Migration of Arsenobetaine from Canned Seafood to Brine

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A study was carried out to determine arsenobetaine (AB) in brines from canned seafood products by coupling high-performance liquid chromatography (HPLC), microwave-assisted oxidation (MO), and hydride generation-atomic absorption spectrometry (HG-AAS). Conditions were established for cleanup of the brines prior to HPLC determination of AB. The analytical features of the method for AB determination in brines were as follows: the detection limit was 2.0 ng g^{-1} As (fresh mass), the mean relative standard deviation 3%, and the mean recovery percentage 99 ± 4%. The proposed procedure was used to analyze AB in brines from canned seafood purchased at local retail outlets. The ranges of total arsenic and AB expressed as As (both in μg g^{-1} of fresh mass) were as follows: total arsenic, 0.06−3.64; AB, 0.05−3.87. The percentage of total arsenic represented by AB in brines varied from 48 to 110%. The migration of AB from muscle tissue to the brine does not rule out the possible presence in brines of other toxic species. Migrations of arsenic to other kinds of brine, oil, or sauce should also be studied.

Keywords: *Arsenobetaine; high-performance liquid chromatography; microwave-assisted oxidation; hydride generation-atomic absorption spectrometry; brines; canned seafood*

Arsenobetaine (AB) is a very widespread and possibly universal component of arsenic in higher aquatic organisms in marine environments (Shiomi, 1994). It also plays an active role in osmoregulation, where it replaces the naturally occurring osmoregulator betaine (Cardinaals et al., 1985), and consequently, it may serve as an osmolyte (Edmonds and Francesconi, 1988). We stated in previous studies (Vélez et al., 1995, 1996a) that AB contents in canned seafood without sauce or brine vary within very broad limits from 0.03 to 5.4 μg g^{-1} As, fresh mass (fm). These studies confirmed that there is generally a lower percentage of total As represented by AB in preserved products (canned or frozen) than in fresh products.

As AB is a water-soluble organoarsenical, AB may leach out into the brine of canned seafoods. Since there is no guarantee that the consumer will discard the brine when consuming the product, the determination of the arsenical species contents in the brine should be investigated. The total As and the percentage of total As represented by AB can be indicators of arsenical toxicity. In cases where the total As content is high and the percentage of total arsenic represented by AB low, it would be necessary to quantify the other arsenical species. Moreover, as indicated previously, the possibility of As migration (in the form of AB or some other arsenical species) could indicate the need to provide legislation for the maximum permitted level of As in the complete product, including both seafood and brine.

The object of this study is, therefore, to examine the possible migration of AB to brines obtained from seafood products acquired in local retail outlets and at the same time to obtain information about the levels of AB in the brines.

The technology used to quantify AB, high-performance liquid chromatography microwave-assisted oxidation, and hydride generation-atomic absorption spectrometry (HPLC-MO-HG-AAS) was previously used by us (Vélez et al., 1996a) to determine AB in canned seafood. In the present study, the quantitative recovery of AB after a brine cleanup procedure prior to HPLC separation was investigated and the detection limit, precision, and accuracy of the method were evaluated.

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MATERIALS AND METHODS

Instrumentation. The equipment used in the hyphenated HPLC-MO-HG-AAS system included a high-performance liquid chromatograph (Hewlett-Packard Model 1050), equipped with a quaternary pump Model HP 79852A with on-line degassing system; a Rheodyne valve fitted with a 100 μL loop and a data station [Hewlett-Packard personal computer, Vectra 486/33N Model 170 with 486 microprocessor rated at 33 MHz (Hewlett-Packard Española, S.A., Madrid, Spain)].

For AB determination, the chromatographic system was connected to a domestic Moulinex Super Crousty microwave oven with a maximum power of 1100 W and an operating frequency of 2450 MHz. A loop of polytetrafluoroethylene (PTFE) tubing was placed inside the microwave through the ventilation holes. An additional oven load of 400 mL of water was placed inside the oven to prevent overheating. The effluent coming from the microwave oven through the PTFE tubing was cooled in an ice bath before it reached the hydride generator, to avoid overpressure and decomposition of the sodium tetrahydroborate. The Perkin-Elmer Model 5000 atomic absorption spectrometer was equipped with a flow injection analysis system (Perkin-Elmer FIAS-400) operating as a hydride generator in continuous-flow mode. A drainage system was incorporated for the waste solution from the hydride generation, working at constant pressure through a peristaltic pump. An electrothermically heated quartz cell was employed. The HG-AAS was controlled by the software of a separate programmable PC system. The spectrometer signal was acquired by a Hewlett-Packard Model 35900 C analogue-to-digital converter, using the chromatograph software. Peak area signals were recorded.

Determination of total arsenic was performed with an atomic absorption spectrometer (Perkin-Elmer Model 5000) equipped with a Perkin-Elmer FIAS-400 operating as a hydride generator in continuous-flow mode.

Reagents. Deionized water (18 MΩ·cm) obtained with a Milli-Q water system (Millipore Inc., Millipore Ibérica, S. A.,

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Table 1. Seafood Products and Source of Brines Analyzed for Arsenobetaine

seafood product	sample	description (source)
		Fish
salmon	01	smoked salmon, skinless and boneless, water, salt, 150 g drained weight (Chile)
tuna	02	white tuna, water, salt, 63 g drained weight (Pontevedra, Spain)
		Lamellibranchs
razor clams	03	pacific razor clams, water, salt, stabilizer (E-450) and antioxidant (H 3246), 65 g drained weight (Chile)
razor clams	04	pacific razor clams, water, salt, 78 g drained weight (Chile)
cockles	05	cockles, water, salt, 130 g drained weight (Holland)
langostillo	06	langostillo, water, salt, 60 g drained weight (Pontevedra, Spain)
mussel	07	mussel, water, salt, 70 g drained weight (Spain)
clams	08	clams, water, salt, 65 g drained weight (Spain)
		Gastropods
snails	09	sea snails, water, salt, stabilizer (E-450) and antioxidant (E 300, H 3246), 110 g drained weight (Chile)
		Crustaceans
shrimp	10	peeled shrimp, water, salt, citric acid, 120 g drained weight (Thailand)
	11	peeled shrimp, water, salt, citric acid, 120 g drained weight (Thailand)
crab	12	fancy pacific crab, water, salt, stabilizer (E-450, E-300) and antioxidant (H 3246), 120 g drained weight (Chile)

Madrid, Spain) 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 G.m.b.H., Hannover, Germany) in 25 mL of 20% (w/v) KOH solution, neutralizing with 20% (v/v) H_2SO_4, and diluting to 1 L with 1% (v/v) H_2SO_4. Arsenate solution As(V) was prepared by dilution of the Titrisol standard (1000 mg L^{-1}) (Titrisol, Merck, Darmstadt, Germany). Solutions of monomethylarsonic acid (MMA) and dimethylarsinic acid (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). AB solution (973 mg L^{-1}) and arsenocholine (AC) solution (1000 mg L^{-1}) were obtained from the Service Central d'Analyse du CNRS-SCA, Vernaisson, France.

Oxidizing potassium persulfate (Probus) solutions (1% w/v) were prepared daily in 2.5% (w/v) NaOH. As reducing solutions for hydride generation coupled to HPLC we used 2% (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 filter paper. Fresh $NaBH_4$ solutions were prepared daily.

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. The brines employed were obtained from canned seafood products purchased at local retail outlets. Descriptions of the seafood products from which the brines were obtained are given in Table 1.

Sample Preparation. The brines in the canned seafood products were removed by the method for determining the drained mass of canned foods (BOE, 1984). The brines collected were placed into a 50 mL centrifuge tube with a screw top and conical base and centrifuged at 4000 rpm (1510*g*) for 10 min. The supernatant liquid was filtered through Whatman No. 1 filter paper, stored in previously decontaminated polyethylene bottles, and kept in the freezer ($T = -14$ °C) until analysis.

Determination of Total As. The brines were dry-ashed, applying the dry mineralization methodology developed previously (Cervera et al., 1989), and total arsenic was determined by HG-AAS. The ash from the mineralized samples was dissolved in 5 mL of 50% (v/v) HCl and washed with water and filtered through Whatman No. 1 filter paper into a 25 mL volumetric flask. The instrumental conditions used for determination of arsenic by HG-AAS in continuous-flow mode were as follows: atomic absorption spectrometer, wavelength (193.7 nm), spectral band-pass (0.7 nm), lamp power (8.5 W) (electrodeless discharge lamp); hydride generation, cell temperature (900 °C), sample solution (1 mL min^{-1} flow rate), 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; 2.5 mL min^{-1} flow rate), carrier gas (argon 45 mL min^{-1} flow rate).

Cleanup. Brine (2.00 ± 0.01 g) was acidified with 25 mL of 0.1 M HCl. The resulting solution was passed through a strong cation exchanger (Dowex 50W-X8, 1 × 6 cm). The column was washed with 25 mL of reagent grade water and then eluted successively with 75 mL of 4 M ammonia, 25 mL of reagent grade water, and 25 mL of 4 M HCl. The AB sorbed was eluted in the ammonia fraction. The ammonia was evaporated until dry at 55 °C because this temperature did not cause losses during the process of evaporating to dryness (Vélez et al., 1996a), and the residue was redissolved in 3 mL of QRG water and filtered through Whatman No. 1 and No. 42 filter paper and a 0.45 μm filter.

AB Determination by HPLC-MO-HG-AAS. For chromatographic separation, samples and standard solutions were loaded into a 100 μL sample loop and injected into the chromatographic column. The eluate from the column was mixed with the persulfate solution before entering the microwave oven. The thermo-oxidized effluent, cooled in an ice bath, was in turn interfaced via PTFE tubing to the continuous HG-AAS system. The operating conditions for HPLC-MO-HG-AAS have been described previously (Vélez et al., 1996a).

The instrumental settings for the high-performance liquid chromatograph were as follows: Hamilton PRP X-100, 10 μm polymer base anionic exchange column (25.0 cm × 4.1 mm i.d.) (Teknokroma, Barcelona); Hamilton PRP X-100, 10−20 μm polymer base anionic exchange guard column (25.0 mm × 2.3 mm i.d.) (Teknokroma, Barcelona), mobile phase, phosphate buffer (Na_2HPO_4/H_3PO_4) 3 mM at pH 5.00, flow rate 1 mL min^{-1}, injection volume 100 μL; temperature 28 °C.

The experimental conditions used for on-line AB microwave oxidation were as follows: power 1100 W, oven load 400 mL of water, digestion coil PTFE tubing (1.6 m, 0.5 mm i.d), refrigeration coil PTFE tubing (0.5 m, 0.5 mm i.d), oxidizing solution 1% (w/v) $K_2S_2O_8$ in 2.5% (w/v) NaOH, flow rate 0.6 mL min^{-1}.

The instrumental conditions selected for determination of arsenobetaine by HG-AAS in continuous-flow mode were as follows: atomic absorption spectrometer, wavelength (193.7 nm), spectral band-pass (0.7 nm), lamp power (8.5 W) (electrodeless discharge lamp); hydride generation, cell temperature (900 °C), sample solution (1 mL min^{-1} flow rate), reducing agent (2% (w/v) $NaBH_4$ in 0.7% (w/v) NaOH, 1.9 mL min^{-1} flow rate), HCl solution (3 M; 1.9 mL min^{-1} flow rate), carrier gas (argon 45 mL min^{-1} flow rate).

Quantifications were made by the method of standard additions and peak area signal was measured. The quantities added were approximately equal to two and three times the AB content determined previously by comparison with the standard curve. The peak areas recorded were the average of at least two injections of each solution. To correct the data for reagent contamination, reagent blanks were taken through the procedure.

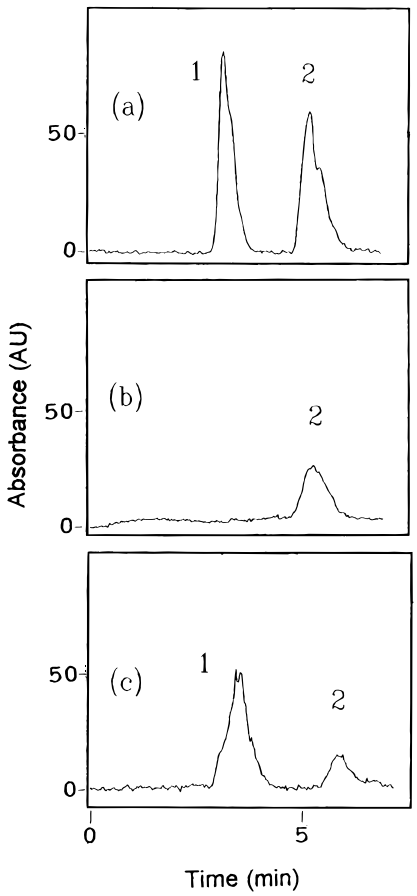

Figure 1. Chromatograms of a standard mixture of AB and DMA (5 ng of As each) obtained by (a) HPLC-MO-HG-AAS and (b) HPLC-HG-AAS. Chromatogram (c): cleaned-up extract of razor clam brine obtained by HPLC-MO-HG-AAS. Peak identification: 1, AB; 2, DMA.

RESULTS AND DISCUSSION

HG-AAS Determination of Total Arsenic in Brines. The brine was mineralized by dry ashing, and $Mg(NO_3)_2$ + MgO was added as an arsenic mineralization coadjuvant and volatilization inhibitor (Cervera et al., 1989). The technique provided a good recovery (96 ± 3%) for a brine from langostillo (a kind of lamellibranch, total As 1.1 μg g^{-1}, fresh mass), spiked with 1.8 μg g^{-1} of As(III), fresh mass.

Cleanup of Brines and HPLC-MO-HG-AAS Determination of AB. The quantitative recovery of 16.52 μg g^{-1} of AB as As (dm) obtained from a sample of DORM-1 after a Dowex cleanup procedure was reported in a previous study (Vélez et al., 1996a), which reveals the high interchange capacity of this cleanup procedure. In the present work, 0.80 μg g^{-1} of AB expressed as arsenic spiked in 2 g of mussel brine (total arsenic 0.77 μg g^{-1}) was quantitatively recovered after passing through the Dowex (recovery percentage 101 ± 1%).

The Dowex cleanup avoided the overlapping of As(III) and AB, tested for by us and frequently described in the literature (Le et al., 1994; López-Gonzálvez et al., 1993; Vélez et al., 1996a), and only AB and DMA were obtained by HPLC-MO-HG-AAS. Figure 1 shows a chromatogram of an aqueous standard mixture of AB and DMA obtained (a) with and (b) without microwave oxidation. Figure 1 c) shows a typical cleaned-up chromatogram (razor clam brine) obtained with microwave oxidation, where AB and DMA are very well separated.

Figure 2 shows chromatograms of (a) cleaned-up extracts of brines from different seafoods (shrimp, mussel, and crab) and the same cleaned-up brines spiked with (b) 2.5 ng and (c) 5 ng of AB as arsenic. The dilutions employed and the contents of AB as As (fresh mass) for each sample were as follows: shrimp (1 + 1), 0.05 μg g^{-1}; mussel (1 + 19), 0.70 μg g^{-1}; crab (1 + 99), 3.87 μg g^{-1}. As these data show, due to the sensitivity of the technique employed, a very wide range of concentrations can be quantified.

Table 2. Analytical Characteristics of the Method

	AB as As
detection limit[a] (ng g^{-1})	2.0 (fm)
precision (RSD %)[b]	
mussel	3% (0.70), n = 6
crab	2% (3.87), n = 3
snail	3% (0.72), n = 3
cockles	5% (0.11), n = 3
recovery[c] (%)	
shrimp	95 ± 3 (0.05, 0.06)
mussel	101 ± 1 (0.70, 0.80)
crab	102 ± 5 (3.87, 4.56)
mean	99 ± 4

[a] Nine reagent blanks were employed; 1 + 1 dilution; results expressed as As fresh mass (fm). [b] Relative standard deviation. Values in parentheses are the average in μg g^{-1} As (fm) for the samples analyzed. [c] Recovery percentages expressed as mean ± standard deviation from three independent analyses. Values in parentheses are the average AB concentration of the unspiked samples (first value) and the concentration of the AB added (second value) in μg g^{-1} As (fm).

Analytical Features of the Method. The analytical characteristics (Table 2), such as detection limit, precision, and recovery, were evaluated in samples of brines.

The detection limit, established as the AB concentration in the brine that provides an absorbance area reading statistically different from that of the blank, was calculated by dividing three times the standard deviation of the absorbance area readings, at the time of retention of AB, of nine reagent blanks by the slope of the standard additions curve and taking into account the sample mass and dilution employed. The dilutions used for the readings of the AB brine water extracts that provided good peak separation for AB ranged from 1 + 1 to 1 + 99, so that the detection limits for AB vary in accordance with the dilution employed (from 2.0 ng g^{-1} As (fm) to 100 ng g^{-1} As). The mean precision of the method, expressed as the relative standard deviation, evaluated by analyzing subsamples of brines from different seafood products, was 3%. The mean recovery percentage from three subsamples of brines from shrimp, mussel, and crab, evaluated by spiking with AB (0.06, 0.80 and 4.56 μg g^{-1} expressed as As, respectively), was 99 ± 4%.

AB Determination in Brines. Table 3 shows the relative percentage (by weight) of brine to muscle tissue for each type of sample, the values of total arsenic, AB as As, and percentages of total arsenic as AB in seafood muscle (data obtained in a previous work, Vélez et al., 1996a), in their brines, and in the canned product (muscle plus brine).

The relative percentage of brine to muscle tissue ranges from 27 to 108%, with a mean value of 67 ± 21%. This shows that, if the entire contents of the can are consumed, the intake of brine is approximately equal to the intake of the product, which explains why it is necessary, as stated earlier, to analyze the levels of arsenical species present in the brine.

In brines, the total arsenic ranges from 0.06 to 3.64 μg g^{-1} (fm). Brines from crab and langostillo gave the highest arsenic concentrations. For AB, the levels found

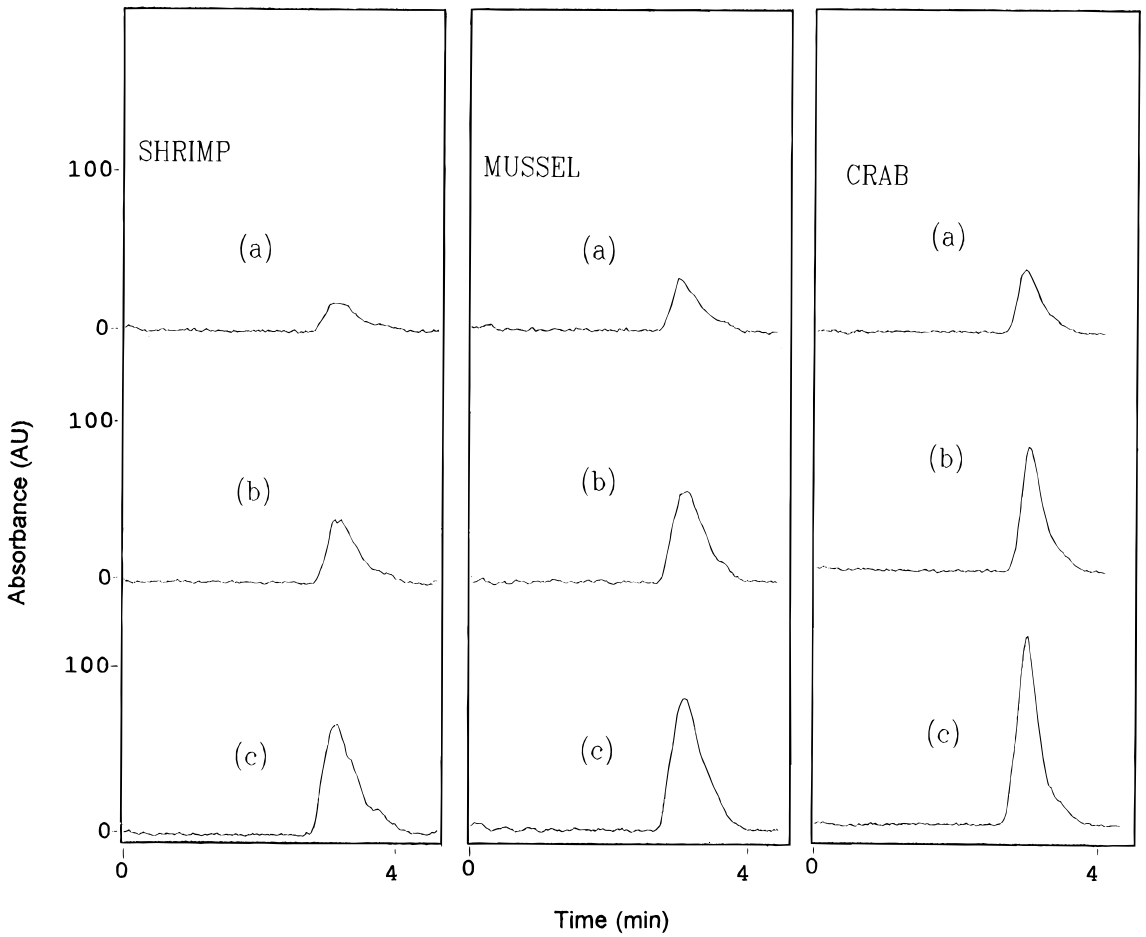

Figure 2. HPLC-MO-HG-AAS chromatograms of brines from shrimp, mussel and crab: (a) cleaned-up extract, (b) cleaned-up extract spiked with 2.5 ng of AB as arsenic, and (c) cleaned-up extract spiked with 5 ng of AB as arsenic. Dilution employed and contents of AB as As (fresh mass) of each sample: shrimp (1 + 1), 0.05 μg g^{-1}; mussel (1 + 19), 0.70 μg g^{-1}; crab (1 + 99), 3.87 μg g^{-1}.

Table 3. Relative Percentage of Brine to Muscle Tissue, Contents of Total Arsenic and Arsenobetaine as As, and Percentages of Total Arsenic as AB in Seafood, Their Brines and the Total Canned Product (muscle plus brine)[a]

		brine			muscle[b]			muscle plus Brine		
seafood product	%[c]	total As	AB	AB (%)	total As	AB	AB (%)	total As	AB	AB (%)
					Fish					
salmon (01)	27	0.08	0.06	75	0.23	0.14	61	0.31	0.20	65
tuna (02)	42	0.10	0.11	110	0.39	0.18	46	0.49	0.29	60
					Lamellibranchs					
razor clams (03)	77	0.27	0.13	48	1.55	0.13	8	1.82	0.26	15
razor clams (04)	77	0.13	0.09	69	0.62	0.07	11	0.75	0.16	21
cockles (05)	108	0.19	0.11	58	2.06	0.10	5	2.25	0.21	9
langostillo (06)	83	1.05	1.08	103	1.92	0.69	36	2.97	1.77	60
mussel (07)	62	0.77	0.70	91	2.65	0.48	18	3.42	1.18	35
clams (08)	71	0.87	0.62	71	2.06	0.44	21	2.93	1.06	36
					Gastropods					
snails (09)	73	0.68	0.72	106	1.27	0.62	49	1.95	1.34	69
					Crustaceans					
shrimp (10)	67	0.55	0.53	96	0.87	0.40	46	1.42	0.93	65
shrimp (11)	67	0.06	0.05	83	0.55	0.03	5	0.61	0.08	13
crab (12)	50	3.64	3.87	106	2.15	1.61	75	5.79	5.48	95

[a] Percentages based on weight and contents expressed in μg g^{-1} (fm). One sample analyzed in triplicate in each category. [b] Values for total arsenic and arsenobetaine in these seafood products were obtained in a previous work (Vélez et al., 1996a). [c] Relative percentage of brine to muscle tissue for each type of sample.

in brines ranged from 0.05 to 3.87 μg g^{-1} As (fm). In all cases, AB levels in the brines were above the detection limit for the method. The percentages of total arsenic represented by these contents are also shown in Table 3. The brines showed an AB percentage ranging from 48 to 110% of total arsenic. The migration of AB to the brine may be due to an osmosis mechanism which could cause the AB solubilized in the intercellular liquids inside the fish to migrate from the fish to the brine. This migration might be enhanced by the weakness of the electrostatic link interaction between AB and fish muscle tissue (Edmonds and Francesconi, 1987). The migration of AB to the brine may explain the finding reported by ourselves (Vélez et al., 1995) and the Ministry of Agriculture, Fisheries and Food in the United Kingdom (MAFF, 1982) that the arsenic content in canned products is lower than in similar unprocessed products.

Table 4. LD_{50} of Arsenic Compounds

arsenic compound	LD_{50} (g kg^{-1})
arsenic trioxide	0.0345
methylarsonic acid	1.8
dimethylarsinic acid	1.2
tetramethylarsonium	0.89
arsenocholine	6.5
trimethylarsine oxide	10.6
arsenobetaine	>10.0

By considering the AB levels and total arsenic contents in the brines we were able to obtain the arsenic levels that would correspond to water-soluble species other than AB. These levels reach a maximum of 0.25 μg g^{-1} As (fm) for a sample of clams. The species coeluted with AB, DMA (dimethylarsinic acid), was found in razor clam, mussel, and clam brines in maximum concentrations of 0.09 μg g^{-1} As (fm). This result falls within the DMA concentration range found by us in seafood in a previous study (Vélez et al., 1996b).

In muscle plus brine the total range found for As was 0.31 to 5.79 μg g^{-1} (fm). Crab and mussel had the highest values. For AB, the levels found ranged from 0.08 to 5.48 μg g^{-1} As (fm). The percentages of total arsenic represented by these contents ranged from 9 to 95%. The percentages of AB for the lamellibranch products group ranged from 9 to 60% and the arsenic content not contributed by AB varying between 1.20 and 2.04 μg g^{-1} (fm). These results are similar to those reported by us for percentages of total arsenic represented by AB in canned lamellibranchs in a previous study (Vélez et al., 1995). The relatively high quantities of arsenic not contributed by AB could belong to other species such as As(III), As(V), MMA (monomethylarsonic acid), DMA (dimethylarsinic acid), AC (arsenocholine), TMAO (trimethylarsine oxide), and TMA^+ (tetramethylarsonium ion) and also to arsenosugars. Shibata and Morita (1992) and Larsen (1995) stated that bivalves generally contain not only arsenobetaine but also arsenic-containing ribofuranosides as the most significant arsenic species.

Table 4 shows the LD_{50} values for the various species of arsenic, obtained by oral administration to mice (Shiomi, 1994). The values given suggest that an increase in the degree of methylation of the arsenic implies a reduction in toxicity, considering the trimethylated species as nontoxic. However, this tendency is not followed by the most highly methylated species of arsenic, TMA^+. Despite the relatively low toxicity of the brines analyzed in this study, in view of the predominant presence of AB in them, it would be desirable to quantify the arsenical species present in a larger number of samples and also to study the effect of storage on the migration of AB. Also, migrations of arsenic to other types of accompanying liquid such as oil or sauce should be studied. This work confirms the recomendations expressed by other authors concerning the special need for chemical studies of arsenic compounds in processed food (Shiomi, 1994).

CONCLUSIONS

The hyphenated technique used in this study confirmed that main migration of arsenic is in the form of AB from canned seafoods to brines. The results obtained indicate the need for further study of arsenical species in canned seafoods, to carry out preliminary research for subsequent regulation of the As content in these products. The HPLC-MO-HG-AAS method employed in this work, which is available in many control laboratories, permits sensitive, precise, and accurate determination of AB in brines and could make a substantial contribution to this objective.

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