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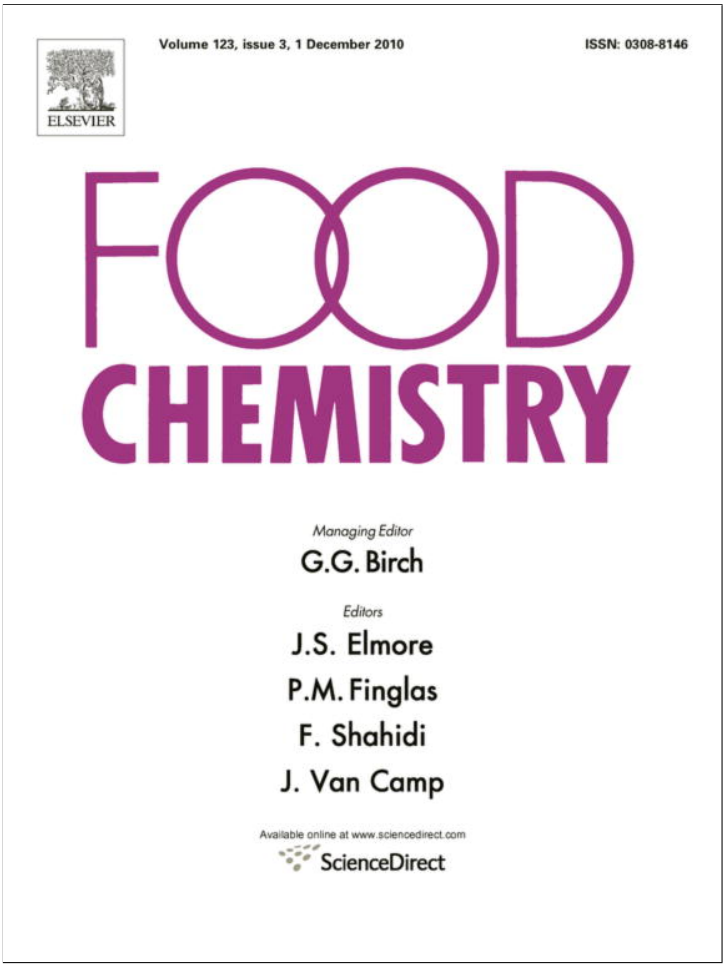

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Stability of arsenic compounds in seafood samples during processing and storage by freezing

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ABSTRACT

In this study, the stability of arsenic compounds in fresh and frozen samples of raw, boiled and fried Atlantic cod (*Gadhus morhua*), Atlantic salmon (*Salmo salar*) and blue mussel (*Mytilus edulis*) were examined. Results show that the total arsenic concentrations of the fresh Atlantic cod and Atlantic salmon samples were not different from the frozen samples within the same seafood type. For blue mussel, the total arsenic concentration decreased significantly after storage. Inorganic arsenic was found only in blue mussels and, importantly, no significant increase of inorganic arsenic was observed after processing or after storage by freezing. The content of tetramethylarsonium ion was generally low in all samples types, but increased significantly in all fried samples of both fresh and frozen seafood. Upon storage by freezing, the arsenobetaine content was reduced significantly, but only in the samples of blue mussels.
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1. Introduction

Seafood contains nutrients associated with various beneficial health effects and is regarded as an important part of a healthy diet. In the Norwegian guidelines for food and nutrition, an increased intake of fish and other seafood is particularly recommended (Norwegian Ministries, 2007). Seafood is a good source of proteins, omega-3 fatty acids, vitamin D, vitamin B_{12}, selenium and iodine (Alexander et al., 2007). Nevertheless, seafood also contributes substantially to dietary arsenic, which is one of the trace elements of concern in relation to food safety (Francesconi, 2007). A wide range of arsenic compounds, including inorganic arsenic, has been reported in marine organisms. Table 1 shows a selection of some of the chemical forms of arsenic typically found in seafood. The inorganic arsenic compounds, arsenate (As(V)) and arsenite (As(III)), are toxic and carcinogenic, whereas the methylated species methylarsonate (MA), dimethylarsinate (DMA) and tetramethylarsonium ion (TETRA) are less toxic. In the recent Scientific Opinion on Arsenic in Food, the European Food Safety Authority (EFSA) set a range of benchmark dose lower confidence limit ($BMDL_{01}$) values between 0.3 and 8 μg/kg bodyweight (bw) per day (EFSA, 2009). The $BMDL_{01}$ values were identified for lung, skin and bladder cancer, as well as skin lesions, with the lowest values being found for lung cancers (EFSA, 2009). The estimated dietary exposures to inorganic arsenic, for average and high level consumers in Europe, are within the range of $BMDL_{01}$ (EFSA, 2009). The Joint FAO/WHO Committee on Food Additives (JECFA) also recently re-evaluated arsenic (WHO, 2010). A benchmark dose lower confidence limit for a 0.5% increased incidence of lung cancer ($BMDL_{0.5}$), from epidemiological studies, was determined to be 3.0 (range 2–7) μg/kg bw per day of inorganic arsenic. The Committee withdrew the previous provisional tolerable weekly intake (PTWI) of 15 μg/kg bw set for inorganic arsenic. Mean dietary exposures to inorganic arsenic ranged from 0.1 to 3.0 μg/kg bw per day in the United States of America (USA) and various European and Asian countries. JECFA noted that more accurate information on the inorganic arsenic content of foods, as they are consumed, is needed to improve assessments of dietary exposures of inorganic arsenic species. The predominant arsenic compound in seafood, arsenobetaine (AB), which is excreted unchanged, is considered non-toxic (Borak & Hosgood, 2007). Other arsenic compounds usually found in

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Table 1
Acronyms and chemical formulae of the arsenic compounds included in the present study. For simplicity the compounds are depicted in their fully deprotonated form. Nomenclature is as proposed by Francesconi and Kuehnelt (2004).

Acronym	Arsenic compounds	Formula
As(V)	Arsenate	$As(O^-)_3$
As(III)	Arsenite	$O{=}As(O^-)_3$
MA	Methylarsonate	$CH_3AsO(O^-)_2$
DMA	Dimethylarsinate	$(CH_3)_2AsO(O^-)$
TMAO	Trimethylarsine oxide	$(CH_3)_3AsO$
TETRA	Tetramethylarsonium ion	$(CH_3)_4As^+$
AB	Arsenobetaine	$(CH_3)_3As^+CH_2COO^-$
TMAP	Trimethylarsoniopropionate	$(CH_3)_3As^+CH_2CH_2COO^-$
AC	Arsenocholine	$(CH_3)_3As^+CH_2CH_2O^-$
DMAA	Dimethylarsinoylacetate	$(CH_3)_2(O)AsCH_2COO^-$
DMAP	Dimethylarsinoylpropionate	$(CH_3)_2(O)AsCH_2CH_2COO^-$

seafood are arsenocholine (AC), trimethylarsoniopropionate (TMAP), trimethylarsine oxide (TMAO) and arsenosugars; the latter are particularly found in marine algae.

The consumption of fish and other seafoods in Norway is high, compared with many other countries (Alexander et al., 2007). Atlantic cod, Atlantic salmon and blue mussels are examples of commonly consumed seafoods in Norway. While arsenic in seafood is usually determined as total arsenic, the primary importance, from a food safety point of view, is the amount of inorganic arsenic in seafood.

Although total arsenic content in the fillet of Atlantic cod varies by several orders of magnitude with concentrations from 0.4 to 220 mg As/kg wet weight (Julshamn, Lundebye, Heggstad, Berntssen, & Bøe, 2004; NIFES), the amount of inorganic arsenic in fillets of cod usually constitutes <1% of total arsenic or <0.01 mg/kg wet weight (Amran, Lagarde, Leroy, & Maier, 1997; Sloth, Larsen, & Julshamn, 2005a). In fillets of farmed Atlantic salmon, total arsenic concentration typically ranges from 0.6 to 4.8 mg/kg wet weight (Julshamn et al., 2004; NIFES) and inorganic arsenic is only found in trace amounts (Sloth et al., 2005a). AB is the predominant form of arsenic in fish fillets (Francesconi & Kuehnelt, 2002). Blue mussels, harvested from various locations along the Norwegian coastline, show concentrations of total arsenic ranging from 1.2 to 13.8 mg As/kg wet weight (Sloth & Julshamn, 2008). In addition to high concentrations of AB and DMA, are arsenosugars, significant arsenicals in blue mussels (Larsen, 1995). In most studies, only relatively low concentrations (<0.1 mg As/kg) of inorganic arsenic in blue mussels have been reported (Francesconi & Kuehnelt, 2002; Munõz et al., 2000; Sörös, Bodó, Fodor, & Morabito, 2003). However, unusually high levels of inorganic arsenic were recently reported in blue mussels from certain locations in Norway (Sloth & Julshamn, 2008). The inorganic arsenic fraction increased with increasing contents of total arsenic, and concentrations as high as 5.8 mg As/kg wet weight of inorganic arsenic, corresponding to 42% of the total arsenic present, were reported (Sloth & Julshamn, 2008). Numerous other studies have also determined arsenic species in raw samples of seafood and they report results similar to those described above (De Gieter et al., 2002; Hirata, Toshimitsu, & Aihara, 2006; Li et al., 2003; Sloth, Larsen, & Julshamn, 2003; Súñer et al., 2002).

Seafood is stored and/or processed (freezing, drying, salting) and is usually consumed after processing, e.g. fried or boiled. Storing and processing of seafood may potentially alter the concentration and/or speciation pattern of arsenic compounds. Consequently, from a food safety point of view, it is important to study the impact of processing on arsenic compounds in seafoods (Devesa, Velez, & Montoro, 2008).

Previous studies on the effect of cooking on total arsenic content in seafood report both decreases and increases of total arsenic concentrations (Dabeka et al., 1993; Devesa et al., 2001a; Ersoy, Yanar, Kücükgülmez, & Celik, 2006). These changes might be due to changes in water content during cooking. Devesa and coworkers (2001b) also found increases in the concentrations of DMA for sardines and bivalves and of TETRA for anchovy, Atlantic horse mackerel, sardine and meagrim after cooking. Cooking procedures (i.e. heat treatment) appear to transform some of the arsenicals present in seafood. As discussed thoroughly by Devesa and colleagues (2008) heat treatment leads to a decarboxylation of AB, forming TETRA, while inorganic arsenic and DMA may be formed by degradation of other arsenic species present. Another study (Devesa et al., 2001b) indicated that, at temperatures between 150 and 190 °C, a partial decomposition of AB to TMAO and/or TETRA was achieved.

Although some information on the effect of storage and processing on arsenic compounds has become available in recent years, there is still inadequate information on the stability of arsenicals in seafood, in particular the impact of storage by freezing. In the present study, we therefore investigate the stability of arsenic compounds in Atlantic cod, Atlantic salmon and blue mussels during storage by freezing and further processing.

2. Material and methods

2.1. Sample collection and preparation

The study included two different species of fish, Atlantic cod (*Gadus morhua,* L.), as a representative of a lean fish species (⩽1% fat in the fillet) and Atlantic salmon (*Salmo salar,* L.), as a representative of a fatty fish species (>10% fat in the fillet), and one type of shellfish, blue mussel (*Mytilus edulis,* L.). The three different types of seafood were purchased at the fish market in Bergen, Norway in May, 2007. Each of the fish weighed ~3.5 kg. The head, tail and intestines were removed from the fish ($n = 3$ for each species) and each fish fillet was divided into nine sub samples of ~150–200 g. Each blue mussel sample ($n = 2$) consisted of 1 kg of blue mussels and was divided into nine subsamples, consisting of 10 specimens each. The total arsenic content and the arsenic compounds were determined in fresh and frozen (stored for one and three months, at −20 °C, respectively) samples of raw, boiled (boiling water (100 °C; seafood was simmered for 10 min) and fried seafood (applied temperature of ~140 °C for 10 min). The processing (Fig. 1) was carried out using a household ceramic electric cooker, a stainless steel cooking pan (diameter 20 cm) and an aluminium frying pan covered with Teflon (diameter 28 cm). Samples ($n = 24$) of the boiling water from the cooking procedure were also included in the study. All seafood samples were freeze-dried to constant weight and subsequently homogenised to a fine pow-

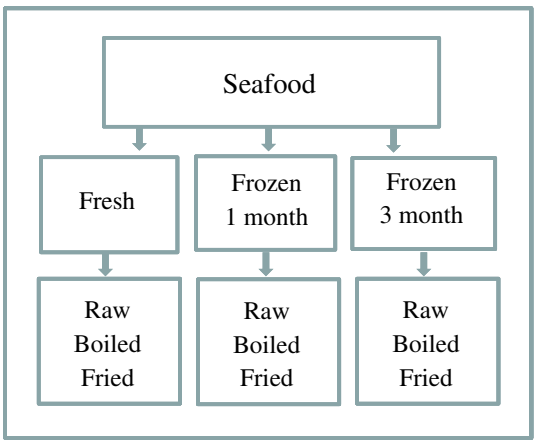

Fig. 1. Schematic overview of sample processing.

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der using a domestic mill, then stored in twist off boxes at room temperature prior to analysis. In total, 72 samples of seafood and 24 samples of boiling water were collected. Determination of organoarsenic species in the boiling water was only performed in the fresh samples ($n = 8$).

2.2. Instrumentation

An Agilent quadrupole ICPMS (Inductively Coupled Plasma Mass Spectrometry) 7500c instrument (Yokogawa Analytical Systems Inc., Tokyo, Japan), equipped with an auto sampler ASX-500 (CETAC Technologies, Omaha, Nebraska, USA), was run in the standard mode (non-cell mode) and used as an arsenic specific detector. For the arsenic speciation analysis, an Agilent 1100 series quaternary HPLC (High Performance Liquid Chromatography) pump, degasser and auto sampler (Agilent Technologies, Waldbronn, Germany) were coupled to the ICPMS instrument. The outlet of the HPLC column was connected to the nebuliser of the ICPMS instrument by a short length of polyethylene tubing. For cation-exchange chromatography, a silica-based strong cation-exchange HPLC column (Chrompack IonoSpher-5C, 100 × 3 mm id, 5 μm particles, Varian, Middelburg, The Netherlands) was used. For determination of inorganic arsenic, a strong anion-exchange HPLC column (ICSep ION-120, 120 × 4.6 mm id, 10 μm particles, Transgenomics, San Jose, CA, USA) was used. Data were collected, using the Agilent Chemstation ICPMS chromatographic software.

2.3. Reagents

All chemicals, including standards and solutions, were of *pro analysi* quality or better. Deionised water (>17 MΩ cm^{-1}, Nanopure-system, Nanopure, Barnsted, UK) was used throughout the work. The mobile phase solution, for anion-exchange chromatography, was prepared by dissolving an appropriate amount of ammonium carbonate (J.T. Baker, Phillipsburg, NJ, USA) in an aqueous 3% (v/v) methanol (Merck, Darmstadt, Germany) solution, followed by adjustment of the pH to 10.3 with 25% (v/v) aqueous ammonia (Merck). The mobile phase solutions for cation-exchange chromatography were prepared by dissolving an appropriate amount of pyridine (Merck) in an aqueous 3% (v/v) methanol solution, followed by adjustment of pH to 2.7 with formic acid (Merck).

2.4. Determination of total arsenic

For the determination of total arsenic, subsamples of ~0.2 g dry weight were submitted to microwave-assisted wet digestion, using 2.0 ml of concentrated nitric acid (Merck) and 0.50 ml of 30% (w/w) hydrogen peroxide (Merck) in an Ethos Pro microwave system (Milestone, Sorisole, Italy). The digests were diluted to a final volume of 25 ml with water. Samples of the boiling water were defrosted in a refrigerator and then centrifuged (5 min, 1900*g*) and filtered through a 0.45 μm single use syringe and disposal filter (Sartorius MiniSart RC25, Sartourius, Göttingen, Germany) prior to analysis. Freshly prepared arsenic standard solutions in 5% (v/v) nitric acid (Merck) were made by appropriate dilution of a 1000 mg/l certified arsenic stock solution (Spectrascan TeknoLab, Drøbak, Norway) and used to construct an external calibration curve. A diluted solution of a 1000 mg/l rhodium stock solution (Spectrascan TeknoLab) was added on-line and served as an internal standard, in order to correct for instrumental drift (Julshamn et al., 2007).

2.5. Determination of inorganic arsenic

Inorganic arsenic was determined as described by Sloth and colleagues (2005a). Briefly, subsamples, of ~0.2 g dry weight each, were placed in a vial of the microwave oven system (CEM Mars5, CEM, Matthews, NC, USA) and 10 ml of 0.9 M sodium hydroxide (Merck) in 50% (v/v) ethanol (Arcus AS, Oslo, Norway) were added. The samples were placed in the oven, which was adjusted to switch off the microwave power when the temperature of the mixture reached 90 °C. No pressure limitation was activated as the pressure only reached a few bars. The microwave treatment achieves both the solubilisation of the sample matrix and oxidation of As(III) to As(V), thus allowing the determination of inorganic arsenic as As(V) (Sloth et al., 2005a). Prior to quantification, the samples were filtered through a 0.45 μm single use syringe and disposal filter. Freshly prepared matrix-matched arsenic standard solutions, in the alkaline-alcoholic mixture, were made by appropriate dilution of a 1000 mg/l certified arsenic stock solution (Spectrascan TeknoLab) and used to construct an external calibration curve.

2.6. Determination of organoarsenic compounds

Organoarsenic compounds were determined as described by Sloth and colleagues (2003). Briefly, subsamples of ~0.25 g dry weight were weighed into 50 ml polyethylene test tubes and 20 ml of 50% (v/v) methanol–water were added. The samples were then vigorously shaken, overnight, followed by centrifugation (10 min., 1900*g*) and the supernatant was transferred to a test tube. The extraction procedure was repeated twice (0.5 h agitation), with 3 ml of 50% (v/v) methanol–water each time, and the supernatants were combined. They were then evaporated at 40 °C under a stream of nitrogen to a volume of ~1 ml and made up to 5 ml with water. Prior to quantification by gradient elution HPLC-ICPMS, all samples were filtered through a 0.45 μm single use syringe filter. Quantification of the content of each of the arsenic compounds in the samples was based on external calibration curves constructed from a multi-species calibration solution made by appropriate dilution of single-species stock solutions. The standard stock solutions were prepared in water using the following chemicals: sodium dimethylarsinic acid (DMA) trihydrate (Merck), trimethylarsine oxide (TMAO), arsenocholine (AC) bromide and tetramethylarsonium (TETRA) iodide (Hot Chemicals, Tokyo, Japan). A certified solution was used for arsenobetaine (BCR CRM626, 1031 ± 6 mg/kg as AB, Institute for Reference Materials and Measurements (IRMM), Geel, Belgium). Analysis of the certified reference material (CRM) DORM-2 (National Research Council Canada (NRCC), Ontario, Canada) was used to qualitatively verify the presence of dimethylarsinoylacetate (DMAA) and dimethylarsinoylpropionate (DMAP) by match in retention times (Sloth, Larsen & Julshamn, 2005b).

2.7. Method figures of merit

The trueness of total arsenic determination was evaluated by the analysis of the CRMs, TORT-2 and DORM-3 (NRCC). The obtained values (20.3 ± 0.8 mg As/kg and 6.44 ± 0.2 mg As/kg, for TORT-2 and DORM-3, respectively) agreed well with the certified values (21.6 ± 1.8 mg As/kg and 6.88 ± 0.30 mg As/kg, respectively). The limit of quantification (LOQ) for total arsenic was estimated at 0.03 mg As/kg dry weight (6 sd) from repeated analyses of blank solutions. For the determination of inorganic arsenic, no CRMs are available. The trueness was estimated from the consecutive analysis of TORT-2 (0.23 ± 0.03 mg As/kg) which agreed well with results previously reported by Sloth and Julshamn (2008) (0.20 ± 0.02 mg As/kg). The LOQ of inorganic arsenic was estimated as six times the baseline noise and calibrated by extrapolation of the calibration curve in the low concentration range at 0.01 mg As/kg dry weight. The speciation determination of organoarsenic compounds was evaluated by using BCR CRM627 Tuna fish tissue

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(IRMM) as a reference material. The obtained results (AB: 4.3 ± 0.6 mg As/kg and DMA: 0.20 ± 0.04 mg As/kg) agreed well with the certified values (AB: 3.9 ± 0.2 mg As/kg and DMA: 0.15 ± 0.01 mg/kg). The obtained results for the determination of other organoarsenic compounds (TMAO: 0.017 ± 0.005 mg As/kg, AC: 0.024 ± 0.009 mg As/kg, TETRA: 0.055 ± 0.002 mg As/kg and TMAP: 0.048 ± 0.003 mg As/kg) were comparable with the results reported by Sloth et al. (2003) (TMAO: 0.010 ± 0.002 mg As/kg, AC: 0.012 ± 0.002 mg As/kg, TETRA: 0.037 ± 0.002 mg As/kg and TMAP: 0.033 ± 0.002 mg As/kg). The LOQs for the individual arsenic species were estimated as 6× the baseline noise and calibrated by extrapolation of the calibration curve in the low concentration range. The following LOQs were obtained: DMA: 4 μg As/kg dry weight, AB: 2 μg As/kg dry weight, TMAO: 8 μg As/kg dry weight, TMAP: 8 μg As/kg dry weight, AC: 5 μg As/kg dry weight, TETRA: 2 μg As/kg dry weight.

2.8. Statistics

All statistical analyses were performed using StatSoft, Inc. (2009) STATISTICA (data analysis software system), version 9.0. www.statsoft.com. Factorial ANOVA (analysis of variance) was used to investigate the effect of processing or storage on the arsenic concentration values, followed by a Fisher LSD test to identify any significant differences. For all analyses, a 95% confidence interval was applied where $p < 0.05$ was considered significant.

3. Results and discussion

3.1. Determination of total arsenic

Total arsenic concentration in the fresh, processed and stored samples of seafood are shown in Table 2. The arsenic concentration in the three fresh Atlantic cod samples varied considerably from 1.7 to 15.3 mg As/kg dry weight. This is in line with previously published data, where the reported arsenic concentrations in fillet of Atlantic cod varied from 0.4 to 52.4 mg As/kg wet weight (Julshamn et al., 2004). The arsenic contents of both the fresh Atlantic salmon and the blue mussel samples had narrow concentration ranges of 3.7–4.2 mg As/kg and 12.9 mg As/kg dry weight, respectively. The arsenic concentrations found in the blue mussels are comparable with levels (1.2–13.8 mg As/kg wet weight) recently reported by Sloth and Julshamn (2008). The arsenic concentrations found in Atlantic salmon are in agreement with concentrations reported for farmed Atlantic salmon in the Norwegian surveillance programme on contaminants in fish (0.6–4.8 mg As/kg wet weight) (NIFES). Processing (i.e. boiling and frying) and storage (one and three months, respectively) only resulted in minor changes of the total arsenic concentrations in the samples (Table 2). The arsenic content of raw samples of Atlantic salmon was significantly higher than those of boiled samples of both fresh ($p = 0.04$) and frozen samples (for one month) ($p = 0.047$) showing that heat processing influenced the total arsenic concentration. In blue mussel, storage by freezing resulted in significantly lower arsenic concentrations in samples that were frozen for one month ($p = 0.0016$) and three months ($p = 0.041$) in comparison with the fresh samples.

3.2. Determination of inorganic arsenic

The quantitative results from the speciation determination of inorganic arsenic and organoarsenic species are shown in Table 3. Inorganic arsenic was only quantified in samples of blue mussels, whereas, for all samples of Atlantic cod and Atlantic salmon, the inorganic arsenic concentration was below the LOQ of 0.01 mg As/kg dry weight. The concentrations of inorganic arsenic in the two fresh samples of blue mussels were 0.02 and 0.01 mg As/kg wet weight, respectively. These were consistent with a recent survey of blue mussels from Norway (i.e. median 0.011 mg As/kg wet weight, range 0.001–5.8 mg As/kg wet weight, $n = 175$) (Sloth & Julshamn, 2008). Neither storage by freezing nor processing by heat treatment of Atlantic cod, Atlantic salmon and blue mussels caused a significant increase in the concentration of inorganic arsenic ($p > 0.05$), indicating that organoarsenic species were not degraded to inorganic arsenic during freezing or heat processing.

Table 2
Total arsenic concentration (mg/kg, dry weight) in samples of cod, salmon and blue mussel. The percentage of dry matter is given in parenthesis.

Seafood	Treatment	Total As (mg/kg, dry weight), (%)		
		Fresh	Frozen 1 month	Frozen 3 month
Cod 1	Raw	6.2 (16.8)	7.3 (19.1)	6.5 (16.6)
	Boiling	6.5 (21.2)	5.9 (20.4)	6.4 (20.2)
	Frying	7.1 (20.8)	8.0 (21.2)	7.9 (21.6)
Cod 2	Raw	15.3 (21.3)	15.0 (18.7)	14.9 (18.8)
	Boiling	11.1 (22.6)	10.8 (23.2)	11.6 (21.6)
	Frying	13.9 (28.7)	13.6 (22.0)	12.3 (25.2)
Cod 3	Raw	1.7 (19.0)	1.8 (18.7)	1.8 (19.3)
	Boiling	1.8 (22.9)	1.7 (21.6)	1.5 (21.7)
	Frying	1.6 (21.4)	1.8 (22.0)	1.6 (24.7)
Salmon 1	Raw	3.7^{a} (38.3)	3.6^{a} (30.5)	3.5^{a} (27.6)
	Boiling	3.0^{b} (39.5)	3.5^{b} (37.6)	3.3^{b} (36.7)
	Frying	3.8^{a} (37.5)	3.5^{a} (34.5)	3.0^{a} (37.2)
Salmon 2	Raw	4.2^{a} (35.0)	3.8^{a} (31.8)	4.1^{a} (32.1)
	Boiling	3.5^{b} (38.2)	3.3^{b} (36.0)	3.9^{b} (33.2)
	Frying	4.1^{a} (42.1)	3.8^{a} (34.0)	4.0^{a} (32.7)
Salmon 3	Raw	3.7^{a} (37.9)	3.1^{a} (29.6)	3.2^{a} (37.9)
	Boiling	3.0^{b} (37.5)	2.8^{b} (34.1)	3.1^{b} (30.9)
	Frying	3.3^{a} (38.1)	3.2^{a} (43.7)	3.3^{a} (41.9)
Blue mussel 1	Raw	12.9^{1} (11.8)	12.6^{2} (16.7)	11.2^{2} (15.1)
	Boiling	13.5^{1} (16.9)	11.4^{2} (25.5)	12.0^{2} (20.8)
	Frying	14.2^{1} (11.1)	11.1^{2} (35.1)	11.3 (35.3)
Blue mussel 2	Raw	12.9^{1} (6.9)	11.0^{2} (16.4)	11.9^{2} (17.6)
	Boiling	12.2^{1} (14.6)	11.3^{2} (25.6)	11.0^{2} (21.8)
	Frying	12.6^{1} (17.0)	11.5^{2} (35.6)	12.1^{2} (36.4)

Values with different superscripts letters denote significant differences ($p < 0.05$) of treatment in salmon ($n = 3$).
Values with different superscripts numbers denote significant differences ($p < 0.05$) of storage in blue mussel ($n = 2$).

3.3. Determination of organoarsenic compounds

3.3.1. General

The results for organoarsenic compounds are presented and discussed separately for each of the three types of seafood.

3.3.2. Atlantic cod

The arsenicals, AB, DMA, TMAO, TMAP, AC and TETRA, were found in samples of Atlantic cod in addition to three unknown peaks, eluting close to the void volume, using cation-exchange chromatography. AB constituted ~97% of the total arsenic in all samples ($n = 27$), while the other species were only found at trace levels. The extraction efficiency was in the range of 90–110% with a median of 102% when comparing the sum of the species with the total arsenic concentration. The concentrations of the various arsenicals were fairly constant during storage (i.e. fresh samples versus samples frozen for one or three months). Weight loss during thawing, due to loss of water, as well as loss of soluble arsenic species in the thawing water, may explain the differences in the dataset between fresh and frozen samples for the various arsenic species. A significant increase in the TETRA concentration was observed after frying ($p = 0.003$), as illustrated in Fig. 2. This is probably due to a degradation of AB, as was recently reported by Devesa and colleagues (2008). The DMA concentration was significantly

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Table 3
Concentration of inorganic As, DMA, AB, TMAO, TMAP, AC and TETRA (mg/kg as As, dry weight) in fresh and frozen samples of cod, salmon and blue mussel.*

Seafood	Treatment	Inorganic As			DMA			AB			TMAO			TMAP			AC			TETRA		
		Fresh	Frozen 1 month	Frozen 3 month	Fresh	Frozen 1 month	Frozen 3 month	Fresh	Frozen 1 month	Frozen 3 month	Fresh	Frozen 1 month	Frozen 3 month	Fresh	Frozen 1 month	Frozen 3 month	Fresh	Frozen 1 month	Frozen 3 month	Fresh	Frozen 1 month	Frozen 3 month
Cod 1	Raw	<LOQ	<LOQ	<LOQ	0.04	0.04	0.03	6.3	7.3	6.5	0.01	0.03	0.01	0.12	0.21	0.09	0.03	0.14	0.02	0.01	<LOD	<LOD
	Boiling	<LOQ	<LOQ	<LOQ	0.04	0.06	0.04	6.5	6.0	6.4	<LOQ	0.03	0.01	0.12	0.19	0.08	0.02	0.06	0.01	0.01	<LOD	<LOD
	Frying	<LOQ	<LOQ	<LOQ	0.04	0.05	0.04	7.0	8.0	8.0	<LOQ	0.03	0.02	0.01	0.2	0.08	0.03	0.08	0.03	0.11	0.06	0.08
Cod 2	Raw	<LOQ	<LOQ	<LOQ	0.03	0.06	0.03	14	15	14	<LOQ	0.06	0.02	0.08	0.06	0.05	0.04	0.06	0.03	0.01	<LOD	<LOD
	Boiling	<LOQ	<LOQ	<LOQ	0.03	0.06	0.04	10	11	11	0.01	0.05	0.02	0.05	0.09	0.04	0.02	0.05	0.02	<LOD	<LOD	<LOD
	Frying	<LOQ	<LOQ	<LOQ	0.03	0.05	0.04	13	14	12	0.01	0.06	0.02	0.07	0.11	0.04	0.03	0.06	0.03	0.36	0.50	0.54
Cod 3	Raw	<LOQ	<LOQ	<LOQ	0.03	0.03	0.03	1.6	1.8	1.7	0.01	0.01	0.01	0.03	0.20	0.02	0.02	0.02	0.01	0.01	0.01	0.01
	Boiling	<LOQ	<LOQ	<LOQ	0.04	0.06	0.05	1.6	1.7	1.5	0.01	0.02	0.01	0.02	0.20	0.01	0.02	0.02	0.01	0.01	<LOD	<LOD
	Frying	<LOQ	<LOQ	<LOQ	0.04	0.05	0.04	1.6	1.8	1.6	0.01	0.02	0.01	0.03	0.20	0.02	0.02	0.03	0.01	0.06	0.07	0.15
Salmon 1	Raw	<LOQ	<LOQ	<LOQ	0.03	0.05	0.04	3.5	3.8	3.4	0.02	0.03	0.02	<LOQ	<LOQ	<LOQ	0.02	0.01	0.01	0.01	0.01	0.01
	Boiling	<LOQ	<LOQ	<LOQ	0.05	0.14	0.08	3.0	3.1	3.8	0.02	0.03	0.01	<LOQ	<LOQ	<LOQ	0.01	0.01	0.01	0.01	<LOD	<LOD
	Frying	<LOQ	<LOQ	<LOQ	0.04	0.26	0.04	3.5	3.4	2.7	0.02	0.03	0.02	<LOQ	<LOQ	<LOQ	0.02	0.01	0.02	0.02	0.01	0.20
Salmon 2	Raw	<LOQ	<LOQ	<LOQ	0.03	0.08	0.07	4.2	3.8	3.8	0.03	0.03	0.05	<LOQ	<LOQ	<LOQ	0.01	0.01	0.02	0.02	0.01	0.02
	Boiling	<LOQ	<LOQ	<LOQ	0.09	0.35	0.07	3.0	3.3	3.5	0.02	0.03	0.03	<LOQ	<LOQ	<LOQ	0.01	0.01	0.01	0.01	0.01	0.01
	Frying	<LOQ	<LOQ	<LOQ	0.09	0.09	0.09	4.0	3.8	3.6	0.02	0.03	0.07	<LOQ	<LOQ	<LOQ	0.02	0.01	0.02	0.14	0.04	0.13
Salmon 3	Raw	<LOQ	<LOQ	<LOQ	0.02	0.26	0.10	3.6	3.1	2.6	0.02	0.01	0.03	<LOQ	<LOQ	<LOQ	0.02	0.01	0.02	0.02	0.01	0.01
	Boiling	<LOQ	<LOQ	<LOQ	0.10	0.26	0.09	3.0	2.9	2.8	0.02	0.02	0.03	<LOQ	<LOQ	<LOQ	0.02	0.01	0.02	0.02	0.01	0.01
	Frying	<LOQ	<LOQ	<LOQ	0.12	0.27	0.13	3.4	2.8	2.6	0.03	0.02	0.03	<LOQ	<LOQ	<LOQ	0.02	0.01	0.02	0.07	0.10	0.23
Blue mussel 1	Raw	0.18	0.16	0.18	0.35	0.57	0.46	8.7	7.5	4.8	0.04	<LOQ	<LOQ	0.09	0.05	0.07	0.73	0.28	0.32	0.19	0.09	0.17
	Boiling	0.14	0.15	0.19	0.66	0.74	0.71	6.9	5.5	4.3	0.04	<LOQ	<LOQ	0.07	0.02	0.04	0.03	0.09	0.17	0.15	0.04	0.09
	Frying	0.20	0.15	0.09	0.76	0.51	0.86	8.1	4.9	4.8	0.06	<LOQ	<LOQ	0.08	0.02	0.05	0.06	0.14	0.26	0.25	0.19	0.38
Blue mussel 2	Raw	0.16	0.17	0.17	0.41	0.42	0.51	8.1	6.1	5.8	0.05	<LOQ	<LOQ	0.08	0.03	0.06	0.74	0.23	0.31	0.15	0.05	0.16
	Boiling	0.19	0.19	0.19	0.43	0.69	0.75	7.2	4.2	3.9	0.06	<LOQ	<LOQ	0.08	0.02	0.04	0.02	0.09	0.07	0.14	0.03	0.08
	Frying	0.16	0.17	0.17	0.63	0.57	0.75	9.5	6.1	5.4	0.04	0.01	<LOQ	0.09	0.04	0.04	0.05	0.18	0.27	0.34	0.11	0.35

* Statistical differences ($p < 0.05$) of storage and/or treatment are given in the text of the specific seafood in Section 3.

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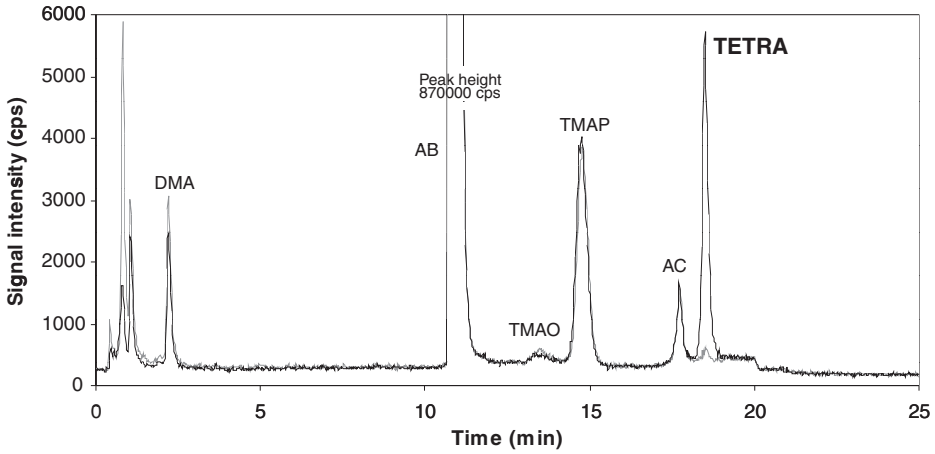

Fig. 2. Overlaid chromatograms illustrating the formation of tetramethylarsonium ion (TETRA) during frying of Atlantic cod. Grey line: raw Atlantic cod fillet, black line: fried Atlantic cod fillet.

lower in raw samples than in boiled samples ($p = 0.0005$) or fried samples ($p = 0.003$). The DMA, TMAO and AC concentrations in the samples frozen for one month were significantly higher than the fresh samples ($p = 0.0001$, $p = 0.0003$ and $p = 0.01$, respectively) and than the samples frozen for three month ($p = 0.0002$, $p = 0.003$ and $p = 0.003$, respectively). Boiling of Atlantic cod released ~5.0% of the total arsenic to the boiling water (data not shown). The boiling water of the fresh samples of cod contained the same species as did the MeOH/H_2O extract, except for TMAP, which was absent.

3.3.3. Atlantic salmon

The arsenicals AB, DMA, TMAO, AC and TETRA (but no TMAP) were found in samples of Atlantic salmon. In addition, one unknown peak eluted very close to the void volume. AB was the predominant species, constituting ~90% of total arsenic, whereas the other arsenic species were found only at trace levels. The extraction efficiency was in the range of 90–120%, with a median of 97% when comparing the sum of the species with total arsenic concentration. Arsenicals not extracted probably include arsenolipids (a collective term for lipid-soluble arsenicals), which may be found at concentrations of 9–14 mg As/kg in fish oils (Sloth, Julshamn, & Lundebye, 2005c) and probably also in fatty fish, such as Atlantic salmon. The concentrations of the different arsenic species were fairly constant during storage by freezing, with the exception of DMA, where a significant increase in the concentration, after one month storage, was observed ($p = 0.0005$), possibly due to degradation of arsenolipids. DMA has previously been identified as a human metabolite of arsenolipids (Schmeisser, Goessler, & Francesconi, 2006). Boiling of Atlantic salmon released approximately 4% of total arsenic to the boiling water and, in general, the same As species were found here as in the fresh samples. In two of the three samples of the boiling water, a small unknown peak, eluting just before DMA, was seen. A peak with the same retention time was also observed in samples of blue mussels. Again, frying of the samples significantly increased the concentration of TETRA ($p < 0.00004$). In addition, storage for three month significantly increased the TETRA concentration ($p = 0.03$). The AC concentration in samples frozen for one month was significantly lower than those of the fresh samples ($p = 0.002$) or samples frozen for three months ($p = 0.002$). Fried samples had significantly higher AC concentration than had boiled samples ($p = 0.02$), but they were not different from the raw samples ($p > 0.05$). In some of the processed samples, small peaks of DMAP were found (Fig. 3). Degradation of TMAP, as suggested by Sloth et al. (2005b), is not a plausible route for the formation of DMAP, since this species was not found in the raw salmon samples. It is more likely that the DMAP origi-

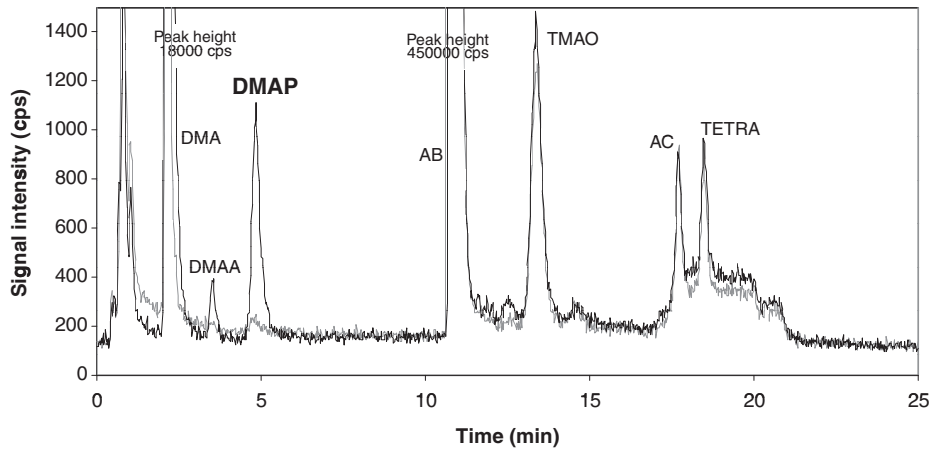

Fig. 3. Overlaid chromatograms illustrating the formation of dimethylarsinoylpropionate (DMAP) during processing (by boiling) of Atlantic salmon. Grey line: raw Atlantic salmon, black line: boiled Atlantic salmon.

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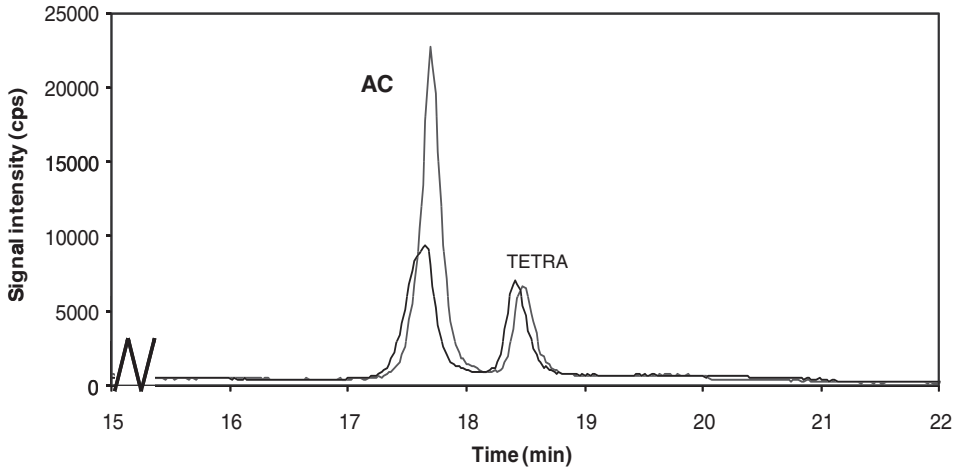

Fig. 4. Overlaid chromatograms illustrating the loss of arsenocholine (AC) in blue mussels following storage (frozen for three month). Grey line: fresh blue mussels; black line: blue mussel frozen for three months. Please note that only the arsenicals eluting after 16 min are shown.

nates from hydrolysis of arsenolipids present in the lipid-rich Atlantic salmon fillet, as previously suggested by Schmeisser et al. (2006).

3.3.4. Blue mussels

The analysis of the blue mussel samples showed a more complex speciation pattern than did Atlantic cod and Atlantic salmon. The arsenic species, DMA, DMAA, AB, TMAO, TMAP, AC and TETRA, were found, in addition to three to four unknown peaks. In raw and fried samples, the unidentified peaks eluted between AB and TMAO. In the boiled samples, only one of the unidentified peaks, between AB and TMAO, was observed. The extraction efficiency of the blue mussels was lower than that for the fish fillets and was in the range 52–71%, with a median of 58% when comparing the sum of the species with total arsenic concentration, which is in accordance with literature data (Kirby & Maher, 2002; Sloth et al., 2005b). AB was the major arsenic species, constituting 51% (±10%) of total arsenic. Unlike Atlantic cod and Atlantic salmon, blue mussels storage by freezing significantly decreased the AB concentration ($p = 0.0002$). Processing also resulted in altered levels of AB. The boiled samples had significantly lower AB concentrations than had raw samples ($p = 0.005$) or fried samples ($p = 0.02$). TMAO was observed in the fresh samples, but not in the frozen samples, showing that storage significantly decreased the TMAO concentration ($p < 0.001$). DMA, AC and TETRA occurred at trace levels, but nevertheless at higher concentrations in the blue mussels than in the fish samples. The amount of unidentified species constituted ~47% (±3%) of total extracted arsenicals in the blue mussel samples. Boiling of blue mussels released ~1.5% of the total arsenic to the boiling water and the same species were found here as in the original sample. The unknown peaks found in the boiled samples were not observed in the boiling water. Both storage ($p = 0.02$) and heat processing ($p = 0.001$) significantly increased the DMA concentration. A significant decrease in the AC levels was observed when comparing the raw with the heat-processed samples ($p = 0.0008$) (Fig. 4). In addition, storage also significantly reduced the AC concentration ($p = 0.04$). As seen for samples of Atlantic cod and Atlantic salmon, frying of the samples also significantly increased the TETRA levels in blue mussels ($p = 0.00005$).

4. Conclusions

The present study has determined the effect of processing (boiling and frying) and storage by freezing on the concentrations and speciation pattern of arsenic compounds in Atlantic cod, Atlantic salmon and blue mussels. Seafood remains a major source of dietary arsenic exposure but, from a food safety aspect, the content and intake of inorganic arsenic are much more important than those of organoarsenic compounds. Food safety assessment of seafood, with respect to arsenic, requires the determination of different arsenic species, especially inorganic arsenic, and their fate during processing and storage. Inorganic arsenic was found above the LOQ only in blue mussels and, importantly, no significant increases in levels of inorganic arsenic were observed after processing or after storage by freezing. Neither processing nor freezing resulted in measurable amounts of inorganic arsenic in the Atlantic cod and Atlantic salmon samples. The processing of the samples caused a limited loss of water, resulting in increased arsenic concentration on a wet weight basis, which is in line with other studies (Devesa et al., 2001a; Ersoy et al., 2006). In general, processing or storage by freezing did not change the total arsenic concentration or alter the speciation pattern greatly; however, in blue mussels, a significant decrease of total arsenic concentration after storage was observed. Although the concentration of TETRA was low, also after processing, significantly increased concentrations of TETRA were observed in all fried samples of both fresh and frozen seafood. This is noteworthy, since TETRA is considered to be more acute toxic than AB (Shiomi, Horiguchi, & Kaise, 1988). In conclusion, processing or storage by freezing did not change the total arsenic content or alter the speciation pattern greatly.

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References

Alexander, J., Frøyland, L., Hemre, G. I., Jacobsen, B. K., Lund, E., Meltzer, H. M., et al. (2007). *A comprehensive assessment of fish and other seafood in the Norwegian diet*. Norwegian Scientific Committee for Food safety. ISBN:978-82-8082-207-9.

Amran, M. B., Lagarde, F., Leroy, M. J. F., & Maier, E. (1997). Determination of arsenic species in marine organisms by HPLC–ICP–OES and HPLC–HG–QFAAS. *Microchimica Acta, 127*, 195–202.

Borak, J., & Hosgood, H. D. (2007). Seafood arsenic: Implication for human risk assessment. *Regulatory Toxicology Pharmacology, 47*, 204–212.

Dabeka, R. W., Mckenzie, A. D., Lacroix, G. M. A., Cleroux, C., Bowe, S., Graham, R. A., et al. (1993). Survey of arsenic in total diet food composites and estimation of

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the dietary intake of arsenic by Canadian adults and children. *Journal AOAC International, 76*, 14–25.
De Gieter, M., Leermarkers, M., Van Ryssen, J., Noyen, L., Goeyens, L., & Baeyens, W. (2002). Total and toxic arsenic levels in North Sea fish. *Archives of Environmental Contamination and Toxicology, 43*, 406–417.
Devesa, V., Macho, M. L., Jalón, M., Urieta, I., Muñoz, O., Súñer, M. A., et al. (2001a). Arsenic in cooked seafood products: Study on the effect of cooking on total and inorganic arsenic contents. *Journal of Agricultural and Food Chemistry, 49*, 4132–4140.
Devesa, V., Martinez, A., Súñer, M. A., Vélez, D., Almela, C., & Montoro, R. (2001b). Effect of cooking temperatures on chemical changes in species of organic arsenic in seafood. *Journal of Agricultural and Food Chemistry, 49*, 2272–2276.
Devesa, V., Velez, D., & Montoro, R. (2008). Effect of thermal treatments on arsenic species content in food. *Food and Chemical Toxicology, 46*, 1–8.
EFSA Panel on Contaminants in the Food Chain (CONTAM) (2009); Scientific Opinion on Arsenic in Food. EFSA Journal; 7(10):1351. Available from:www.efsa.europa.eu. doi:10.2903/j.efsa.2009.1351.
Ersoy, B., Yanar, Y., Kücükgülmez, A., & Celik, M. (2006). Effects of four cooking methods on the heavy metal concentration of sea bass fillets (*Dicentrarhus labrax* Linne, 1785). *Food Chemistry, 99*, 748–751.
Francesconi, K. A., & Kuehnelt, D. (2002). Arsenic compounds in the environment. In W. T. Frankenberger, Jr. (Ed.), *Environmental chemistry of arsenic*. New York: Marcel Dekker.
Francesconi, K. A., & Kuehnelt, D. (2004). Determination of arsenic species: A critical review of methods and applications, 2000–2003. *Analyst, 129*, 373–395.
Francesconi, K. A. (2007). Toxic metal species and food regulations – Making a healthy choice. *Analyst, 132*, 17–20.
Hirata, S., Toshimitsu, H., & Aihara, M. (2006). Determination of arsenic species in marine samples by HPLC–ICP–MS. *Analytical Sciences, 22*, 39–43.
Julshamn, K., Lundebye, A. K., Heggstad, K., Berntssen, M. H., & Bøe, B. (2004). Norwegian monitoring programme on the inorganic and organic contaminants in fish caught in the Barents Sea, Norwegian Sea and North Sea, 1994–2001. *Food Additives and Contaminants, 21*, 365–376.
Julshamn, K., Maage, A., Norli, H. S., Grobecker, K. H., Jorheim, L., & Fecher, P. (2007). Determination of arsenic, cadmium, mercury, and lead by inductively coupled plasma/mass spectrometry in foods after pressure digestion: NMKL interlaboratory study. *Journal of AOAC International, 90*, 844–856.
Kirby, J., & Maher, W. (2002). Measurement of water-soluble arsenic species in freeze-dried marine animal tissues by microwave-assisted extraction and HPLC–ICPMS. *Journal of Analytical Atomic Spectrometry, 17*, 838–843.
Larsen, E. H. (1995). Speciation of dimethylarsinyl-riboside derivatives (arsenosugars) in marine reference materials by HPLC–ICPMS. *Fresenius' Journal of Analytical Chemistry, 352*, 582–588.
Li, W., Wei, C., Zhang, C., Van Hulle, M., Cornelis, R., & Zhang, X. (2003). A survey of arsenic species in Chinese seafood. *Food and Chemical Toxicology, 41*, 1103–1110.
Munõz, O., Devesa, V., Suñer, M. A., Vélez, D., Montoro, R., Urieta, I., et al. (2000). Total and inorganic arsenic in fresh and processed fish products. *Journal of Agricultural and Food Chemistry, 48*, 4369–4376.
NIFES, seafood database: www.nifes.no (accessed on March 2010).
Norwegian Ministries (2007). *The Norwegian Action Plan on Nutrition (2007–2011) Recipe for a healthier diet.* Report from Ministry of Health and Care Services. Publication code: I-1128 E. Short version in English retrieved from:<http://www.helsedirektoratet.no/vp/multimedia/archive/00026/IS-0238_26559a.pdf>.
Schmeisser, E., Goessler, W., & Francesconi, K. A. (2006). Human metabolism of arsenolipids present in cod liver. *Analytical and Bioanalytical Chemistry, 385*, 367–376.
Shiomi, K., Horiguchi, Y., & Kaise, T. (1988). Acute toxicity and rapid excretion in urine of tetramethylarsonium salts found in some marine animals. *Applied Organometallic Chemistry, 2*, 385–389.
Sloth, J. J., Larsen, E. H., & Julshamn, K. (2003). Determination of organoarsenic species in marine samples using gradient elution cation exchange HPLC–ICPMS. *Journal of Analytical Atomic Spectrometry, 18*, 452–459.
Sloth, J. J., Larsen, E. H., & Julshamn, K. (2005a). Survey of inorganic arsenic in marine animals and marine certified reference materials by anion exchange HPLC–ICPMS. *Journal of Agricultural and Food Chemistry, 53*, 6011–6018.
Sloth, J. J., Larsen, E. H., & Julshamn, K. (2005b). Report on three aliphatic dimethylarsinoyl compounds as common minor constituents in marine samples. An investigation using high-performance liquid chromatography/inductively coupled plasma mass spectrometry and electrospray ionisation tandem mass spectrometry. *Rapid Communications in Mass Spectrometry, 19*, 227–235.
Sloth, J. J., Julshamn, K., & Lundebye, A. K. (2005c). Total and inorganic arsenic content in Norwegian fish feed products. *Aquaculture Nutrition, 11*, 61–66.
Sloth, J. J., & Julshamn, K. (2008). Survey of total and inorganic arsenic content in blue mussels (*Mytilus edulis* L.) from Norwegian fiords: Revelation of unusual high levels of inorganic arsenic. *Journal of Agricultural and Food Chemistry, 56*, 1269–1273.
Sörös, C., Bodó, E. T., Fodor, P., & Morabito, R. (2003). The potential of arsenic speciation in molluscs for environmental monitoring. *Analytical and Bioanalytical Chemistry, 377*, 25–31.
Súñer, M. A., Devesa, V., Clemente, M. J., Vélez, D., Montoro, R., Urieta, I., et al. (2002). Organoarsenical species contents in fresh and processed seafood products. *Journal of Agricultural and Food Chemistry, 50*, 924–932.
WHO (2010). <http://www.who.int/entity/foodsafety/chem/summary72_rev.pdf>.