Percentages of Total Arsenic Represented by Arsenobetaine Levels of Manufactured Seafood Products

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The aim of the present study is to establish whether arsenobetaine (AB) is the principal species in manufactured seafood products. Total As and AB were analyzed in a wide range of seafood products using hydride generation–atomic absorption spectrometry (HG–AAS) and liquid chromatography–inductively coupled argon plasma atomic emission spectrometric detection (HPLC–ICP-AES). Our findings shows that total As contents were in general of the order of those found by the U.K. Ministry of Agriculture, Fisheries and Food (MAFF). The range for AB is <0.1–5.4 $\mu g\ g^{-1}$ of fresh weight (expressed as As) or <0.2–12.8 $\mu g\ g^{-1}$ of fresh weight (expressed as AB). The mean values ± SD of percentages of total As represented by AB are 81 ± 21 in fresh seafood, 42 ± 22 in frozen seafood, and 28 ± 17 in preserved seafood. This confirms that there is a lower percentage of As as AB in frozen or preserved products than in fresh seafoods. Further research is to be carried out to confirm whether AB is degraded during manufacturing processes or leached out into liquor, oil, or sauce of canned seafoods.

Keywords: *Arsenobetaine levels; arsenic levels; high-performance liquid chromatography–inductively coupled argon plasma atomic emission spectrometric detection; hydride generation–atomic absorption spectrometry; manufactured seafood*

Marine fishery products may contain high levels of arsenic as muscle fish tissues are as a rule a site of rather high arsenic accumulation (Sekulic et al., 1993). This can adversely affect marketability of seafood products and requires the evaluation of their toxicity or harmlessness. Since arsenic can occur in the form of various chemical species that differ considerably in terms of the degree of toxicity they present, it is necessary not only to determine total As in seafood but also to quantify the various chemical species (Beauchemin et al., 1988).

Among the main species of arsenic, arsenobetaine (AB), which is the most frequently reported organoarsenical in marine animals, has been shown to be relatively nontoxic (FAO/WHO, 1989). Moreover, AB is reported as being the major form of arsenic in fish and crustaceans (IUPAC, 1992). This has led to the conviction that arsenicals in traditionally eaten marine-derived foodstuffs do not pose any hazard to human health. In this connection, in 1984 the U.S. Environmental Protection Agency established that dietary arsenic intake consisted of organoarsenic species that are not thought to have any toxic properties (Atallah and Kalman, 1991). The aim of the present study is to establish whether AB is the principal species in seafood products and also to evaluate the percentage of total As represented by AB in these products. For this purpose, total As and AB were analyzed in a wide range of popular seafood products using methods developed by us. The total As and AB values found when these methods were applied agree with total As levels in standard reference material from the National Institute of Standards and Technology, Washington, DC, 1566a oyster tissue (Ybáñez et al., 1992), and the AB values obtained by Larsen et al. (1993) and Branch et al. (1994) in the Canada National Research Council Reference Material, DORM-1 dogfish muscle (Ybáñez et al., 1995).

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The total As and the percentage of total As represented by AB cannot, alone, evaluate the toxicity or harmlessnes of seafood products but both can be indicators of their possible toxicity. In cases when the total As content is high and the percentage of total As represented by AB is low, it is clearly necessary to quantify the toxic species and especially As(III) and As(V).

MATERIALS AND METHODS

Instrumentation. A Perkin-Elmer Model 6000 ICP-AES spectrometer, equipped with an ICP source Plasmatherm (2.5 kW, 27.13 MHz) controlled by a Perkin-Elmer 7500 laboratory computer and with a Perkin-Elmer PR-210 printer (Perkin-Elmer Hispania, S.A., Madrid, Spain), was used as an HPLC detector. A Perkin-Elmer Model 5000 atomic absorption spectrometer equipped with an MHS-10 hydride generation attachment and an 056 stripchart recorder was used to determine total arsenic. We used a high-performance liquid chromatograph (Hewlett-Packard Model 1050), equipped with a Model HP 79852A quaternary pump, with on-line degassing via vacuum chamber and membranes semipermeable to gases and impermeable to liquids; a Model 79855A automatic injector; Hewlett-Packard personal computer data station, Vectra 486/33N Model 170 with 486 microprocessor rated at 33 MHz (Hewlett-Packard Española, S.A., Madrid, Spain); and a column (25.0 cm × 4.6 mm i.d.) packed with Supelcosil LC-SAX (5 μm) (Supelco, Rohm and Haas Co., Bellefonte, PA). A Heraeus Model 1100/3 muffle furnace (Heraeus, S.A., Madrid, Spain) fitted with a Jumo Model DPG-44/1 digital microprocessor (Heraeus) was used to dry ash the seafood products to determine total As content. An FTS lyophilizer system was equipped with a microprocessor controlling the lyophilization process. This microprocessor was connected to an Epson Equity I+ (Giralt, S.A., Madrid, Spain).

Other equipment used included a homogenizer, a Janke and Kunkel Model A10 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); a P-Selecta Vibromatic 340 mechanical arm shaker (Selecta, Barcelona, Spain); a Gallenkamp Model INR-200 orbital incubator mechanical shaker (Gallenkamp, Loughborough, England); and a Büchi Rotavapor rotary vacuum vaporizer (Afora, Valencia, Spain).

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Reagents. All chemicals including standards and solutions were of Pro Analyse quality or better. All glassware was treated with 10% (v/v) HNO_3 for 24 h and then rinsed three times with analytical reagent grade water (QRG water) before being used for the first time. Between uses glassware was placed in HNO_3 for 24 h.

Reagent grade water had a metered resistivity of 1.8 MΩ·mm (Milli-Q water system, Millipore). Argon: 99.995% (Sociedad Española de Oxígeno, Madrid, Spain). Potassium iodide: Panreac (Montplet and Esteban, S.A., Montcada i Reixac, Barcelona, Spain). A stock solution of As(III) was prepared by dissolving 1.320 g of Riedel de Haën arsenic trioxide (Riedel de Haën AG, 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. A stock solution of AB was prepared as an aqueous solution of 973 μg mL^{-1} of AB (Service Central d'Analyse du CNRS-SCA, Vernaisson, France). HCl (d = 1.19 g mL^{-1}), $Mg(NO_3)_2$ (98%), and MgO (90%): Panreac (Montplet and Esteban). HNO_3 (d = 1.38 g mL^{-1}) and $NaBH_4$: Probus (Probus, S.A., Badalona, Barcelona, Spain). Acetone, methanol: Scharlau HPLC grade (Scharlau, S.A., Barcelona, Spain). Ammonia solution 32% extra pure Merck (Igoda, Barcelona, Spain).

For the dry ashing of samples an ashing aid suspension was prepared by stirring 10 g of $Mg(NO_3)_2{\cdot}6H_2O$ and 1 g of MgO in 100 mL of water until homogeneous. Sodium tetrahydroborate(III) solution (3% w/v) was prepared in 100 mL (1% w/v) of NaOH solution and filtered through Whatman No. 42 paper. The potassium iodide (2% w/v) solution and the $NaBH_4$ solution were kept under refrigeration. "Econopack" columns, 30 mL volume (Durviz, Valencia, Spain), were packed with cation exchange resin Dowex 50W-X8, H+ form, 1 × 6 cm Bio-Rad (Bio-Rad, Barcelona, Spain). Biomedical Package statistical software was from Statistical Software Ltd. (Cork Technology Park, Cork, Ireland).

Commercial Samples. Various fresh, frozen, and canned seafood products were purchased at local retail outlets. For determination of total arsenic, 82 samples were analyzed. Table 1 shows the different seafood products, their presentation, and the number of samples. For the determination of AB, 32 samples were analyzed. Descriptions are given in Table 2.

Sample Preparation. The fresh samples were prepared as they are normally eaten. Head, viscera, and tail were removed (also the skin was removed from the sole). The frozen seafood products were lyophilized. The brine or sauce in the canned seafood products was removed by the method for determining the drained weight of canned foods. The total contents of each package were emptied onto a sieve with a 5 mm stainless steel mesh made of 1 mm gauge wire, the sieve being tilted slightly to facilitate drainage. To ensure total drainage, the seafood products were allowed to drain for 5 min at room temperature. Afterwards, the samples manufactured in oil sauce were pressed between two sheets of filter paper, as the oily residue can produce lyophilized samples that tend to cake. The clean samples of fresh, frozen, and drained products were cut into pieces and frozen at −20 °C and then freeze-dried for 20 h at a chamber pressure of 0.225 Torr. Sublimation heat was supplied by conduction from heating plates at 20 °C. The lyophilized samples were crushed and homogenized to a fine powder in a water-refrigerated mill. The resulting powder was stored in previously decontaminated twist-off flasks and kept in the freezer until the analysis.

Determination of Total As. The seafood products were dry ashed by applying the methodology developed previously, and the total As was determined by HG−AAS (Ybáñez et al., 1992). The recommended procedure is as follows: Take 0.250 g (dry weight) of seafood, add 5 mL of 50% (v/v) HNO_3 and 1 mL of ashing aid suspension containing 20% (w/v) $Mg(NO_3)_2$ and 2% (w/v) MgO, and mix well. After evaporation in a sand bath until totally dry, the dry residue undergoes a first careful ashing process in a muffle furnace: 150 °C for 1 h; 200 °C for 30 min; 250 °C for 1 h; 300 °C for 3 h; 350 °C for 30 min; and 450 °C for 12−14 h. Generally it is necessary to wet the ash with 50% (v/v) HNO_3, evaporate in a sand bath, and perform a second shorter ashing process (150 °C for 1 h; 300 °C for 30 min; and 450 °C for 12−14 h)—once or twice—until the ash is completely white. The ash is dissolved with 10 mL of 50% (v/v) HCl, washed with water, and filtered through Whatman No. 1 filter paper into a 50 mL standard flask. The solution is stored in a polyethylene bottle. Triplicate blanks are prepared by taking 5 mL of ashing aid solution and HNO_3 through the digestion procedure. Analysis of the mineralized samples by HG−AAS was performed by pipetting samples of the mineralized solution (containing <60 ng of arsenic) in triplicate into hydride reaction flasks, adding 1 mL of 2% (w/v) KI solution, and allowing to stand for 5 min. The analytical features of the method are as follows: detection limit, 0.017 μg g^{-1} fresh mass (fm); relative standard deviation (n = 8), 3%; percentage recovery, 102 ± 3. The analysis of standard reference material from the National Institute of Standards and Technology, Washington, DC, 1566a oyster tissue, provided an As value of 13.7 ± 1.2 μg g^{-1} (dm), agreeing with the certified value of 14.0 ± 1.2 μg g^{-1}.

Table 1. Arsenic in Seafood (Fresh Weight)

seafood product	no. of samples	range (μg/g)	mean (μg/g)
fish			
anchovy (fresh)	2	3.9−4.5	4.5
(in vinegar)	5	1.0−4.5	2.6
(smoked)	1		2.5
cod (frozen)	1		<0.1
(salted)	3	2.4−4.0	3.0
cod eggs (canned)	1		0.4
cod liver (smoked)	1		1.5
hake (frozen)	3	0.7−1.3	1.1
lompo eggs (canned)	1		0.4
plaice (frozen)	1		6.5
salmon (canned)	1		0.7
(frozen)	1		0.5
(smoked)	1		1.5
sardine (canned)	3	1.4−2.1	1.8
(fresh)	2	4.0−4.0	4.0
sole (fresh)	1		4.0
(frozen)	3	4.1−9.0	6.0
tuna (canned)	3	0.9−1.3	1.1
lamellibranchs			
clams (canned)	1		2.2
(fresh)	5	1.8−1.9	1.8
cockles (canned)	3	1.5−2.8	2.0
(fresh)	3	2.7−4.6	3.6
mussels (canned)	11	2.1−3.3	2.5
(fresh)	4	1.5−2.1	1.8
cephalopods			
octopus (canned)	3	2.3−16.5	7.2
(frozen)	1		1.4
squid (canned)	4	0.3−1.4	0.9
(fresh)	4	0.1−4.7	1.8
(frozen)	1		0.3
gastropods			
whelks	1		12.4
crustaceans			
shrimps (canned)	2	0.4−1.5	1.0
(frozen)	2	0.4−1.0	0.7
crabs (frozen)	1		0.3
(canned)	1		1.0
echinoderms			
sea urchin eggs (canned)	1		7.1

Extraction, Cleanup, and HPLC−ICP-AES Determination of AB. Prior to the extraction, the lyophilized samples were defatted with acetone. Lyophilized and defatted samples (2.000 ± 0.001 g) were agitated mechanically with 40 mL of methanol. After extraction, the mixture was centrifuged and the supernatant liquid separated by aspiration. A second extraction with 40 mL of methanol was performed on the residue. The mixture was centrifuged and the floating liquid separated by aspiration with a syringe. The two methanol extracts were combined.

Before injection into the chromatographic system, the methanol extract of the lyophilized and defatted samples was evaporated (T^a < 40 °C) until dry, redissolved in 25 mL of 0.1 N HCl, and adjusted to a pH less than 2 (1.8 ± 0.2) with 4M

Table 2. Seafood Products Analyzed for Arsenobetaine

seafood product	sample	description (source)
fish		
anchovy (fresh)	01	complete fish (Valencia, Spain)
(fresh)	02	complete fish (Valencia, Spain)
(in vinegar)	03	anchovies, vinegar, and salt packed in plastic, 200 g drained weight (Madrid, Spain)
(in vinegar)	04	anchovies, vinegar, water, and salt canned, 135 g drained weight (Valencia, Spain)
cod (salted)	05	boneless salted fillets packed in a plastic wrapping, 400 g net weight (Soria, Spain)
(salted)	06	boneless salted fillets packed in a plastic wrapping, 400 g net weight (Valencia, Spain)
hake (frozen)	07	hake middles packed in plastic, 350 g net weight (Valencia, Spain)
plaice (frozen)	08	boneless fillets packed in plastic and cardboard pack, 300 g net weight (Pontevedra, Spain)
sardine (fresh)	09	complete fish (Valencia, Spain)
(fresh)	10	complete fish (Valencia, Spain)
(canned)	11	sardines, olive oil, and salt canned, 88 g drained weight (Vigo, Spain)
(canned)	12	sardines, vegetable oil, and salt canned, 80 g drained weight (Canarias, Spain)
sole (fresh)	13	complete fish (Holland)
(frozen)	14	boneless fillets in cardboard pack, 400 g net weight (Denmark)
(frozen)	15	boneless fillets in cardboard pack, 400 g net weight (Denmark)
tuna (canned)	16	white tuna, olive oil, and salt canned, 82 g drained weight (Vigo, Spain)
(canned)	17	white tuna, vegetable oil, and salt canned, 65 g drained weight (Pontevedra, Spain)
lamellibranchs		
cockles (fresh)	18	complete mollusk (Valencia, Spain)
(fresh)	19	complete mollusk (Valencia, Spain)
(fresh)	20	complete mollusk (Valencia, Spain)
(canned)	21	cockles, water, and salt canned, 65 g drained weight (Pontevedra, Spain)
(canned)	22	cockles, water, and salt canned, 135 g drained weight (Holland)
cephalopods		
octopus (canned)	23	octopus, olive oil, and salt canned, 72 g drained weight (Vigo, Spain)
(canned)	24	octopus, vegetable oil, and salt canned, 60 g drained weight (Pontevedra, Spain)
squid (fresh)	25	peeled squids, without ink, eyes, tentacles, and plume (Valencia, Spain)
(fresh)	26	peeled squids, without ink, eyes, tentacles, and plume (Valencia, Spain)
(canned)	27	pieces of squids, ink, vegetable oil, tomato, onion, spices, and salt canned, 64 g drained weight
(canned)	28	whole squids, olive oil, and salt canned, 135 g drained weight (Vigo, Spain)
small squid (fresh)	29	complete mollusk (Valencia, Spain)
(fresh)	30	complete mollusk (Valencia, Spain)
(canned)	31	squids, olive oil, and salt canned, 120 g drained weight (Vigo, Spain)
(canned)	32	squids, vegetable oil, water, and salt canned, 120 g drained weight (Thailand)
crustaceans		
shrimps (frozen)	33	peeled shrimp with antioxidant (E-223) packed in plastic, 200 g net weight (United Kingdom)
(frozen)	34	peeled shrimp packed in plastic, 200 g net weight (Pontevedra, Spain)

HCl. The acidified solution was cleaned by being passed through a strong cation exchanger of Dowex 50W-X8. The AB was eluted with 50–75 mL of 4 M ammonia. The ammonia was evaporated to dryness (T^a < 40 °C), and the residue was redissolved in 1 mL of QRG water. AB was determined in the water extract by using an HPLC system interfaced via Teflon tubing to the nebulizer of the ICP-AES spectrometer. The HPLC and ICP-AES conditions have been described previously (Ybáñez et al., 1995). The HPLC separation was performed isocratically with 5 mM phosphate buffer at pH 3.75. The mobile flow rate was 1 mL/min. The arsenic emission wavelength selected for determination by ICP-AES was 193.696. Arsenic was monitored under the following conditions: viewing height, 10 mm; outer Ar flow, 17 L/min; inner Ar pressure, 12 psi; inner Ar flow, 0.5 L/min; intermediate Ar flow, 0.4 L/min; radio frequency power, 1.1 kW; sample solution flow, 1.0 mL/min. All chromatograms were recorded using a stripchart recorder (Perkin-Elmer Model 056), and the peak height signal was measured. Determinations were made by the method of standard additions. The quantities added were approximately 2 and 3 times the AB content determined previously by comparison with the standard curve. The analytical features of the method are as follows: detection limit, 0.51 $\mu g\ g^{-1}$ of As dry mass (dm) or 0.13 $\mu g\ g^{-1}$ of As fresh mass (fm); relative standard deviation ($n = 6$), 6% and 2% for the Canada National Research Council Reference Material, DORM-1 (dogfish muscle) ($n = 3$). The percentage recovery for AB is 86 ± 6. The analysis of DORM-1 provided an AB value of 16.5 ± 0.9 μg of As/g (dm), agreeing with results obtained by other authors using HPLC–ICP-mass spectrometry (HPLC–ICP-MS).

RESULTS AND DISCUSSION

Determination of Total Arsenic in Seafood Products. The moisture content of the seafood products is shown in Table 3. The mean moisture contents varied from 62 to 84%. The canned products generally had less moisture than the fresh and frozen products. The arsenic contents found for the various kinds of seafood are shown in Figure 1. The total range found was <0.1–16.5 $\mu g\ g^{-1}$ of fresh weight. The ranges found for the fish are approximately the same as those obtained by the U.K. Ministry of Agriculture, Fisheries and Food (MAFF, 1982), except that they found a higher level (34 $\mu g\ g^{-1}$ of fresh weight) for plaice. The ranges of values we found for lamellibranchs and cephalopods closely overlap the MAFF values for these groups. The ranges of values we found for crustaceans are much lower than the values found by the MAFF.

Table 3. Moisture Content of the Different Classes of Seafood Product

seafood product	no. of samples	range (%)	medium (%)
fish (canned)	6	57–73	62
(fresh)	5	74–79	76
(frozen)	4	77–82	79
lamellibranchs (canned)	5	60–76	70
(fresh)	3	78–80	79
cephalopods (canned)	6	58–70	67
(fresh)	4	80–86	83
crustaceans (frozen)	2	83–85	84

Table 1 shows the individual values obtained for each of the seafoods analyzed. Samples of octopus, whelks, and sea urchin eggs gave the highest values for the total selection. These values—expressed in micrograms per gram of fresh weight—are approximately the same as those found in the literature for these seafoods. Octopus: found, 16.5; reported, 15 (MAFF, 1982). Whelk: found, 12.4; reported, 16 (MAFF, 1982). The next

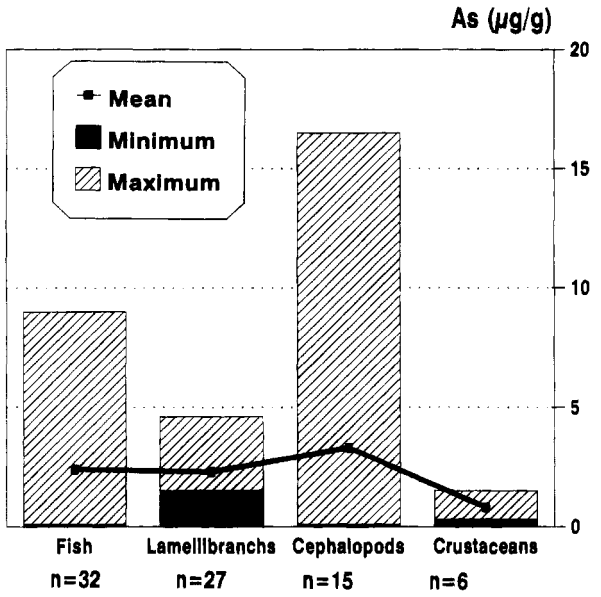

Figure 1. Arsenic levels in different classes of fresh and processed seafoods.

highest values occur among the fish. The highest values, expressed in microgram per gram of fresh weight, were for sole (9.0) and plaice (6.5). High values for these flatfish have been also reported previously [sole, 4.0; plaice, 34.0 (MAFF, 1982)]. Similarly, Branch et al. (1994) found concentrations in sole of 41.6 and 34.5 $\mu g\ g^{-1}$ of fresh weight and in plaice of 37.3 and 47.6 $\mu g\ g^{-1}$ of fresh weight. They explained the high arsenic content in this kind of fish as being due to the fact that these fish are carnivorous bottom feeders, existing on diets of mollusks and bivalves which filter feed on the sea bed. In the fish group, fresh anchovy and anchovy in vinegar, fresh sardine, and salted cod also had a fairly high content (4.5, 4.5, 4.0, and 4.0 $\mu g\ g^{-1}$ of fresh weight, respectively). The content for cod is close to that reported by the MAFF (4.8 $\mu g\ g^{-1}$ of fresh weight).

With the exception of fresh cockles, the lamellibranchs had arsenic contents of around 2 $\mu g\ g^{-1}$ fresh weight. The values found for clams, cockles, and mussels were of the order of those found by the MAFF.

Crustaceans generally have the lowest levels of arsenic, and in this class the lowest value was for the frozen prawns. The shrimps had a lower mean content (0.7 $\mu g\ g^{-1}$ of fresh weight) than that reported by the MAFF (1982) for cooked shrimps (2.2 $\mu g\ g^{-1}$ of fresh weight). The content for crabs (1.0 $\mu g\ g^{-1}$ of fresh weight) was in the range reported by the MAFF for these seafoods.

As is well-known, the wide variability of As contents for a given species due to intrinsic factors, such as age, sex, and metabolic state, and external factors, such as place of origin, time of year, and water salinity and temperature, makes it difficult to observe differences for a limited number of analyses. However, from the results shown in Table 1 it can be concluded that in the case of sardines, cockles, and squid, the arsenic content of the canned product was half that of the fresh product, which agrees with the conclusions of the MAFF (1982). The MAFF appreciates that the difference is unlikely to be caused by processing but is due to the fact that generally fish that are canned are often different in species and size from unprocessed fish. Nevertheless, there exists the possibility of As leaching out into liquor/oil/sauce of cans.

Table 4. Arsenic Contents in Seafood Products in Terms of Presentation (Fresh Weight)

seafood product	no. of samples	range (μg/g)	mean ± SD (μg/g)
fresh			
fish	5	3.9–4.5	4.1 ± 0.3
lamellibranchs	12	1.5–4.6	2.3 ± 0.9
cephalopods	4	0.1–4.7	1.8 ● 2.1
total	21	0.1–4.7	**2.6 ± 1.4**
frozen			
fish	9	<0.1–9.0	3.2 ± 3.2
cephalopods	2	0.3–1.4	0.9 ± 0.8
crustaceans	3	0.3–1.0	0.6 ± 0.4
total	14	<0.1–9.0	**2.3 ± 2.8**
preserved			
fish	18	0.4–3.9	1.6 ± 0.8
lamellibranchs	15	1.6–3.3	2.4 ± 0.5
cephalopods	9	0.3–16.5	2.9 ± 5.1
crustaceans	3	0.4–1.5	1.0 ± 0.6
gastropods	1		12.4
echinoderms	1		7.1
total	47	0.3–16.5	**2.4 ± 2.9**
total	82	<0.1–16.5	**2.4 ± 2.5**

Table 4 shows arsenic levels for the various seafood products, fresh, frozen, or preserved in vinegar, salted or canned, depending on the particular manufacturing process. As can be readily seen, there are no significant differences in the confidence ranges for the mean values of total As in the various seafood products, fresh, frozen, and preserved using various manufacturing processes. The mean values found for all of the products overlap [mean ± SD (μg/g): fresh, 2.6 ± 1.4; frozen, 2.3 ± 2.8; preserved 2.4 ± 2.9], showing the difficulty of trying to evaluate the effect of the manufacturing process on arsenic contents on the basis of an analysis of the final product. In principle, therefore, it can be concluded that industrial processing of seafood products is not a determining factor for the total As these products contribute to the diet. Nevertheless, this statement should be confirmed by wider studies with statistically significant values.

Determination of Arsenobetaine in Seafood Products. The levels of AB and the percentages of As as AB expressed in fresh weight for all of the samples of seafood analyzed are given in Table 5. Table 6 shows AB contents (expressed as percent of As in fresh weight) in terms of class of fish and manufacturing process. The total range for AB is between <0.1 and 5.4 $\mu g\ g^{-1}$ of fresh weight (Table 5). The percentages of total As represented by these contents vary from 50 to 119% for fresh seafood and in the case of manufactured products from <18 to 82% for frozen seafood and from <5 to 71% for preserved seafood (Table 6).

Table 7 shows the percentages of As represented by AB as reported in the literature for the same types of seafood as those we analyzed. All of the data reported by Cardinaals et al. (1985), Lawrence et al. (1986), Larsen et al. (1993), and Branch et al. (1994) are for fresh seafoods with the exception of one sample of canned tuna, and the percentages range between 56 and 115%. The percentages we found for fresh seafoods, with the exception of the lamellibranchs we analyzed, overlap the values reported in the literature and are in line with the general consensus that the dominant arsenic compound in fish muscle is AB (Leah et al.,

Table 5. Arsenic and Arsenobetaine in Seafood (Fresh Weight)

seafood product	sample	As total (μg/g)	AB as As (μg/g)	AB/As (%)
fish				
anchovy (fresh)	01	3.9	3.7	94
(fresh)	02	4.5	3.6	81
(in vinegar)	03	2.1	0.3	14
(in vinegar)	04	1.5	<0.1	<9
cod (salted)	05	2.4	1.0	43
(salted)	6	3.9		
hake (frozen)	07	1.3	0.4	31
plaice (frozen)	08	6.5	5.4	82
sardine (fresh)	09	4.0	3.1	77
(fresh)	10	4.0	3.7	93
(canned)	11	2.1	0.5	23
(canned)	12	1.2	0.5	41
sole (fresh)	13	3.9		
(frozen)	14	9.0	4.2	47
(frozen)	15	4.1	1.4	35
tuna (canned)	16	0.9	<0.2	<24
(canned)	17	1.2	0.3	21
lamellibranchs				
cockles (fresh)	18	2.7	1.7	66
(fresh)	19	4.6	2.5	53
(fresh)	20	3.5	1.7	50
(canned)	21	2.8	0.8	28
(canned)	22	1.6	0.6	38
cephalopods				
octopus (canned)	23	2.3	1.6	71
(canned)	24	2.9	1.2	42
squid (fresh)	25	0.3	0.3	119
(fresh)	26	0.1	<0.2	
(canned)	27	0.8	<0.2	<21
(canned)	28	1.3	<0.2	<16
small squid (fresh)	29	2.0	1.6	82
(fresh)	30	4.7	4.4	94
(canned)	31	0.8	<0.2	<19
(canned)	32	2.9	<0.1	<5
crustaceans				
shrimps (frozen)	33	0.4	<0.07	<18
(frozen)	34	1.0	0.4	37

Table 6. Contents of AB in Seafood Products in Terms of Class of Fish and Manufacturing Process (Expressed as Percent of As in Fresh Weight)

seafood product	no. of samples	range (%)	mean ± SD (%)
fresh			
fish	4	81–94	86 ± 9
lamellibranchs	3	50–66	56 ± 9
cephalopods	4	82–119	98 ± 19
total	11	50–119	**81 ± 21**
frozen			
fish	4	31–82	49 ± 23
crustaceans	2	<18–37	28 ± 13
total	6	<18–82	**42 ± 22**
preserved			
fish	7	<9–43	25 ± 13
lamellibranchs	2	28–38	33 ± 7
cephalopods	6	<5–71	29 ± 24
total	15	<5–71	**28 ± 17**
total	32	<5–119	**48 ± 30**

1992) and that the fraction of the total arsenic that is present as AB in seafood is high, 80% or more (Larsen et al., 1993). As for the percentages of As as AB obtained for fresh lamellibranchs (50–66%), in the literature there is a precedent for even lower values as reported by Larsen et al. (1993) for shellfish reference samples (NIES mussel and NIST SRM 1566a). This author states that the arsenic present as AB in these samples accounts for only 19 and 28% of the total arsenic.

Table 7. Percentages of As Represented by AB Reported in the Literature

seafood	(%) AB/As	reference
fish		
cod	>67	Cardinals et al. (1985)
	80–84	Lawrence et al. (1986)
	96–115	Branch et al. (1994)
hake	78	Lawrence et al. (1986)
	60–65	Branch et al. (1994)
plaice	>67	Cardinals et al. (1985)
	74	Lawrence et al. (1986)
	94.5	Larsen et al. (1993)
	56–96	Branch et al. (1994)
sole	>68–>74	Cardinals et al. (1985)
	80–88	Lawrence et al. (1986)
	69–84	Branch et al. (1994)
tuna	72	Larsen et al. (1993)
crustaceans		
shrimp	>79	Cardinals et al. (1985)
	8–76	Lawrence et al. (1986)
	73.5–109	Larsen et al. (1993)

The percentages for preserved products, shown in Table 5, are very low in some cases, such as anchovy in vinegar, which has an AB percentage of less than 9%, and small squid in vegetable oil, with a percentage of less than 5%, and are without precedent in the literature. Also, the confidence ranges for the means of the percentages of As as AB in fresh and preserved seafood do not overlap, and the percentages for fresh and frozen seafood overlap only slightly (Table 6). This confirms that in general there is a lower percentage of As as AB in frozen or preserved products than in fresh seafoods. This all goes to show that in many manufactured products of marine origin AB may not be the dominant arsenic species, going against the predictions based on fresh seafood products.

Recently, Hanaoka et al. (1991) showed the conversion of AB to less methylated arsenic compounds by microorganisms associated with marine mollusks. Edmonds and Francesconi (1988) also considered the possible decomposition of AB in frozen fish, although evidence for the decomposition of AB in frozen fish is not available. Further research is to be carried out to confirm whether AB is degraded during seafood manufacturing processes. Otherwise, the possibility of AB leaching out into liquor, oil, or sauce of canned seafoods must be investigated.

The Food and Agriculture Organization/World Health Organization (FAO/WHO, 1989) established a provisional tolerable weekly intake (PTWI) of arsenic (15 μg of inorganic arsenic/kg of body weight) which, translated to a daily basis, is 2.1 μg/kg. In the case of the seafoods we analyzed, assuming in a worst case scenario that the arsenic not found as AB is inorganic As (Table 5), this intake would be reached, for a person weighing 70 kg, by the following weights: frozen sole, 31 g; canned small squid, 53 g; anchovy in vinegar, 82 g; canned sardine, 92 g; and salted cod, 105 g. These quantities can easily be reached in a normal diet. Consequently, in the case of manufactured seafoods which present high contents, in accordance with the recommendations of the IUPAC (1992) and for reasons of toxicological reassurance, the concentrations of AB should be determined. In cases when the percentage of As expressed as AB is very low, the possible toxicity of the product should be evaluated by analyzing the other arsenic species. Following the recommendations of the above-mentioned organization,

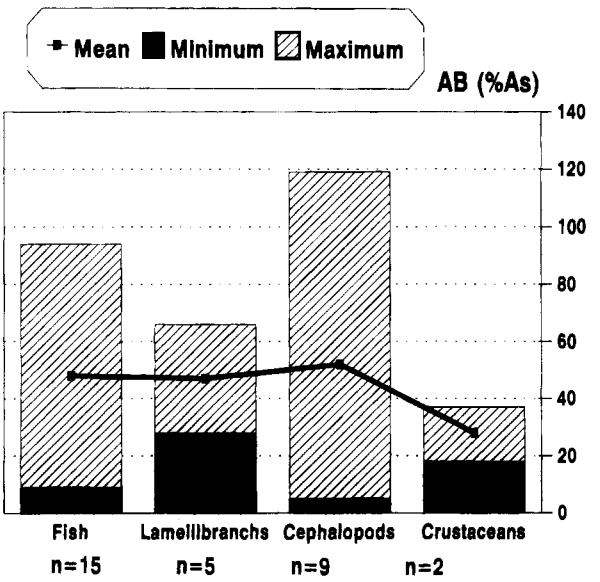

Figure 2. Percentage of total As represented by AB in different classes of fresh and processed seafoods.

this should be done by using methods that can be routinely applied and which have been validated, as was the case for our analysis of AB.

Further, the ranges for the percentages of As represented by AB for the various classes of fresh and manufactured fish overlap in all cases, as can be seen in Figure 2. The values range from <9 to 94% for fish, from 28 to 66% for lamellibranchs, from <5 to 119% for cephalopods, and from <18 to 37% for crustaceans. Consequently, it seems unlikely that the class of fish could affect the percentage of As represented by AB in the various manufactured products. In the case of processed crustaceans, the tendency we found for AB not to be the dominant arsenic species (percent total As as AB, 18–37) must be confirmed with a larger number of samples. This tendency is in line with that described by other authors for processed prawns, for which no AB at all was found (Lawrence et al., 1986).

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