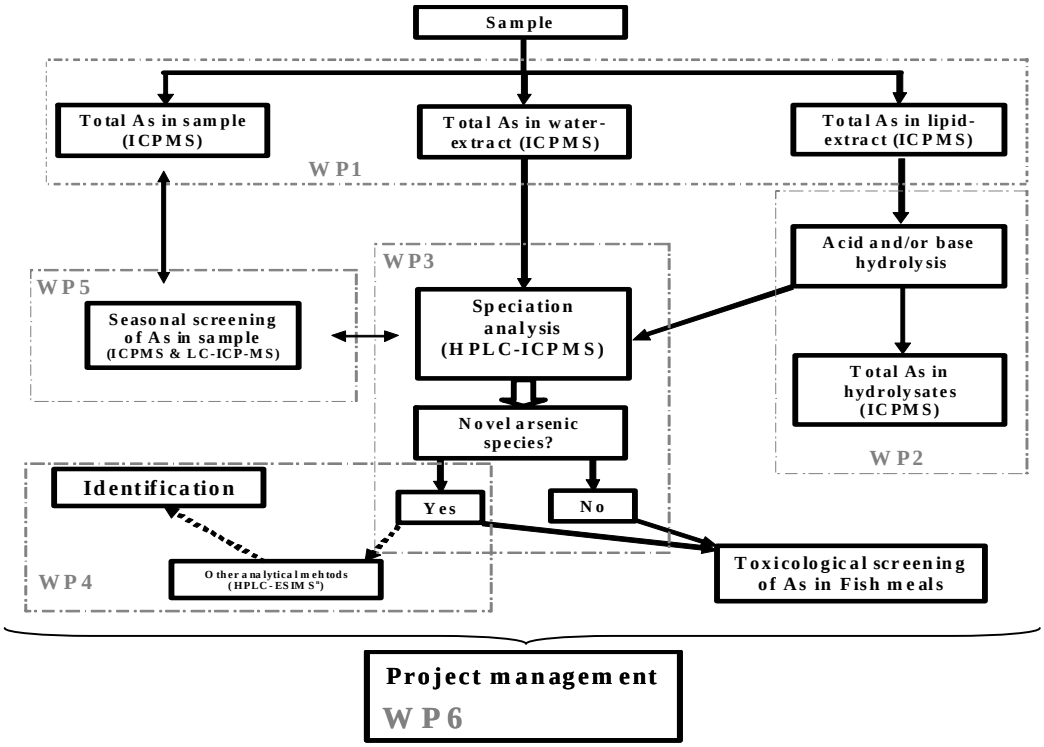


Figure 1: Schematic layout of the Work Packages

3 RESULTS AND DISCUSSION

Aim: To determine the total arsenic concentration in Icelandic fish meal and extraction of water – and lipid-soluble arsenic

3.1 Total element analysis and arsenic extraction

3.1.1 Analysis of total arsenic

Approximately 100 different fish meal samples from three species, blue whiting, capelin and herring, were analysed for total arsenic concentration. This is shown for each species in Figure 2, Figure 3 and Figure 4.

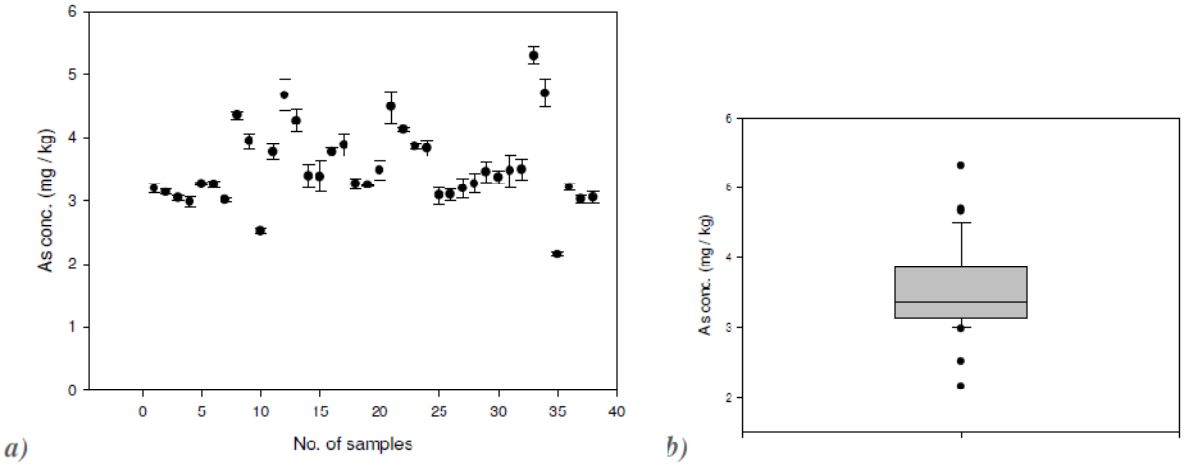


Figure 2: a) Distribution of the total arsenic concentration for all herring samples. b) Box plot of the herring samples

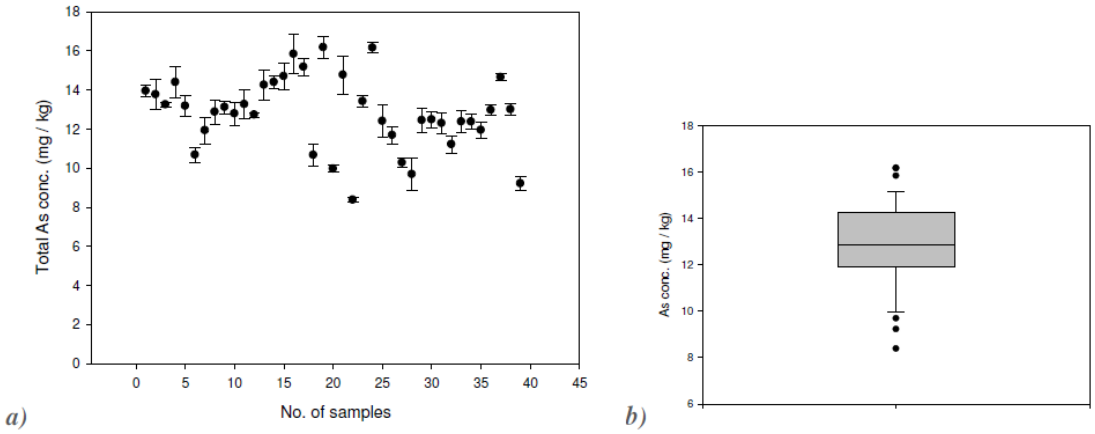


Figure 3: a) Distribution of the total arsenic concentration for all the blue whiting samples. b) Box plot for the blue whiting samples.

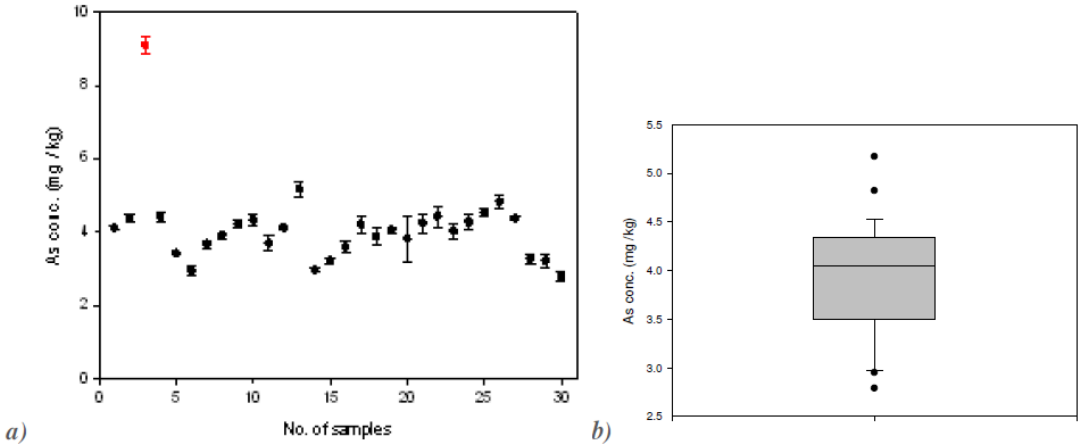


Figure 4: a) Distribution of the total arsenic concentration for all the capelin samples. b) Box plot for the capelin samples.

One capelin samples was classified as a outlier with a Q-test (p<0,01) and dismissed from the dataset.

These results provide reliable reference data about the average arsenic concentration in fish meal samples from each species.

3.1.2 Extraction of water and lipid soluble arsenic:

A sequential extraction method was developed as described in Figure 5. A detailed method description is in the supporting document for this report [1].

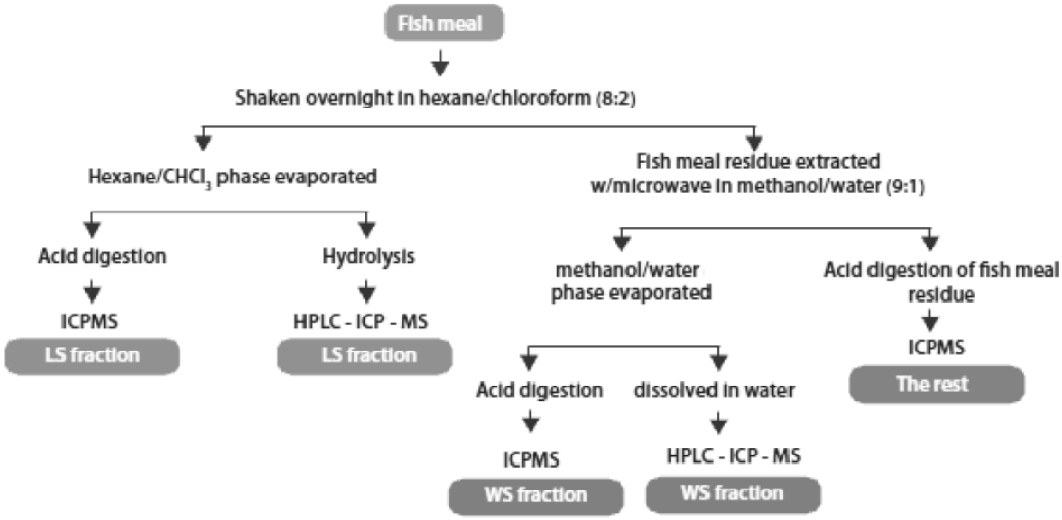


Figure 5: A scheme of the sequential extraction method.

Each residue was analysed with ICP-MS to determine the arsenic concentration in each fraction as described in Table 2.

Table 2: The concentration of each arsenic fraction extracted from 15 fish meal samples using sequential extraction.

Samples[a]	Total As	Concentration As extracted from total As [mg/kg]				
	[mg/kg]	Hexane/ $CHCl_3$	H2O/ MeOH	Acid digestion of the rest	Sum of steps	Recovery (%)
	(acid digestion)	Step I (Lipid sol.)	Step II (Water sol.)	Step III (The rest)	(I+II+III)	
Herring						
H1	4.35 ± 0.06	0.84 ± 0.06	3.20 ± 0.02	0.56 ± 0.01	4.55 ± 0.08	106 ± 2
H2	5.31 ± 0.15	1.09 ± 0.05	3.27 ± 0.03	0.49 ± 0.03	4.85 ± 0.04	91 ± 1
H3	3.48 ± 0.15	0.81 ± 0.02	2.18 ± 0.03	0.45 ± 0.01	3.44 ± 0.02	99 ± 1
H4	3.45 ± 0.15	1.15 ± 0.04	1.50 ± 0.03	0.51 ± 0.01	3.16 ± 0.14	92 ± 1
H5	2.51 ± 0.04	0.71 ± 0.04	1.44 ± 0.01	0.36 ± 0.01	2.51 ± 0.03	100 ± 1
Blue whiting						
B1	14.0 ± 0.3	0.19 ± 0.01	12.4 ± 1.7	1.25 ± 0.04	13.9 ± 1.6	99 ± 12
B2	16.2 ± 0.6	0.23 ± 0.01	14.7 ± 0.5	1.09 ± 0.01	16.0 ± 0.5	99 ± 3
B3	12.4 ± 0.6	0.13 ± 0.03	10.6 ± 0.2	0.76 ± 0.03	11.5 ± 0.2	93 ± 2
B4	8.4 ± 0.1	0.29 ± 0.07	8.3 ± 0.4	0.69 ± 0.01	9.3 ± 0.3	111 ± 4
B5	13.3 ± 0.7	0.17 ± 0.01	11.9 ± 0.1	0.89 ± 0.04	12.9 ± 0.1	97 ± 1
Capelin						
C1	4.11 ± 0.06	1.32 ± 0.13	2.35 ± 0.04	0.74 ± 0.16	4.41 ± 0.33	107 ± 8
C2	5.17 ± 0.20	0.52 ± 0.04	3.29 ± 0.04	0.57 ± 0.03	4.38 ± 0.05	85 ± 1
C3	4.20 ± 0.22	1.10 ± 0.02	1.97 ± 0.03	0.53 ± 0.01	3.60 ± 0.02	86 ± 1
C4	4.42 ± 0.29	1.11 ± 0.08	1.97 ± 0.05	0.55 ± 0.01	3.63 ± 0.06	82 ± 2
C5	2.95 ± 0.11	0.87 ± 0.02	1.62 ± 0.05	0.54 ± 0.04	3.03 ± 0.06	103 ± 2

[a]Water content of the fish meal samples ranged from 4.5-8.3%, concentration given on a product weight basis. Details about the fish meal samples is described elsewhere [1].

Table 3: The mass balance between the three fractions for 15 fish meal samples.

Samples	Lipid soluble fraction (% of total As conc.) Step I	Water soluble fraction (% of total As conc.) Step II	The rest (% of total As conc.) Step III
Herring			
H1	19.3	73.6	12.9
H2	20.5	61.6	9.2
H3	23.3	62.6	13.0
H4	33.3	43.5	14.8
H5	28.3	57.4	14.3
Average:	*25 ± 6*	*60 ± 11*	*13 ± 2*
Blue whiting			
B1	1.4	89.0	9.0
B2	1.4	90.7	6.7
B3	1.1	85.4	6.1
B4	3.5	98.8	8.2
B5	1.3	89.4	6.7
Average:	*2 ± 1*	*91 ± 5*	*7 ± 1*
Capelin			
C1	32.1	57.2	18.0
C2	10.1	63.6	11.0
C3	26.2	46.9	12.6
C4	25.1	44.6	12.4
C5	29.5	54.9	18.3
Average:	*25 ± 9*	*53 ± 8*	*15 ± 3*

3.2 Hydrolysing of lipid soluble arsenicals

Aim: To hydrolyse lipid-soluble arsenic compounds systematically.

A hydrolysis process was developed, Figure 6.

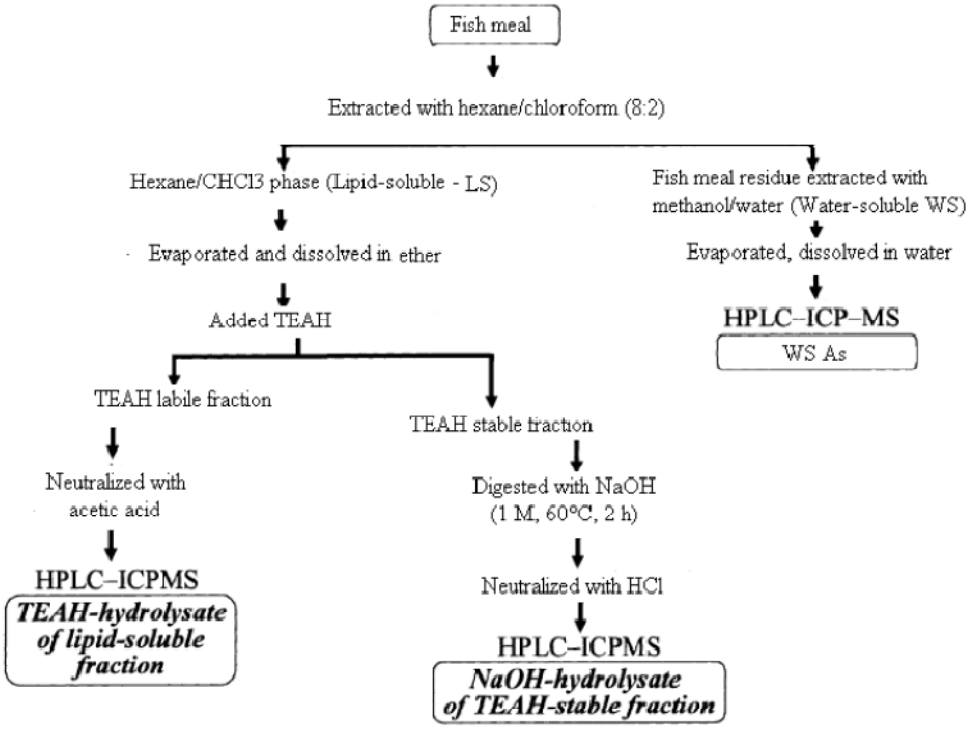


Figure 6: Schema for the hydrolysis.

Three meal samples from each species were tested with the method described in Figure 6. Somewhat contradicting results were achieved indicating that further development is needed as described by Pétursdóttir [1].

3.3 Speciation analysis with HPLC-ICP-MS

Aim: To carry out arsenic speciation of fish meal samples using HPLC-ICP-MS.

Speciation was performed on different fractions with HPLC-ICP-MS and compared to authentic reference standards. Different fractions are described in Figure 5. Detailed description of procedures and methods are found elsewhere [1]. A summarisation of main arsenic species in both water soluble fraction and lipid soluble fraction is shown in Table 4.

Table 4: The detected arsenic species in the three fish meal types.

	Capelin	Herring	Blue whiting	DORM-2
AB	Major	Major	Major	Major
TMAO[a]	Minor			
TMAP[a]			Trace	Minor
AC[a]			Trace	
TETRA[a]			Trace	Minor
As(V)[a]	Trace			
Inorganic	Minor	Minor	Trace	Minor
U / non-polar	Minor	Minor		

[a]Verification by e.g. spiking is needed

The method development for speciation analysis turned out to be very laborious and time consuming. Therefore, the main effort was concentrated on the method development for toxic inorganic arsenic. A detailed description of the method is found elsewhere [1]. The main results are shown in Table 5. The results show that even if the total arsenic concentration is high, as is in the case of blue whiting meal, there is no relationship to the inorganic arsenic concentration.

Table 5: Total arsenic and inorganic arsenic in 12 fish meal samples and two Certified Reference Materials

Samples[a,c]	Conc. As(V) (µg/kg)	n	Total As conc. (mg/kg)	%As(V) of total
Herring				
H1	37 ±18	5	4.35 ± 0.06	0.8
H2	37 ± 9	3	5.31 ± 0.15	0.7
H4	29 ± 3	3	3.45 ± 0.15	0.8
H5	82 ± 9	3	2.51 ± 0.04	3.3
Blue whiting				
B1	41 ±16	9	13.95 ± 0.29	0.3
B2	72 ± 3	3	16.18 ± 0.57	0.4
B3	51 ± 4	3	12.38 ± 0.55	0.4
B4	51 ± 3	3	8.38 ± 0.13	0.6
Capelin				
C1	50 ±18	9	4.11 ± 0.06	1.2
C2	198 ± 8	3	5.17 ± 0.20	3.8
C4	36 ± 9	3	4.42 ± 0.29	0.8
C5	47 ± 5	3	2.95 ± 0.11	1.6
TORT-2	639 ± 81	4	19.6 ± 0.4 [21.6 ± 1.8][b]	3.3
DORM-3	283 ± 27	3	6.2 ± 0.1 [6.88 ± 0.30][b]	4.5

[a] Water content of fish meal samples ranges from 4.5-8.3%, concentration given on a product weight basis. [b]Numbers in brackets are certified value of total arsenic concentration [c]LOD ≥ 3σ and LOQ ≥ 0.014 mg/kg.

3.4 Identification of novel arsenic species in fish meal samples

Aim: To identify novel arsenic species.

It is very important to obtain more information about novel arsenic species because the toxicity of these is unknown and various chemical forms of arsenic have different toxicity. Therefore, if novel arsenic species are found, the overall aim is to identify them. As recently discussed [12] structural assignments based solely on HPLC data with atomic mass spectrometric detection may not always provide sufficient proof of structure, particularly when novel compounds are involved.

As mentioned in Table 4, there was an unidentified peak in the chromatogram for capelin and herring fish meal samples when the water soluble fraction (Figure 5) was analysed on a reversed phase HPLC column.

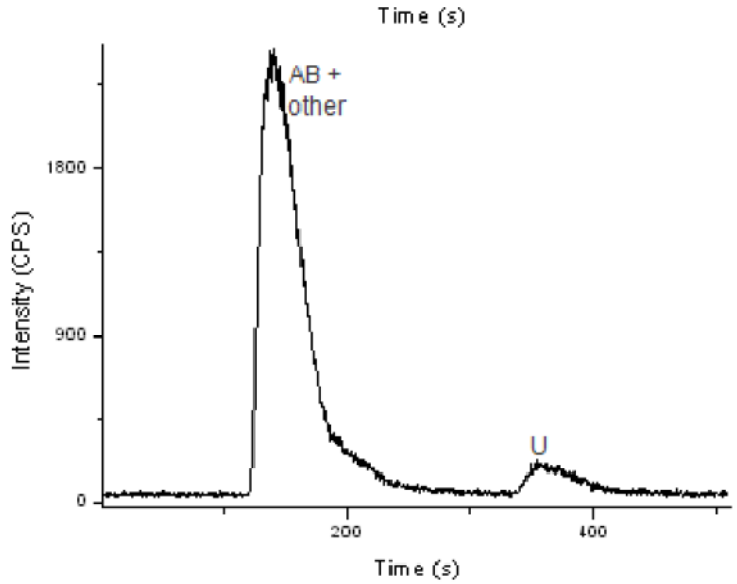


Figure 7: Unidentified arsenic species detected in both capelin and herring fish meal samples.

There was not enough time in the project to fully identify this compound. Nevertheless, there are certain information available, e.g. it is probably water soluble, still less polar that AB and probably not very large. Further, work of identifying this compound should be carried out in future studies.

3.5 Seasonal screening of total arsenic as well as toxic and non-toxic arsenic species in fish meal samples

Aim: To distinguish arsenic levels and also arsenic species in the fish meal samples among different seasons.

As mentioned in Section 3.1.1, approximately 100 samples were analysed for total arsenic. Seasonal variation was noticed for herring and blue whiting (Figure 8 and Figure 9). The capelin samples were not well enough distributed over the year to give any clear seasonal variation. This is illustrated in Figure 10. This is because capelin is only fished at certain seasons. No statistical difference was between the two groups shown in Figure 10.

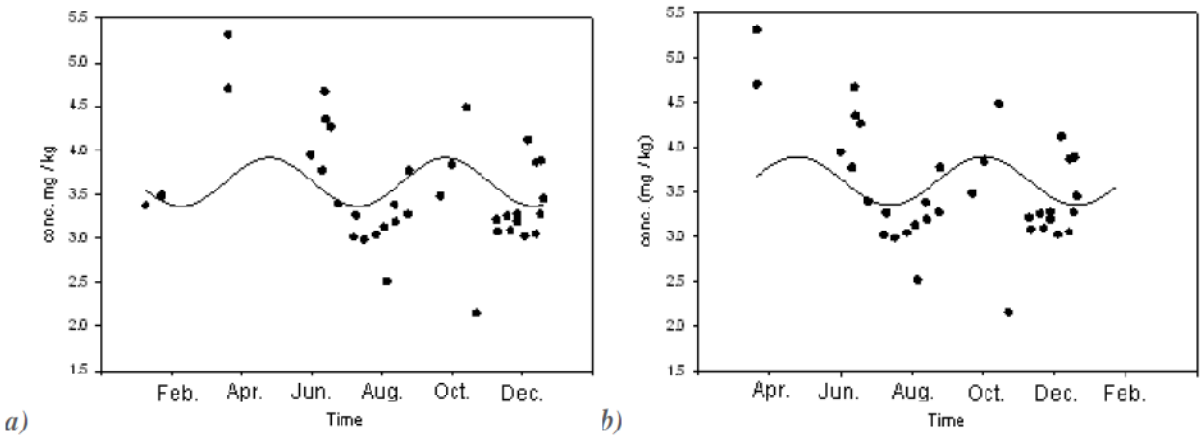


Figure 8: Herring samples plotted as total As concentration vs. time with a simple sinus regression, a) January – December, b) March – January.

There is an negative linear relationship between the total arsenic concentration and the lipid content [1] and the fish is commonly leanest just after spawning. Figure 8 indicates two maximums in the total arsenic concentration in herring meal samples. This could be explained by the fact that there are two herring populations around the country which spawn at different time periods.

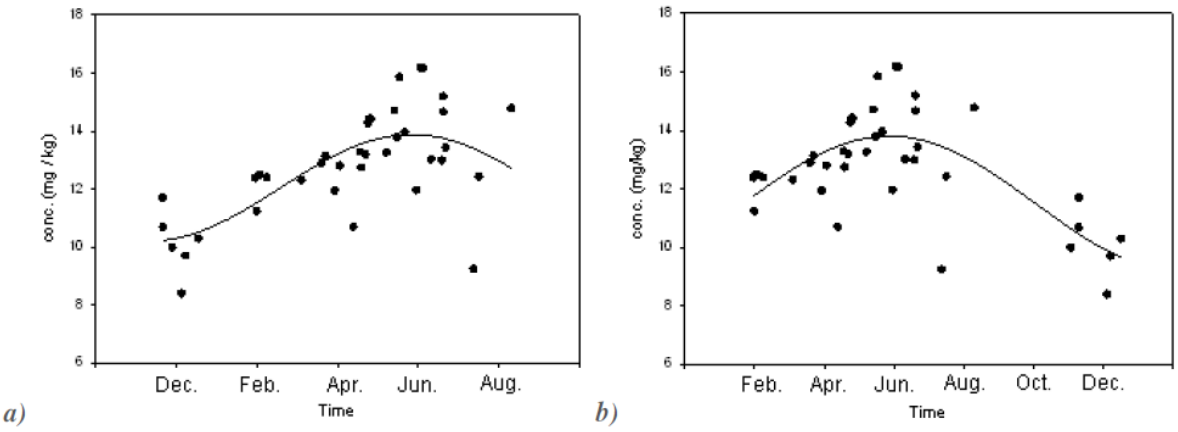


Figure 9: Blue whiting samples plotted as total As concentration versus time with a simple since regression, a) November – August, b) January – December.

Figure 9 indicates a single maximum in the seasonal change for blue whiting, in June. A negative linear relationship between lipids and arsenic concentration has been shown for blue whiting as well, as for the herring. The blue whiting meal has a minimum in the lipid concentration in May-June, after the fish spawns, which correlates with the maximum in arsenic concentration [1].

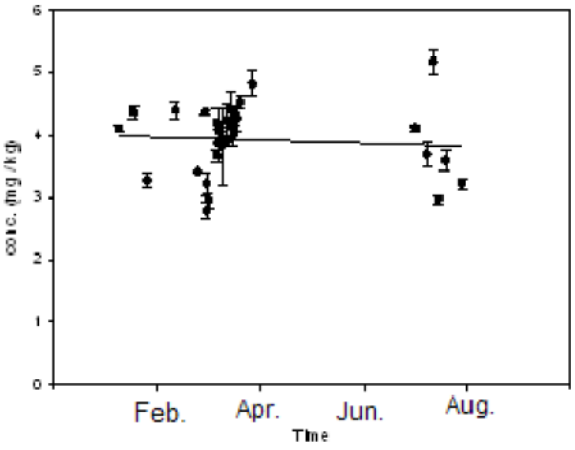


Figure 10: Capelin samples plottet as total As concentration versus time (January – July)

The project was unsuccessful in detecting seasonal difference in toxic and non-toxic arsenic species because of difficulties in method development and shortage of time. On the other hand, no linear relationship was found between the concentration of inorganic arsenic and the concentration of total arsenic in the samples analysed so far (12/102) [1]. Future work will include the seasonal difference of toxic and non-toxic arsenic species.

4 CONCLUSIONS

Total arsenic concentration ranges from 2.2 – 16.2 mg/kg for over 100 samples of three types of Icelandic fish meal; capelin, herring and blue whiting. The blue whiting meal has approximately three times higher total arsenic concentration compared to herring and capelin meal. This could be due to the physiological differences of the fish species, the food source of the fish and could also be dependent on the fish meal production process where different parts of the tissues/organs are used for the meal. For herring and blue whiting meal a correlation between fishing time and the total arsenic concentration was observed, while this was not the case for the capelin meal. The total arsenic concentration seems to be higher when the fish is leaner, e.g. just after spawning, as there are indications of a negative correlation between the lipid content and the arsenic content. No difference in arsenic content was found dependent on the fishing location.

The sequential method developed yielded good extraction efficiencies. Analyses on cation exchange chromatography showed arsenobetaine to be the predominant peak in all fish meal types and when calculated based as percentage of total area AB was found to be 70 - 96% of the water soluble fraction. Other arsenicals were present either as trace or minor constituents. Different arsenic species were detected for the three fish meal types as illustrated in Table 4. The alkaline-alcoholic extraction method for extraction of inorganic arsenic showed promising results. However, suspicion was raised when a double peak detected for a herring sample, when the PRP-X100 Hamilton column was new, eluted in later experiments as a single well defined peak. The decrease in retention time indicates that the degree of matrix effects depends on the wear of the column. Based on a spiking experiment alone the inorganic arsenic concentration would have been overestimated by 6% of total arsenic concentration for the herring sample. For quality control reasons, it is therefore necessary to verify results with at least two separate analytical methods. This can be accomplished with a verification of selected samples by e.g. hydride generation. For detection limit purposes hydride generation with ICP-MS as a detector would be more suitable than HG-AFS. The measured inorganic concentration in the CRMs; DORM-3 and TORT-2 was 0.283 ± 0.027 and 0.639 ± 0.081, respectively. Although a suspicion of a co-elution exists, especially for the TORT-2 sample, were approximately three times lower concentration was measured on HPLC-HG-AFS. The values measured with both methods are close to previously reported values of inorganic arsenic in TORT-2, even though they vary by a factor of three. These results stress the need for a certified value of inorganic arsenic and other species of interest in certified reference materials in order to check the robustness of developed methods. The alkaline-alcoholic extraction method, however, gives convincing upper limits of the inorganic concentration in the fish meal. Using this extraction method the results show that for 12 fish meal samples, most have an inorganic arsenic concentration close to or under 1% of the total arsenic concentration and all below 4%. This is in accordance with previously reported values in the literature for seafood. No correlation between inorganic arsenic concentration and total arsenic concentration was noticed.

Four samples of the blue whiting meal analyzed exceed the previous EU maximum level of 15 mg/kg total arsenic concentration in feedingstuff and several come close to the level. All samples in this study,

however, fall below a recently set maximum level of total arsenic concentration that was raised for feedingstuffs obtained from the processing of fish or other marine animals of 25 mg/kg. Feedingstuffs high in total arsenic and low in inorganic were previously excluded from the market, however, the recent EU directive reduces the risk of such unjust exclusion [8]. Raising the maximum limits of total arsenic should, however, be considered a short term solution as total arsenic concentration is not a good indication of exposure to the undesirable substance. Future legislation should rather aim for maximum levels of arsenic in food- and feedingstuff to be set in respect to toxic species (inorganic arsenic, and e.g. arsenic species of medium toxicity). EFSA has recommended that dietary exposure to inorganic arsenic should be reduced and that more extensive speciation data for different food commodities is needed for risk assessment of inorganic arsenic. To evaluate the inorganic arsenic concentration in biological samples robust analytical methods are needed and further work lies ahead for the scientific community in this area.

5 FUTURE WORK

To refine this study a separation of the unknown arsenical co-eluting with the arsenate would be the next step. In the work by Sloth et al. [13], which the alkaline-alcoholic extraction was based on, for separation of inorganic arsenic from organoarsenic species, an isocratic mobile phase ammonium carbonate, was found to be sufficient [13, 14]. However, a first step to get separation might be to try a gradient mobile phase. Identification of the unknown co-eluting cationic species and the unknown species found on the reversed phase chromatography would be of interest. In that regard, comparison of other known arsenic compounds would be the first step, and then it might be possible to consider measurements with ESI-MS. A clean up of the sample before analysis on the column would also be greatly beneficial as to minimize the effect of the sample matrix and strain on the column. A sequential extraction method, to look further into the lipid soluble fraction and evaluate the organoarsenicals in the water soluble fraction, would be of interest in combination with extraction of inorganic arsenic. Extracting the lipids first could be a partial cleanup procedure. Further steps of cleanup would be advisable. In general the need for a validated value of inorganic arsenic in a CRM is crucial, but in order to achieve this, different analytical methods must all reach the same value. However, the current status in the field is that different analysts and different methods do not yield the same values. The methods therefore must be refined and further developed. This is a realistic goal as today lower detection limits than before are achievable and better equipment is on the market. There are also many unanswered questions regarding the arsenolipids, where much work lays ahead in that area. Identification of new arsenolipids is needed and the chronic toxicity must be further evaluated.

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