Monomethylarsonic and Dimethylarsinic Acid Contents in Seafood Products

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The aim of the present study is to obtain information about the levels of monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in popular seafood products and to study the possible effect of the manufacturing process on the levels of arsenical species. The methodology employed couples high-performance liquid chromatography with hydride generation atomic absorption spectrometric detection. Among all of the samples analyzed (n = 29), only 3 mollusks presented MMA levels above the limit of detection for the method, 0.6−3.7 ng g^{-1}, fresh mass (fm), expressed as arsenic compound, and according to the dilution employed. The DMA levels ranged between <2 and 475 ng g^{-1} (fm), with the highest values occurring in the fish group. In 10 of the samples analyzed the total arsenic (fm) represented by unknown species [other than arsenobetaine (AB), MMA, and DMA] was around 1 μg g^{-1} or considerably higher (1.7−4.8 μg g^{-1}) mostly in frozen and preserved fish. In the fish group the highest level of DMA expressed as a percentage of total arsenic was found in the preserved fish. More research is needed to identify all arsenic compounds in seafood and to confirm whether AB is degraded during the manufacturing processes, producing DMA and other arsenical compounds.

Keywords: *Monomethylarsonic and dimethylarsinic acid levels; arsenic levels; high-performance liquid chromatography−hydride generation atomic absorption spectrometry; manufactured seafood*

It has long been known that seafood products are the most significant source of arsenic in our dietary intake (MAFF, 1982; Larsen, 1993), and thus the total amount of As ingested daily by humans is strongly influenced by the amounts of seafood included in the diet (Anke, 1986). Conversions of arsenic between oxidation states and organometalloid forms alter the binding affinities of arsenic for different proteins, thus altering the relative toxicities of the various arsenic species (Thompsom, 1993). Therefore, it is important to determine the amounts of individual compounds rather than just the total arsenic concentrations. Among the arsenic compounds that have so far been most frequently reported in marine animals are monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Morita and Edmonds, 1992). They present a toxicity situated between the most toxic inorganic forms—AsIII, LD_{50} (oral administration to rats), 0.0345 (g/kg)—and the organic forms—arsenobetaine (AB), LD_{50} >10.0 g/kg, practically nontoxic (Shiomi, 1994). These arsenical species present toxicological problems. Among those described for DMA are damage to DNA (Yamanaka et al., 1989a, 1991) and mutagenicity (Yamanaka et al., 1989b). Moreover, DMA enhances carcinogenesis in the urinary bladder, kidney, liver, and thyroid gland of treated rats (Yamamoto et al., 1994). These authors speculated that DMA is a carcinogen or carcinogen promoter for these organs.

MMA and DMA are the most commonly detected species in marine waters (Millward et al., 1993), mainly as a consequence of phytoplankton activity, DMA being the dominant organoarsenic compound (Cullen and Reimer, 1989). MMA was generally present in smaller concentrations, existing probably as an intermediate in the arsenic methylation sequence (Braman and Foreback, 1973). Marine animals contain traces of these methylated forms, although very few data on the concentrations of these species have been reported.

Previous studies in which we quantified total As and AB in a wide range of seafood products showed that in some manufactured products AB was not the dominant arsenic species (Velez et al., 1995), going against the general consensus established for seafood samples which holds that AB is the dominant species (Leah et al., 1992; Larsen et al., 1993). This could be due to a metabolic degradation of AB to less methylated arsenic compounds [degradation of AB to trimethylarsine oxide (TMAO) or DMA by microorganisms is a ubiquitous phenomenon in the marine environment; Hanaoka et al., 1991a] or the presence of the other arsenical species in significant concentrations before the process of conservation. These results, together with the limited availability of data on MMA and DMA levels in the literature, show that there is a general need to confirm the presence of the "minor" arsenic compounds in marine animals and to determine their levels, as has been noted by other authors (Larsen et al., 1993), especially if one wishes to evaluate the potential toxicity or harmlessness of seafood products for humans.

The aim of the present study is to obtain information about levels of MMA and DMA in popular seafood products and to study the possible effect of the manufacturing process on the levels of the arsenical species. The methodology used for quantifying the species [high-performance liquid chromatography coupled with hydride generation atomic absorption spectrometry (HPLC−HG-AAS)] was optimized by us previously (Velez et al., 1996). In this methodology the species are extracted quantitatively, and the detection limit, precision, and accuracy of the method have been evaluated.

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Table 1. Description of Seafood Products Analyzed in This Work

seafood product		sample	description (source)
fish			
anchovy	fresh	1	complete fish (Valencia, Spain)
	fresh	2	complete fish (Valencia, Spain)
	in vinegar	3	anchovies, vinegar, and salt, packed in plastic, 200 g drained wt (Madrid, Spain)
	in vinegar	4	anchovies, vinegar, water, and salt, canned, 135 g drained wt (Valencia, Spain)
cod	salted	5	boneless salted fillets, packed in a plastic wrapping, 400 g net wt (Valencia, Spain)
hake	frozen	6	hake middles, packed in plastic, 350 g net wt (Valencia, Spain)
sardine	fresh	7	complete fish (Valencia, Spain)
	fresh	8	complete fish (Valencia, Spain)
	canned	9	sardines, olive oil, and salt, canned, 88 g drained wt (Vigo, Spain)
	canned	10	sardines, vegetable oil, and salt, canned, 80 g drained wt (Canarias, Spain)
sole	frozen	11	boneless fillets, in cardboard pack, 400 g net wt (Denmark)
	frozen	12	boneless fillets, in cardboard pack, 400 g net wt (Denmark)
tuna	canned	13	white tuna, olive oil, and salt, canned, 82 g drained wt (Vigo, Spain)
	canned	14	white tuna, vegetable oil, and salt, canned, 65 g drained wt (Pontevedra, Spain)
lamellibranchs			
cockles	fresh	15	complete mollusk (Valencia, Spain)
	fresh	16	complete mollusk (Valencia, Spain)
	canned	17	cockles, water, and salt, canned, 65 g drained wt (Pontevedra, Spain)
cephalopods			
octopus	canned	18	octopus, olive oil, and salt, canned, 72 g drained wt (Vigo, Spain)
	canned	19	octopus, vegetable oil, and salt, canned, 60 g drained wt (Pontevedra, Spain)
squid	fresh	20	peeled squid, without ink, eyes, tentacles, and plume (Valencia, Spain)
	fresh	21	peeled squid, without ink, eyes tentacles, and plume (Valencia, Spain)
	canned	22	pieces of squid, ink, vegetable oil, tomato, onion, spices, and salt, canned, 64 g drained wt
	canned	23	whole squid, olive oil, and salt, canned, 135 g drained wt (Vigo, Spain)
small squid	fresh	24	complete mollusk (Valencia, Spain)
	fresh	25	complete mollusk (Valencia, Spain)
	canned	26	squid, olive oil, and salt, canned, 120 g drained wt (Vigo, Spain)
	canned	27	squid, vegetable oil, water, and salt, canned, 120 g drained wt (Thailand)
crustaceans			
shrimps	frozen	28	peeled shrimp with antioxidant (E-223), packed in plastic, 200 g net wt (United Kingdom)
	frozen	29	peeled shrimp, packed in plastic, 200 g net wt (Pontevedra, Spain)

MATERIALS AND METHODS

Instrumentation. The equipment used included a high-performance liquid chromatograph (Hewlett-Packard Model 1050), equipped with a quaternary pump Model HP 79852A with on-line degassing system, an automatic injector Model 79855A, and a Hewlett-Packard personal computer, Vectra 486/33N Model 170 with 486 microprocessor rated at 33 MHz (Hewlett-Packard Española, S.A., Madrid, Spain) as data station.

For DMA and MMA determination the chromatographic system was connected to a Perkin-Elmer Model 5000 atomic absorption spectrometer equipped with a Perkin-Elmer FIAS-400 in continuous flow controlled by the software of a separate programmable PC system. The spectrometer signal was picked up by a Hewlett-Packard Model 35900C digital analogical converter, using the chromatograph software.

Determination of total As was performed, in previously dry-ashed samples, with a Perkin-Elmer Model 5000 or 3300 atomic absorption spectrometer equipped with an FIAS-400 in continuous flow.

A lyophilizer equipped with a microprocessor controlling the lyophilization process was employed. This microprocessor was connected to an Epson Equity I+ computer (FTS Systems, Giralt, S.A., Madrid, Spain).

Other equipment used included a Janke & Kunkel Model A10 homogenizer water-refrigerated mill (Schott Iberica, Barcelona, Spain), an MSE Minor centrifuge (Pacisa, Madrid, Spain), a Millipore Inc. Milli-Q water purification system (Millipore Ibérica, S.A., Madrid, Spain), and a P-Selecta Vibromatic 340 mechanical arm shaker (Selecta, Barcelona, Spain).

Reagents. Deionized water (18 MΩ cm) obtained with the Milli-Q water system was used for the preparation of reagents and standards. All chemicals including standards and solutions were of Pro Analysi quality or better: hydrochloric acid (ρ = 1.19 g mL^{-1}) (Panreac, Montplet & Esteban, S.A., Montcada i Reixac, Barcelona, Spain); nitric acid (ρ = 1.38 g mL^{-1}) (Probus, Probus S.A., Badalona, Barcelona, Spain); ammonia solution, 32% extra pure (Merck, Igoda, Barcelona, Spain); HPLC phosphate buffers $Na_2HPO_4 \cdot 2H_2O$ (Scharlau, S.L., Barcelona, Spain) and H_3PO_4 (ρ = 1.70 g mL^{-1}) 85% (Panreac).

The stock standard solutions were a solution of As(III) (1000 mg L^{-1}) prepared by dissolving 1.320 g of arsenic trioxide (Riedel de Haën, Riedel de Haën GmbH, Hannover, Germany) in 25 mL 20% (m/v) KOH solution, neutralized with 20% (v/v) H_2SO_4 and diluted to 1 L with 1% (v/v) H_2SO_4. Solutions of MMA and DMA were prepared by dissolving in water appropriate amounts of $CH_3AsO(ONa)_2 \cdot 6H_2O$ (Carlo Erba, Farmitalia Carlo Erba, S.p.a., Milano, Italy) and $(CH_3)_2AsNaO_2 \cdot 3H_2O$ (Fluka, Fluka Chemika Biochemika, Alcobendas, Madrid, Spain).

As reducing solutions for hydride generation coupled to HPLC, we used 1.5 (w/v) sodium tetrahydroborate(III) (Probus) solution prepared by dissolving $NaBH_4$ powder in 0.7 (w/v) NaOH solution, filtered through Whatman No. 42 paper. $NaBH_4$ solutions were prepared freshly every day. For the determination of total arsenic a prereducing solution, containing 5% (w/v) IK (Panreac) and 5% (w/v) ascorbic acid (Merck), was used prior to hydride generation.

Reference Material DORM-1 (dogfish muscle) was obtained from the National Research Council Canada, Institute for Environmental Chemistry, Ottawa, Canada.

All glassware was treated with 10% (v/v) HNO_3 for 24 h and then rinsed three times with Milli-Q water before being used for the first time. Between uses glassware was placed in 10% (v/v) HNO_3 for 24 h.

Commercial Samples. Various fresh, frozen, and canned seafood products were purchased at local retail outlets. For the determination of total As, MMA, and DMA, 29 samples were analyzed. Descriptions and sample sources are given in Table 1.

Sample Preparation. The samples were prepared according to the methodology developed previously (Velez et al., 1995).

Determination of Total As. The seafood products were dry-ashed by applying the methodology of dry mineralization developed previously (Ybáñez et al., 1992), and the total As was determined by HG-AAS. The mineralized samples were

Table 2. Total Arsenic, MMA, and DMA in Seafood Samples Purchased at Local Retail Market Outlets[a]

			total As		MMA		DMA	
seafood product		sample	dm	fm	dm	fm	dm	fm
fish								
anchovy	fresh	1	14930	3880	<2.2	<0.6	517	134
	fresh	2	18800	4470	<2.2	<0.6	462	110
	in vinegar	3	6330	2060	<4.5	<1.5	1455	475
	in vinegar	4	5720	1520	<4.5	<1.1	1110	294
cod	salted	5	6560	3900	<2.2	<1.3	160	96
hake	frozen	6	5610	1280	<2.2	<0.6	110	202
sardine	fresh	7	16720	3990	<2.2	<0.6	504	120
	fresh	8	15500	3970	<2.2	<0.6	550	142
	canned	9	4850	2090	<2.2	<0.9	896	393
	canned	10	3270	1360	<4.5	<1.9	878	364
sole	frozen	11	43660	8990	<2.2	<0.4	92	18
	frozen	12	22980	4130	<2.2	<0.4	42	7
tuna	canned	13	2050	870	<4.5	<1.9	131	55
	canned	14	3040	1210	<2.2	<0.9	70	28
lamellibranchs								
cockles	fresh	15	20710	4640	<2.2	<0.6	112	26
	fresh	16	17700	3490	101	21	414	81
	canned	17	11740	2800	<2.2	<0.6	791	190
cephalopods								
octopus	canned	18	6860	2280	<2.2	<0.7	53	18
	canned	19	9470	2930	<2.2	<0.7	90	28
squid	fresh	20	1900	260	<2.2	<0.4	<9	<2
	fresh	21	890	140	<2.2	<0.4	<9	<2
	canned	22	1940	810	<2.2	<0.9	33	15
	canned	23	3740	1250	<2.2	<0.7	<9	<4
small squid								
	fresh	24	9850	1970	103	21	136	28
	fresh	25	26210	4650	37	8	228	40
	canned	26	2610	820	<2.2	<0.7	<9	<4
	canned	27	9780	2900	<2.2	<0.7	<9	<2
crustaceans								
shrimps	frozen	28	2400	350	<4.5	<0.6	29	4
	frozen	29	6000	1050	<3.4	<0.6	<7	<2

[a] Results expressed in ng g^{-1} of total arsenic and of arsenic compound.

prereduced prior to analysis by HG-AAS in continuous flows by adding 1 mL of concentrated HCl and 1 mL of prereducing solution to 1 mL of sample, waiting 45 min at ambient temperature, and diluting to 10 mL for analysis. The instrumental conditions used for determination of arsenic were the following: atomic absorption spectrometer, wavelength (193.7 nm), bandpass (0.7 nm), lamp power (8.5 W) (electrodeless discharge lamp); hydride generation, cell temperature (900 °C), reducing agent [1.5% (w/v), $NaBH_4$ in 0.7% (w/v) NaOH, 1 mL min^{-1} flow rate], HCl solution (1.5 M; 1.2 mL min^{-1} flow rate), carrier gas (argon, 45 mL min^{-1} flow rate).

Extraction and HPLC−HG-AAS Determination of MMA and DMA. MMA and DMA were extracted by employing the modified Shibata and Morita extraction developed elsewhere (Velez et al., 1996). Lyophilized sample (2.00 ± 0.01 g) was weighed into a 50 mL centrifuge tube with screw top and conical base. Methanol/water (40 mL, 1:1 v/v) was added, and the tube was agitated for 15 min in a mechanical arm shaker. The extract was collected after centrifugation at 2000 rpm for 10 min. The extraction process was repeated three times, and the extracts were combined, evaporated to dryness, dissolved in 3 mL of water, and filtered through Whatman No. 1 and No. 42 papers and a 0.45 μm filter.

Before injection into the chromatographic system, the sample extracts were evaporated (T = 55 °C) until dry and redissolved in water; MMA and DMA were determined in the water extract by using an HPLC system interfaced via Teflon tubing to the continuous HG-AAS. The HPLC separation and HG-AAS conditions have been described previously (Velez et al., 1996). The HPLC separation was performed with phosphate buffer at pH 5.75 in gradient concentration elution. Determinations were made according to the method of standard additions, and peak area signal was measured. The quantities added were approximately equal to 2 and 3 times MMA and DMA content determined previously by comparison with the standard curve.

The instrumental settings for the high-performance liquid chromatograph were the following: 10 μm polymer base anionic exchange column Hamilton PRP X-100 (25.0 cm × 4.1 mm i.d.) (Teknokroma, Barcelona); 10−20 μm polymer base anionic exchange guard column Hamilton PRP X-100 (25 × 2.3 mm i.d.) (Teknokroma); mobile phase, phosphate buffer (Na_2HPO_4/H_3PO_4) at pH 5.75; solvent A 20 mmol L^{-1}, solvent B 100 mmol L^{-1}, gradient elution 100% solvent A for 2 min, decreasing to 50% solvent A/50% solvent B in 0.1 min, and maintaining this ratio for 3 min, then reaching 100% solvent A again in 0.1 min and maintaining for 7 min; flow rate, 1 mL min^{-1}; injection volume, 100 μL; temperature, 28 °C.

The instrumental conditions selected for hydride generation were the same as those used to determine total arsenic. The analytical features of the HPLC−HG-AAS method are as follows: detection limits expressed as nanograms per gram of arsenic compound, taking the average moisture of seafood products to be 75% (Velez et al., 1995), which vary in accordance with the dilution employed: for MMA, 2.2−13.1, dry mass (dm) and 0.6−3.7 (fm); for DMA, 4.8−29.4 (dm) and 1.2−7.4 (fm). Relative standard deviations were 3% for MMA and 2% for DMA. The percentage recoveries were 103 ± 6 for MMA and 99 ± 5 for DMA. The analysis of DORM-1 provided a DMA value of 440 ± 40 ng g^{-1} As (dm), very close to that obtained by other authors using HPLC−ICP mass spectrometry.

RESULTS AND DISCUSSION

Determination of MMA and DMA in Seafood Products. The levels of total arsenic, MMA, and DMA, expressed in terms of dry mass and fresh mass for all of the samples of seafood analyzed, are given in Table 2. For arsenic the total range found was 0.14−8.99 μg g^{-1} As (fm). Samples of frozen sole, fresh anchovy, fresh small squid, and fresh cockles gave values over 4 μg g^{-1} (fm).

Table 3. Arsenobetaine, MMA, and DMA in Seafood Samples Purchased at Local Retail Market Outlets[a]

seafood product		sample	AB (%)	MMA (%)	DMA (%)	others (%)
fish						
anchovy	fresh	01	94	<0.008	1.9	4
	fresh	02	81	<0.006	1.3	18
	in vinegar	03	14	<0.04	12.5	74
	in vinegar	04	<9	<0.04	10.5	>80
cod	salted	05	na[b]	<0.02	1.3	nd[c]
hake	frozen	06	31	<0.02	1.1	68
sardine	fresh	07	77	<0.007	1.6	21
	fresh	08	93	<0.008	1.9	5
	canned	09	23	<0.03	10.0	67
	canned	10	41	<0.07	14.6	44
sole	frozen	11	47	<0.003	0.1	53
	frozen	12	35	<0.005	0.1	65
tuna	canned	13	<24	<0.1	3.5	>72
	canned	14	25	<0.04	1.3	74
lamellibranchs						
cockles	fresh	15	53	<0.006	0.3	47
	fresh	16	50	0.3	1.3	49
	canned	17	28	<0.01	3.7	68
cephalopods						
octopus	canned	18	71	<0.02	0.4	29
	canned	19	42	<0.01	0.5	57
squid	fresh	20	119	<0.08	<0.3	0
	fresh	21	na	<0.1	<0.6	nd
	canned	22	<21	<0.06	0.9	>78
	canned	23	<16	<0.03	0.1	>84
small squid						
	fresh	24	82	0.6	0.8	17
	fresh	25	94	0.08	0.5	5
	canned	26	<19	<0.05	<0.2	>81
	canned	27	<5	<0.01	<0.1	>95
crustaceans						
shrimps	frozen	28	<18	<0.1	0.7	>81
	frozen	29	37	<0.03	<0.1	63

[a] Results expressed as percentages of arsenic in fresh mass. [b] na, not analyzed. [c] nd, not determined.

For MMA the levels found ranged from <2.2 to 103 ng g^{-1} (dm) or from <0.4 to 21 ng g^{-1} (fm). Of all the samples analyzed, only three had MMA levels higher than the limit of detection, which varies for each sample taking into account the moisture and dilution employed: two samples of fresh small squid [8 and 21 ng g^{-1} (fm)] and one of fresh cockles [21 ng g^{-1} (fm)]. The quantities of MMA found by other authors in other mollusks, 30−290 ng g^{-1} (fm), (Howard and Arbab-Zavar, 1981), in muscle of shrimp, crab, and plaice, <47 ng g^{-1} (dm), and in oyster, 70 ng g^{-1} (dm), (Larsen et al., 1993), are practically within the same range of levels as we found for this species.

The DMA levels ranged from <7 to 1455 ng g^{-1} (dm), or from <2 to 475 ng g^{-1} (fm). The highest DMA levels were detected in the two samples of anchovy in vinegar [294 and 475 ng g^{-1} (fm)], followed by that found in the two samples of canned sardines [364 and 393 ng g^{-1} (fm)]; in the fish group the lowest DMA level corresponded to a sample of frozen sole [7 ng g^{-1} (fm)]. In the other samples not belonging to the fish group, the highest level was in the canned and fresh cockles [81 and 190 ng g^{-1} (fm)], followed by the fresh small squid [28 and 40, ng g^{-1} (fm)]. In the cephalopod group, for 50% of the samples ($n = 5$) the level was below the limit of detection, while the other 50% of the samples presented a mean level of 26 ng g^{-1} (fm). In the two samples of frozen shrimps analyzed, the highest level was 4 ng g^{-1} (fm). The DMA levels detected are similar to those found by other authors—Branch et al. (1994) found in fish <300−500 ng g^{-1} (dm)—and are below those found by Howard and Arbab-Zavar (1981) in mollusks [1.26−1.74 μg g^{-1} (fm)] and by Larsen et al. (1993) in muscle of mussel [4 μg g^{-1} (dm)] and oyster [3.1 μg g^{-1} (dm)].

Table 3 shows the percentages of MMA and DMA, expressed as percentage of arsenic (fm), together with the percentages of AB also expressed as percentage of arsenic (fm), obtained by us in a previous work (Velez et al., 1995). By taking these percentages into account, we have been able to obtain the percentages that would correspond to MMA, DMA, and unknown species (other than AB, MMA, and DMA). The MMA percentages are insignificant in all of the samples with the exception of the fresh cockles (0.3%) and the fresh small squid (0.6−0.08%). The highest percentages of DMA were found in the fish group, in the anchovy in vinegar (10.5−12.5%), and in canned sardine (10−14.6%), while in the other fish samples the range was 0.1−3.5%. In the remaining samples analyzed not belonging to the fish group, only two had a DMA percentage level higher than 1% (1.3%, fresh cockles; 3.7%, canned cockles). From the results shown in Table 3 and from the total arsenic levels expressed in Table 2 we can deduce that in 10 of the 29 samples analyzed the total arsenic represented by unknown species is around 1 μg g^{-1} (fm) (anchovy in vinegar, fresh sardine, canned squid) or considerably above this level (frozen sole, 2.7−4.8 μg g^{-1}; fresh cockles, 1.7−2.2 μg g^{-1}; canned cockles, 1.9 μg g^{-1}; canned octopus, 1.7 μg g^{-1}; canned small squid, >2.75 μg g^{-1}). This confirms the view expressed by other authors that more research is needed to identify all arsenic compounds in the diet, especially in seafood (Vather, 1994).

DMA contents [expressed as percentage of As (fm)] are shown in Table 4 in terms of class of fish and manufacturing process. The total percentage range for DMA is between <0.1% and 14.6%. For fresh seafood, the total percentages of arsenic represented by DMA vary from <0.3% to 1.9% and in the case of manufac-

Table 4. Contents of DMA in Seafood Products in Terms of Class of Fish and Manufacturing Process[a]

seafood product	no. of samples	range (%)	mean[b] ± SD (%)
fresh			
fish	4	1.3−1.9	1.7 ± 0.3
lamellibranchs	2	0.3−1.3	0.8 ± 0.7
cephalopods	4	<0.3−0.8	0.6 ± 0.2
total[b]	**10**	**<0.3−1.9**	**1 ± 0.4**
frozen			
fish	3	0.1−1.1	0.4 ± 0.5
crustaceans	2	<0.1− 0.7	0.4 ± 0.4
total[b]	**5**	**<0.1−1.1**	**0.4 ± 0.5**
preserved			
fish	7	1.3−14.6	7.7 ± 5.5
lamellibranchs	1	3.7	3.7
cephalopods	6	<0.1−0.9	0.4 ± 0.3
total[b]	**14**	**<0.1−14.6**	**4 ± 2.9**
total[b]	**29**	**<0.1−14.6**	**1.8 ± 1.3**

[a] Results expressed as percentages of arsenic in fresh mass. [b] Totals and means were calculated from absolute values without considering the sign <.

Table 5. Contents of AB, MMA, DMA, and Other Compounds in Seafood Products in Terms of Class of Fish and Manufacturing Process[a]

seafood product	no. of samples	AB (%)	MMA (%)	DMA (%)	others (%)
fresh					
fish	4	86	<0.007	1.7	12
lamellibranchs	2	52	<0.15	0.8	48
cephalopods	3[b]	98	<0.25	<0.5	11
total[c]	**9**	**79**	**<0.14**	**<1.0**	**<24**
frozen					
fish	3	38	<0.009	0.4	>62
crustaceans	2	<28	<0.07	<0.4	>72
total[c]	**5**	**<33**	**<0.040**	**<0.4**	**>67**
preserved					
fish	6[b]	<23	<0.05	8.7	>69
lamellibranchs	1	28	<0.01	3.7	68
cephalopods	6	<29	<0.03	<0.4	>71
total[c]	**13**	**<27**	**<0.03**	**<4.3**	**>69**
total[c]	**27**	**<46**	**<0.07**	**<1.9**	**>54**

[a] Results expressed as percentages of arsenic in fresh mass. [b] Samples for which no AB data are available have been eliminated. [c] Means have been calculated from absolute values without considering the signs > or <.

tured products from <0.1% to 1.1% for frozen seafoods and from <0.1% to 14.6% for preserved seafood. In the fish group we see that the highest level of DMA as a percentage of total arsenic—7.7% of total arsenic—occurs in the preserved fish, with 1.7% in the fresh fish and 0.4% in the frozen fish. This tendency in the results seems to be confirmed when we consider the two species of fish analyzed (anchovy and sardine), both fresh and preserved; in these two species DMA represents a mean percentage of 1.7% of total As for the fresh fish and 12% for the preserved fish.

Table 5 shows the percentages of AB, MMA, DMA, and other unknown species, expressed as percentage of arsenic (fm), in terms of class of fish and manufacturing process. From the values found we can deduce that the highest levels of unknown species (other than AB, MMA, and DMA) occur in the frozen and preserved fish. This tendency, together with the higher levels of DMA found in preserved fish, points to the possibility that arsenobetaine may be degraded to other species, including DMA, in preserved products which may previously have been subjected to a process of refrigeration or freezing. This degradation could have been carried out by endogenous enzymes or enzymes connected with bacterial activity. This tendency is in line with that described by other authors for microorganisms associated with marine sediments (Hanaoka et al., 1988), marine macroalgae (Hanaoka et al., 1989), and intestine of marine animals (Hanaoka et al., 1991a). DMA is found among the products resulting from the degradation of AB (Hanaoka et al., 1991b). More research is needed to confirm arsenobetaine microbial degradation in real seafood samples.

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