Arsenic in Cooked Seafood Products: Study on the Effect of Cooking on Total and Inorganic Arsenic Contents

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Total and inorganic arsenic contents were analyzed in cooked seafood products consumed in Spain during the period July 1997−June 1998: hake, meagrim, small hake, anchovy, Atlantic horse mackerel, sardine, bivalves, crustaceans, squid, and salted cod. Various cooking treatments were used (grilling, roasting, baking, stewing, boiling, steaming, and microwaving). The results obtained were compared statistically with those found previously in the same products raw, and they showed that after cooking there was a significant increase in the concentration of total arsenic for salted cod and bivalves, and in the concentration of inorganic arsenic for bivalves and squid. The mean content of inorganic arsenic was significantly higher in bivalves than in any other type of seafood. For the Spanish population, the mean intake of total arsenic estimated on the basis of the results obtained in this study is 245 μg/day. The intake of inorganic arsenic (2.3 μg/day) represents 1.7% of the World Health Organization provisional tolerable weekly intake (PTWI), leaving an ample safety margin for this population, which has a very high consumption of seafood.

Keywords: *Arsenic; inorganic arsenic; seafood; cooking; hydride generation atomic absorption spectrometry*

INTRODUCTION

Because of the ubiquity of arsenic (As) in the environment it is habitually present in air, soil, and water, and human consumption of food and water results in chronic exposure to low levels of arsenic (*1*). Total diet studies carried out in various countries have revealed the variability of arsenic intake, which ranges between 20 μg day^{-1} for the inhabitants of the Czech Republic (*2*) and 345 μg day^{-1} for the population of Japan (*3*). In all cases, the greatest contribution to the intake of arsenic is from seafood products, which is why it is considered that in areas not exposed to natural or man-made pollution the total quantity of arsenic ingested by humans depends on the seafood consumed. However, the bioavailability and toxicity of arsenic depends largely on the chemical form in which it is found. As(III) and As(V), which together constitute inorganic arsenic, are the most toxic species. The toxicity of the organoarsenical species is lower, and arsenobetaine (AB), a trimethylated species, is recognized to be the least toxic (*4*).

In the majority of arsenic dietary intake studies (*5*−*7*), total arsenic is estimated instead of inorganic arsenic. Therefore, the results cannot be evaluated in relation to the toxicological reference value, which exists only for inorganic arsenic (provisional tolerable weekly intake (PTWI) of 15 μg of inorganic arsenic/kg of body weight/week, *8*). Also, specific studies have been conducted on levels of inorganic arsenic in seafood products (*9*−*11*), but with the analyses being performed on the raw product. This method does not provide a good estimate of the real intake, since most of these foods are cooked before consumption, and the results would not reflect the changes in the concentration of the various arsenic species that could take place during the cooking treatment. In this connection, there are several studies describing the changes that take place in the concentrations of various metals in foods during cooking processes (*12*, *13*). Jorhem et al. (*14*) report the cooking-induced changes in the Cd, Ni, Co, Pb, Cu, and Mn present in crayfish, with the behavior varying according to the metal and the organ considered. Atta et al. (*15*) describe a decrease in the concentrations of Cd, Cu, Pb, and Zn in fish (*Tilapia nilotica*) after steaming or baking. However, the state of our knowledge about arsenic is very different from what is known about other elements, with very few studies having been performed in seafood on changes in the levels of total arsenic as a result of cooking (*6*, *13*, *14*). In studies carried out on aqueous standards subjected to a temperature of 160 °C for periods of 30 min and 24 h, Van Elteren and Šlejkovec (*16*) observed the transformations of arsenobetaine (AB) into trimethylarsine oxide (TMAO) and tetramethylarsonium ion (TMA^+), dimethylarsinic acid (DMA) into monomethylarsonic acid (MMA), and MMA into As(III) and As(V). Studies carried out in our laboratory (*17*), using a wider range of temperatures (85−190 °C) and various times (15−44 min), revealed the transformation of AB standards into TMAO at temperatures of 150 °C or above, and transformation of AB into TMA^+ at temperatures of 160 °C or above.

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In real samples, Devesa et al. (*18*) demonstrated the transformation of AB into TMA^+ in cooked seafood products. However, no studies have been made on the effect of cooking on levels of inorganic arsenic in real samples.

In the present work, the concentrations of total and inorganic arsenic were determined in individual cooked seafood samples acquired in connection with the Total Diet Study carried out by the Basque Government's Health Department as part of the Food Chemical Safety Surveillance Program (*19*). To evaluate the possible effect of cooking on the contents of total and inorganic arsenic, the concentrations obtained in the cooked products were compared with the concentrations found in the same products in the raw state (*11*). From the data for the concentrations of total and inorganic arsenic in the various cooked seafoods and data concerning the consumption of these foods in Spain, it is possible to make a good estimate of their intake by the Spanish population.

MATERIALS AND METHODS

Instrumentation. Determination of total and inorganic arsenic was performed with a 3300 atomic absorption spectrometer (AAS) (Perkin-Elmer, PE, Madrid, Spain) with hydride generated by a flow injection system (PE FIAS-400) with an autosampler (PE AS-90). For MMA determination, a Hewlett-Packard model 1050 high performance liquid chromatograph (HPLC) (Hewlett-Packard, Barcelona, Spain) was employed, connected to a Perkin-Elmer model 5000 AAS equipped with a PE FIAS-400 to provide hydride generation in continuous flow mode. The chromatograph column employed was a Hamilton PRP-X100 (anionic exchange column, 10-μm polymer base, 250 mm $\times$ 4.1 mm, Teknokroma, Barcelona, Spain).

A lyophilizer equipped with a microprocessor controlling the lyophilization process was employed (FTS Systems, Stone Ridge, NY). Other equipment used included a PL 5125 sand bath (Raypa, Scharlau, S. L., Spain), a K 1253 muffle furnace equipped with a Eurotherm Controls 902 control program (Heraeus S. A., Madrid, Spain), a KS 125 Basic mechanical shaker (IKA Labortechnik, Merck Farma y Química, S. A., Barcelona, Spain), and an Eppendorf 5810 centrifuge (Merck).

Reagents. Deionized water (18 MΩ cm) was used for the preparation of reagents and standards. All chemicals, including standards and solutions, were of at least *pro analysi* quality or better.

A standard solution of As(V) (1000 mg L^{-1}) was used (Merck). Calibration standard solutions of As(III) were prepared from a reduced standard solution of As(V). The standard solution of MMA (1000 mg L^{-1}) was prepared by dissolving in water appropriate amounts of $CH_3AsO(ONa)_2$ $6H_2O$ (Carlo Erba, Italy). An aqueous solution of 5% m/v KI + 5% m/v ascorbic acid was used to reduce the standard of As(V) to As(III) and to prereduce the sample solutions before total and inorganic arsenic quantification. All glassware was treated with 10% v/v HNO_3 for 24 h, and then rinsed three times with deionized water before use.

Sample Collection and Preparation. In total, 123 samples of seafood products (classified into 10 different types of seafood) were analyzed. Eight types consisted of a single seafood product: meagrim, hake, small hake, anchovy, Atlantic horse mackerel, sardine, salted cod, and squid. The remaining two types included various similar types of seafood products whose individual contribution to the total diet is low: bivalves (clam and mussel) and crustaceans (scampi, shrimp, and prawn). Meagrim, hake, and small hake are white fish, with a fat content <1% and demersal and/or benthic habits. Anchovy, Atlantic horse mackerel, and sardine are blue fish, pelagic, with a fat content >1%. Bivalves and squid (both mollusks) and crustaceans are shellfish, and salted cod is a preserved fish.

The edible portions of seafood samples were rinsed with distilled water and dried. Each type of seafood was minced and blended to give a homogeneous sample, using an ordinary domestic mixer. Subsequently, the individual samples were frozen at −20 °C and afterward they were freeze-dried. The lyophilized samples were crushed and homogenized to a fine powder in a domestic mill. The resulting powder was stored in previously decontaminated twist-off flasks and kept at 4 °C until analysis.

The samples were purchased from retail outlets throughout the Basque Country and collected at regular intervals through the year in different locations. Each sample was prepared in such a way that inedible portions were not included. The head, tail, and digestive tract were removed from the fish samples. The shell and exoskeleton were removed from the bivalves and crustaceans, respectively. The squid were cleaned and thoroughly rinsed in deionized water. After removal of nonedible portions, part of the sample was assigned to be analyzed raw and the rest was cooked. Subsequently, the individual subsamples were homogenized, frozen, and lyophilized. Total and inorganic arsenic were determined in raw and cooked subsamples. The concentrations in the raw products were reported in a previous study (*11*). The cooking processes most commonly used by consumers for cooking each kind of seafood were employed (grilling, roasting, baking, stewing, boiling, steaming, or microwaving). Consequently, the 10 different types of seafood were not all submitted to the same cooking treatments. Each sample was cooked using only one particular treatment selected at random among those used for that particular fish item. For instance, hake could be boiled, stewed, or steamed, but anchovies were always grilled because they are never consumed boiled or stewed in our culture.

Determination of Total Arsenic (*11*). The samples (0.25 g), treated with nitric acid (5 mL of 50% v/v) and ashing aid (20% m/v $MgNO_3$ + 2% m/v MgO), were evaporated to dryness and mineralized at 450 °C with a gradual increase in temperature. The ash was dissolved in hydrochloric acid (6 mol L^{-1}) and prereduced (5% m/v ascorbic acid + 5% m/v KI). The analytical conditions used for arsenic determination by flow injection−hydride generation−atomic absorption spectrometry (FI−HG−AAS) were the following: loop sample, 0.5 mL; reducing agent, 0.2% (m/v) $NaBH_4$ in 0.05% (m/v) NaOH, 5 mL min^{-1} flow rate; HCl solution 10% (v/v), 10 mL min^{-1} flow rate; carrier gas argon, 100 mL min^{-1} flow rate. Conditions for AAS were the following: wavelength 193.7 nm; spectral band-pass 0.7 nm; electrodeless discharge lamp system 2, lamp current setting 400 mA; and cell temperature 900 °C.

Determination of Inorganic Arsenic (*20*). The lyophilized sample (0.50 ± 0.01 g) was weighed into a 50-mL screw-top centrifuge tube, 4.1 mL of water was added, and the sample was agitated until it was completely moistened. Then 18.4 mL of concentrated HCl was added, and the sample was agitated again for 1 h, and then left to stand for 12−15 h (overnight). The reducing agent (1 mL of 1.5% m/v hydrazine sulfate solution and 2 mL of HBr) was added, and the sample was agitated for 30 s. Then 10 mL of $CHCl_3$ was added and the sample was agitated for 3 min. The phases were separated by centrifuging at 2000 rpm for 5 min. The chloroform phase was separated by aspiration and poured into another tube. The extraction process was repeated two more times. The chloroform phases were combined and centrifuged again. The remnants of the acid phase were completely eliminated by aspiration (acid-phase remnants in the chloroform phase cause substantial overestimates of inorganic arsenic). Possible remnants of solid material in the chloroform phase were eliminated by passing it through Whatman GD/X syringe filters with a 25-mm PTFE membrane (Merck Farma y Química S. A., Barcelona, Spain).

The inorganic As in the chloroform phase was back-extracted by agitating for 3 min with 10 mL of 1 mol L^{-1} HCl. The phases were separated by centrifuging at 2000 rpm, and the aqueous phase was then aspirated and poured into a beaker. This stage was repeated once again, and the back-extraction phases obtained were combined. The determination of inorganic arsenic in the back-extraction phase was per-

Table 1. Total Arsenic, Inorganic Arsenic, and Moisture Contents in Cooked White Fish (Expressed in $\mu g\ g^{-1}$ (dry wt))[a]

hake ($n = 10$)				meagrim ($n = 12$)				small hake ($n = 18$)			
total As	inorg. As	H (%)	treatment	total As	inorg. As	H (%)	treatment	total As	inorg. As	H (%)	treatment
26.9	0.026	78.0	microwaving	21.1	0.030	72.1	grilling	4.8	0.030	77.5	stewing
5.4	0.034	75.4	microwaving	11.2	0.024	76.7	grilling	17.4	0.030	78.5	stewing
6.0	0.021	78.4	microwaving	31.5	0.068	70.9	grilling	10.0	0.024	77.0	stewing
6.6	0.029	77.7	microwaving	28.0	0.040	76.3	grilling	6.5	0.031	76.2	stewing
15.2	0.016	79.4	microwaving	33.4	0.042	74.5	grilling	4.4	0.013	80.4	stewing
11.3	0.037	78.0	microwaving	21.4	0.053	72.2	roasting	6.8	0.031	77.7	stewing
7.6	0.041	78.0	stewing	4.5	0.037	72.8	roasting	10.0	0.031	76.8	microwaving
4.9	0.011	82.2	stewing	3.6	0.098	62.6	roasting	3.7	0.017	79.7	microwaving
3.0	0.023	75.1	boiling	13.2	0.064	70.0	baking	14.1	0.032	77.3	microwaving
3.8	0.033	76.7	boiling	18.9	0.027	74.5	baking	15.9	0.027	78.2	microwaving
				27.4	0.026	73.9	baking	17.7	0.051	76.6	microwaving
				13.9	0.048	73.8	baking	12.4	0.039	79.2	microwaving
								5.1	0.016	76.4	baking
								23.1	0.017	75.7	baking
								7.1	0.029	76.6	baking
								8.1	0.011	76.4	baking
								8.3	0.047	75.1	baking
								8.5	0.047	72.7	baking
ranges of levels found in the samples analyzed											
3.0−26.9	0.011−0.041			3.6−33.4	0.024−0.098			3.7−23.1	0.011−0.051		

[a] n, number of samples analyzed. H, humidity.

formed by means of the following procedure: 2.5 mL of ashing aid suspension and 10 mL of concentrated HNO_3 were added to the combined back-extraction phases. The result was evaporated and treated in the same way as for total arsenic.

Determination of MMA. The lyophilized sample was extracted with methanol/water (1:1 v/v) and the extract was collected after centrifugation. This process was repeated three times, and the extracts were evaporated to dryness and redissolved in 3 mL of water. MMA was determined in the water extract by using HPLC−HG−AAS. The analytical conditions employed have been described in a previous paper (*21*).

Validation of the Analytical Procedures. Analytical characteristics for total arsenic, inorganic arsenic, and MMA were evaluated in previous papers. Total As (*22*): detection limit 0.026 $\mu g\ g^{-1}$ dry wt; precision 2%; accuracy DORM-2 certified reference material, found value ($17.9 \pm 0.5\ \mu g\ g^{-1}$ dry wet), certified value ($18.0 \pm 1.1\ \mu g\ g^{-1}$ dry wet). Inorganic arsenic (*20*): detection limit $0.013\ \mu g\ g^{-1}$ dry wt; precision 4%; recovery 99%. MMA (*21*): detection limit 0.2 ng g^{-1} wet wt; precision 3%; recovery 103%.

Statistical Analysis. To find out whether the cooking of fish could change the initial concentration of total and inorganic arsenic present in the raw product (expressed in wet weight), a repeated measures ANOVA model was used. "Type of seafood" and cooking "treatment" were between-subjects factors and "cooking" was a within-subjects factor. The "type of seafood" factor had 10 levels. "Treatment" was nested in "type of seafood", having different levels for each level of "type of seafood" as stated earlier. The "cooking" factor had two levels (cooked state and raw state). All effects were fixed effects and all proper interactions were included. Specific contrasts of the "cooking" effect for each level of "type of seafood" were calculated (*23*). Because some contrasts were not orthogonal, a Bonferroni correction of their significance was performed.

Additionally, to find out whether there was some type of seafood that had higher contents of inorganic arsenic in the cooked state (expressed in wet weight), an ANOVA model was applied. Factors were "type of seafood" and "treatment", with the same characteristics as in the previous model. Because we were interested in every pair of comparisons, multiple comparisons were made between every pair of "type of seafood" means using Tukey tests.

For both models, the experimental values did not pass a Hartley test (*24*) for homogeneity of variances between "type of seafood" groups, although they did for variances between "treatment" groups and interaction groups. To cope with this problem, the ANOVA models were finally calculated using a weighting factor proportional to the inverse of each "type of seafood" variance. Type III errors and 5% significance levels were considered in the F tests for every effect. Statistical analyses of the data were performed using the GLM procedure in the SAS/STAT statistical package (*25*).

RESULTS AND DISCUSSION

Tables 1 to 4 show the levels of total and inorganic arsenic [$\mu g\ g^{-1}$, dry weight (dry wt)], the ranges in which they lie, the percentages of humidity, and the type of cooking to which the samples were subjected. Figure 1 shows, for each type of seafood, the differences in the concentrations of total arsenic (Figure 1a) and inorganic arsenic (Figure 1b) in cooked and raw products, expressed as wet weight (wet wt). The box and whisker plot system of representation is employed in Figure 1. The concentrations of total and inorganic arsenic in the raw products were determined in a previous study (*11*).

Total Arsenic Contents in Cooked Seafood. The concentrations of total arsenic found in the cooked products expressed as dry wt (Tables 1−4) present a high variability between the specimens analyzed for each type of seafood. Comparison of the results obtained with those previously quantified in raw seafood products by Muñoz et al. (*11*) shows that in the white fish, both cooked and raw, the highest levels of total arsenic were in the samples of meagrim, with lower levels in hake and small hake. In the blue fish, the highest levels of arsenic were detected in samples of cooked anchovy and cooked sardine, as was also the case with the raw products (*11*). In the cooked shellfish group, the levels of total arsenic found were, in increasing order, squid<bivalves<crustaceans. The widest spread of results was found in the cooked crustaceans, similar to the situation found with raw crustaceans (*11*). This spread was predictable in view of the heterogeneity of the group, in which scampi and shrimp presented higher values than prawn. In the preserved fish group, the samples of cooked salted cod showed very similar concentrations, and these were the lowest in the whole study, as was also the case with the raw products (*11*).

Comparison of Total Arsenic Contents in Cooked and Raw Seafood. The ANOVA table and contrast results of the "cooking" effect for each "treatment" and "type of seafood" are shown in Table 5. The global

Table 2. Total Arsenic, Inorganic Arsenic, Monomethylarsonic Acid (MMA), and Moisture Contents in Cooked Blue Fish[a]

anchovy ($n = 10$)					Atlantic horse mackerel ($n = 12$)				sardine ($n = 10$)			
total As	inorganic As	MMA	H (%)	treatment	total As	inorganic As	H (%)	treatment	total As	inorganic As	MMA	H (%)	treatment
2.5	0.056	<LOQ	66.6	grilling	3.3	0.139	73.7	baking	9.6	0.177	0.024	56.7	roasting
10.7	0.049	<LOQ	68.9	grilling	2.8	0.105	68.6	baking	18.6	0.166	<LOQ	61.0	roasting
6.1	0.171	0.020	63.7	grilling	4.0	0.083	70.5	baking	19.6	0.186	0.021	66.8	roasting
28.4	0.050	<LOQ	67.1	grilling	5.1	0.113	71.1	baking	13.0	0.36	0.017	67.3	roasting
22.2	0.21	<LOQ	64.2	grilling	2.7	0.088	70.2	baking	10.5	0.175	0.012	64.4	roasting
21.4	0.161	0.017	63.3	grilling	3.4	0.105	67.6	baking	13.5	0.137	0.034	60.1	grilling
38.2	0.44	<LOQ	67.7	grilling	9.2	0.190	69.7	baking	6.0	0.138	0.018	76.2	grilling
19.1	0.127	<LOQ	64.0	grilling	13.7	0.068	73.3	baking	5.8	0.160	<LOQ	68.0	grilling
11.0	0.131	<LOQ	67.6	grilling	6.1	0.074	73.2	roasting	29.1	0.25	0.023	68.7	grilling
5.5	0.066	0.009	72.8	grilling	5.3	0.166	72.6	roasting	11.4	0.181	0.016	64.6	grilling
					3.9	0.120	65.1	grilling					
					2.7	0.118	68.6	grilling					
					ranges of levels found in the samples analyzed								
2.5−38.2	0.049−0.44	<LOQ-0.020			2.7−13.7	0.068−0.190			5.8−29.1	0.137−0.36	<LOQ-0.034		

[a] Total and inorganic arsenic expressed in $\mu g\ g^{-1}$ (dry wt) and MMA results expressed as As in $\mu g\ g^{-1}$ (dry wt). *n*, number of samples analyzed. H, humidity. LOQ, limit of quantification of the method (0.009 $\mu g\ g^{-1}$, dry wt).

"cooking" effect was not significant. Both a global "type of seafood" effect and the "cooking*type of seafood" interaction were significant, showing that the effect of cooking on the concentration of total arsenic depends on the type of seafood considered. The contrasts showed that these differences were significant for salted cod and bivalves, with mean increases after cooking of 151 and 858 ng g^{-1} wet wt, respectively. Expressed as percentages, the gains were 27% in salted cod and 37% in bivalves. The difference of 1326 ng g^{-1} wet wt for anchovies was almost significant. Both the absolute values and the group variances affect the final significance of the various contrasts. A graphical representation of the differences between cooked and raw total arsenic content by type of seafood can be seen in Figure 1a.

The opinion expressed by the U.S. EPA guideline in 1989 that cooking had no effect on contaminants (*12*) agrees with our results concerning the nonsignificance of a global "cooking" effect, common to all types of seafood, for total arsenic content. However, we observed a significant effect in certain types of seafood, and therefore we must assume that the U.S. EPA results did not take this interaction effect into account or their results were obtained from data of cooked samples with a low proportion of these types of seafood.

In our opinion, the variations in the concentration of total arsenic in seafood products after cooking may be the result of the sum of two contrary effects: (a) concentration of the metalloid due to the decrease in weight resulting from loss of water, volatiles, and to a lesser extent the other gross sample constituents (lipids, carbohydrates, and proteins), and (b) loss of total arsenic as a result of volatilization or solubilization.

The effect of the concentration of arsenic after cooking was indicated earlier in a Canadian survey of arsenic in total diet food composites (*6*). This study reported a mean increase in the concentration of total arsenic in seafood after cooking (raw, 2466 ng g^{-1}, wet wt; cooked, 3048 ng g^{-1}, wet wt), and the authors indicated that this increase generally agreed closely with the decrease in weight.

With respect to the existence of losses of total arsenic, no data are available concerning their possible volatilization during the cooking of food products. However, several studies report that there is solubilization of organoarsenical species during the cooking process. This solubilization may be due to the lability of the bond (electrostatic link) between AB and the proteins in fish muscle. Solubilization of arsenic species (AB and DMA) in the accompanying liquid was shown in a previous study carried out with canned fish (*26*). In vegetables, loss of arsenic during cooking has been found by other authors (*27*), although there is no indication as to whether this arsenic loss is due to volatilization or solubilization. To confirm losses of arsenic by solubilization, we studied the presence of organoarsenical species in accompanying liquids by carrying out an experiment with samples of crustaceans and bivalves, baked and steamed with deionized water. Total arsenic was detected in all the accompanying liquids. Speciation of the arsenic present in these liquids by means of HPLC−microwave-assisted oxidation−HG−AAS, following the methodology of Vélez et al. (*26*), showed the existence of AB in the liquid in which the crustaceans had been cooked, and AB and small quantities of DMA in the liquid resulting from the cooking of the bivalves.

Table 3. Total Arsenic, Inorganic Arsenic, Monomethylarsonic Acid (MMA), and Moisture Contents in Cooked Shellfish[a]

bivalves ($n = 12$)					crustaceans ($n = 10$)				squid ($n = 11$)			
total As	inorganic As	MMA	H (%)	treatment	total As	inorganic As	H (%)	treatment	total As	inorganic As	H (%)	treatment
22.8[b]	0.97[b]	0.069	75.2	steaming	18.2[d]	0.37[d]	73.7	stewing	3.1	0.047	73.0	boiling
13.9[b]	1.08[b]	0.019	75.5	steaming	33.5[d]	0.40[d]	74.5	stewing	3.5	0.023	71.2	boiling
10.8[c]	0.29[c]	0.037	73.5	steaming	73.8[e]	0.22[e]	72.5	stewing	9.2	0.020	72.7	boiling
8.9[c]	0.36[c]	0.029	74.8	steaming	21.3[e]	0.113[e]	72.7	stewing	3.3	0.050	73.3	boiling
10.7[c]	0.45[c]	0.034	70.7	steaming	14.7[f]	0.21[f]	73.8	stewing	22.3	0.060	73.2	boiling
8.7[c]	0.37[c]	0.066	75.9	steaming	1.4[f]	0.089[f]	70.3	stewing	4.9	0.054	73.8	boiling
10.0[c]	0.28[c]	0.042	62.6	steaming	18.7[f]	0.170[f]	71.2	stewing	7.8	0.028	70.8	boiling
8.6[c]	0.38[c]	0.020	65.3	steaming	2.6[f]	0.069[f]	70.8	stewing	2.7	0.045	67.8	grilling
13.3[c]	0.47[c]	0.014	71.3	steaming	2.2[f]	0.161[f]	78.5	stewing	8.4	0.014	72.0	grilling
12.9[c]	0.41[c]	0.055	72.6	steaming	6.6[f]	0.300[f]	72.8	stewing	3.5	0.049	67.4	stewing
14.0[c]	0.33[c]	0.013	74.3	steaming					8.0	0.041	71.0	stewing
8.9[c]	0.22[c]	0.009	74.5	steaming								
ranges of levels found in the samples analyzed												
8.6−22.8	0.22−1.08	0.009−0.069			1.4−73.8	0.069−0.40			2.7−22.3	0.014−0.060		

[a] Total and inorganic arsenic expressed in μg g^{-1} (dry wt). MMA results expressed as arsenic in μg g^{-1} (dry wt). *n*, number of samples analyzed. H, humidity. [b] Clam ($n = 2$). [c] Mussel ($n = 10$). [d] Scampi ($n = 2$). [e] Shrimp ($n = 2$). [f] Prawn ($n = 7$).

Table 4. Total Arsenic, Inorganic Arsenic, and Moisture Contents in Cooked Preserved Fish[a]

salted cod ($n = 18$)			
total As	inorganic As	H (%)	treatment
1.6	0.058	65.2	stewing
3.1	0.083	71.4	stewing
2.3	0.057	68.3	stewing
1.8	0.026	70.4	stewing
1.8	0.033	71.2	stewing
1.5	0.019	62.0	stewing
3.1	0.035	67.7	stewing
4.0	0.061	63.0	microwaving
1.6	0.020	68.0	microwaving
2.3	0.008	70.6	microwaving
1.2	0.038	74.0	microwaving
1.1	0.020	75.5	microwaving
3.3	0.049	68.0	microwaving
3.0	0.021	69.0	grilling
1.6	0.015	68.9	grilling
1.8	0.052	65.8	grilling
1.5	0.035	69.8	grilling
4.1	0.048	66.0	grilling
ranges of levels found in the samples analyzed			
1.1−4.1	0.008−0.083		

[a] Results of total and inorganic arsenic expressed in μg g^{-1} (dry wt). *n*, number of samples analyzed. H, humidity.

Given that solubilization of arsenic does take place, we assume that the final increase in the levels of total arsenic in bivalves and salted cod after cooking is due to the fact that the gains in arsenic through concentration are greater than the losses through solubilization.

Inorganic Arsenic Contents in Cooked Seafood. The method employed for determination of inorganic arsenic (*20*) also provides a quantitative determination of the MMA present in the seafood product. Considering the low levels of MMA detected in seafood products (*21*), the possible overestimation of inorganic arsenic attributable to MMA was considered negligible in previous studies (*28*). The analyses of seafood samples in the present study confirmed this assumption in 7 of the 10 types of seafood. However, in the samples of anchovy, sardine, and bivalves the concentrations of MMA detected varied between 0.009 and 0.069 μg g^{-1} (dry wt), and consequently the concentrations of inorganic arsenic for these samples were corrected to allow for the concentration of MMA (Tables 2 and 3).

The concentrations of inorganic arsenic (Tables 1−4) varied between 0.008 and 1.08 μg g^{-1} dry wt, representing between 0.1% and 8% of the total arsenic. This is similar to the range found by Muñoz et al. (*11*) in raw products (0.02−7%), which indicates that the percentage of inorganic arsenic varies between the same levels in both the raw and the cooked product.

In the white fish group, the levels of inorganic arsenic were similar in all the types of fish studied, being generally low and not exceeding 0.1 μg g^{-1} dry wt in any of the samples (Table 1). In the blue fish group, as also in the case of the corresponding raw products (*11*), it was the samples of sardine that, for the majority of the population, lying between percentiles 10 and 90, presented the highest concentrations of inorganic arsenic in the entire group, with values ranging between 0.137 and 0.36 μg g^{-1} dry wt.

In the case of shellfish, both the cooked and raw products (*11*) presented great heterogeneity in the concentrations of inorganic arsenic. The values in the cooked shellfish ranged between 0.014 (squid) and 1.08 μg g^{-1} dry wt (bivalves), with clam samples having the highest concentrations in the present study (Table 3). In preserved fish, the concentrations observed in cooked salted cod were slightly higher than those found in raw salted cod (Table 4).

To make a comparison of the inorganic arsenic contents in the various types of seafood, the concentrations of inorganic arsenic in wet wt were analyzed by means of an unbalanced repeated-measures ANOVA model and Tukey comparisons between means for each "type of seafood" (Table 6). The cooking "treatment" effect was not significant, but there was a significant "type of seafood" effect. The Tukey comparisons showed that the mean content of inorganic arsenic was significantly higher in bivalves (0.124 μg g^{-1} wet wt) than in any other type of seafood. A group of midrange types of seafood (ranging from 0.034 to 0.066 μg g^{-1} wet wt), without significant differences between them, consisted of sardines (with the highest level), crustaceans, anchovies, and Atlantic horse mackerel (with the lowest level). The remaining types of seafood had lower values without significant differences between them. Because bivalves seafood presented clear differences with respect to the other seafood samples analyzed, it would be desirable to control them from both a legislative and a toxicological viewpoint.

Comparison of Inorganic Arsenic Contents in Raw and Cooked Seafood. ANOVA table and contrast results of the "cooking" effect for each "treatment" and "type of seafood" are shown in Table 7. The global "type of seafood" effect, global "cooking" effect and "cooking*type of seafood" interaction were significant,

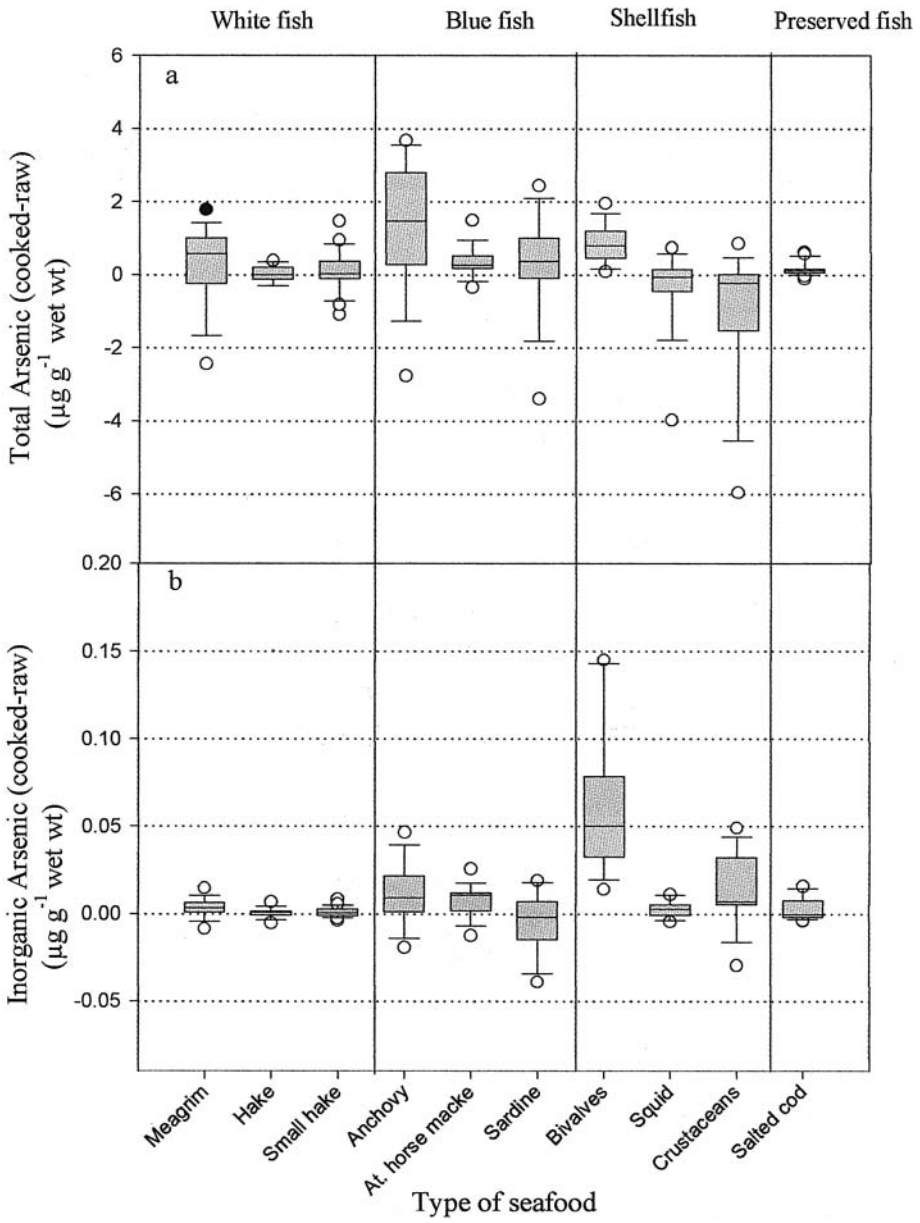

Figure 1. Differences in the concentrations of (a) total arsenic, and (b) inorganic arsenic, in cooked and raw seafood products. The vertical lines that divide the graph separate the seafood products into the four groups analyzed. The shaded boxes represent the concentrations in the population found between percentiles 25 and 75. The line dividing each box represents the value of the median. The whiskers below and above the box comprise the concentrations situated between percentiles 10 and 90. The dots represent the outlying data beyond percentiles 10 and 90.

so it was concluded that the effect of cooking on the concentration of inorganic arsenic depends on the type of seafood considered. The contrasts showed that these differences were generally very low (from 1 to 62 ng g^{-1}), and were only significant for bivalves and squid, with mean increases after cooking of 0.062 and 0.004 μg g^{-1} wet wt, respectively. The difference of 0.003 μg g^{-1} wet wt for salted cod was almost significant. Whereas in bivalves the differences are considerable and represent a percentage increase of 99%, these small differences for squid and salted cod have no practical consequences despite their significance or near significance. A graphic representation of the differences between the two concentrations (cooked−raw) for all the samples of seafood analyzed, expressed in wet wt, is given in Figure 1b.

As in the case of total arsenic, the variations in inorganic arsenic could be explained in terms of various mechanisms which act during the cooking process and are responsible for losses or gains. Losses of inorganic arsenic might be caused by volatilization or solubilization. For As(III), these mechanisms seem unlikely, in view of the strong bond that exists between this species and the thiol groups of proteins (*29*); for As(V), it is not known whether such losses are possible. As for gains, they might result from degradation of organoarsenical species to inorganic arsenic during the cooking process. A third mechanism which would lead to an increase in the concentrations of inorganic arsenic after cooking might be attributed, as in the case of total arsenic, to the weight reduction during cooking as a result of loss of water, volatiles, and to a lesser extent the other gross sample constituents (lipids, carbohydrates, and proteins).

In the case of the bivalves, although a clearly significant increase in inorganic arsenic during cooking was shown, the factors responsible for the increase are not

Table 5. Unbalanced Repeated Measures ANOVA Results for Total Arsenic Content (ng g^{-1} wet wt)

source	DF[a]	SS[b]	MS[c]	F	Pr > F
type of seafood	9	199.64	22.18	21.28	0.0001
treatment (type)	13	8.77	0.67	0.65	0.8086
sample (type*treatment)	100	104.23			
cooking	1	0.17	0.17	1.80	0.1832
cooking*type	9	3.00	0.33	3.49	0.0009
cooking*treatment (type)	13	0.61	0.05	0.49	0.9234
error	100	9.55	0.10		

Contrasts

cooking effect in	estimate[d]	DF	SS	MS	F	Bonferroni t[e]
hake	−4	1	0.000	0.000	0.00	0.00
meagrim	269	1	0.027	0.027	0.29	0.54
small hake	90	1	0.026	0.026	0.27	0.52
anchovy	1326	1	0.465	0.465	4.86	2.20
Atlantic horse mackerel	296	1	0.310	0.310	3.24	1.80
sardine	302	1	0.066	0.066	0.69	0.83
bivalves	858	1	3.870	3.870	40.52	6.37
crustaceans	−1153	1	0.072	0.072	0.76	0.87
squid	−35	1	0.001	0.001	0.01	0.10
salted cod	151	1	1.417	1.417	14.84	3.85

[a] DF, degrees of freedom. [b] SS, sum of squares. [c] MS, mean square. [d] Cooking effect in ng g^{-1} wet wt. [e] Bonferroni critical values: 2.626 (alpha = 0.05, 10 comparisons, 100 df); 3.174 (alpha = 0.01, 10 comparisons, 100 df); 3.391 (alpha = 0.005, 10 comparisons, 100 df).

Table 6. Unbalanced Repeated Measures ANOVA Results for Inorganic Arsenic Contents in Cooked Samples (ng g^{-1} wet wt)

source	DF[a]	SS[b]	MS[c]	F	Pr > F
type of seafood	9	169.93	18.88	19.93	0.0001
treatment (type)	13	8.59	0.66	0.70	0.7619
error	100	94.74	0.95		

Tukey's Studentized range (HSD) comparisons[d]

Tukey grouping	mean (ng g^{-1} wet wt)	N	type of seafood
A	124.84	12	bivalves
B	66.26	10	sardines
B	55.57	10	crustaceans
B C	49.52	10	anchovies
B C D	33.88	12	Atlantic horse mackerel
C D	13.40	12	meagrim
C D	11.99	18	salted cod
C D	11.16	11	squid
D	6.72	18	small hake
D	5.87	10	hake

[a] DF, degrees of freedom. [b] SS, sum of squares. [c] MS, mean square. [d] Means with the same letter are not significantly different. Alpha = 0.05. Critical value of Studentized range = 4.577. Minimum significant difference = 38.983.

known. We assume that it is attributable to the decrease in weight that takes place during cooking. Other transformations that might indirectly increase the concentrations of inorganic arsenic in the cooked product could be the increases in MMA and/or TMAO, codetermined species, of 100% and 10% respectively with the analytical method used for the quantification of inorganic arsenic. In the present study, MMA was quantified and the values of inorganic arsenic were corrected in both the raw and the cooked products. Consequently, MMA cannot be responsible for the variations observed after cooking. As for TMAO, the studies carried out by Van Elteren and Šlejkovec (*16*) and Devesa et al. (*17*) on standards revealed that AB is not transformed into TMAO in heat treatments which

Table 7. Unbalanced Repeated Measures ANOVA Results for Inorganic Arsenic Content (ng g^{-1} wet wt)

source	DF[a]	SS[b]	MS[c]	F	Pr > F
type of seafood	9	284.64	31.63	30.48	0.0001
treatment (type)	13	9.23	0.71	0.68	0.7748
sample (type*treatment)	100	103.77			
cooking	1	5.75	5.75	14.81	0.0002
cooking*type	9	9.99	1.11	2.86	0.0048
cooking*treatment (type)	13	6.57	0.51	1.30	0.2244
error	100	38.82	0.39		

contrasts

cooking effect in	estimate[d]	DF	SS	MS	F	Bonferroni t[e]
hake	−0.82	1	0.154	0.154	0.40	0.63
meagrim	3.64	1	0.639	0.639	1.65	1.28
small hake	1.06	1	1.099	1.099	2.83	1.68
anchovy	10.34	1	0.246	0.246	0.63	0.79
Atlantic horse mackerel	4.44	1	0.298	0.298	0.77	0.88
sardine	−4.91	1	0.212	0.212	0.55	0.74
bivalves	62.02	1	5.457	5.457	14.06	3.75
squid	3.52	1	4.657	4.657	11.99	3.46
crustaceans	13.32	1	0.870	0.870	2.24	1.50
salted cod	2.63	1	1.778	1.778	4.58	2.14

[a] DF, degrees of freedom. [b] SS, sum of squares. [c] MS, mean square. [d] Cooking effect in ng g^{-1} wet wt. [e] Bonferroni critical values: 2.626 (alpha = 0.05, 10 comparisons, 100 df); 3.174 (alpha = 0.01, 10 comparisons, 100 df); 3.391 (alpha = 0.005, 10 comparisons, 100 df).

do not exceed 100 °C; for such a transformation, temperatures above 150 °C are required. Such temperatures are presumably not attained in the cooking process to which the bivalves were subjected (steaming). Therefore, it seems unlikely that TMAO is the species that causes the increased concentrations of inorganic arsenic detected in cooked seafood.

Estimation of Mean Intake of Total and Inorganic Arsenic for the Spanish Population. In the Basque Country a total diet study (TDS) was set up in 1990 (*30*). This study enables the estimation of the intake of specific contaminants through the diet. The intake of total arsenic is calculated by means of its determination in the "fish" group in the TDS, since it is only in this group that results exceeding the limit of determination have been obtained (*19*). The "fish" group includes 13 food items, 10 of which are considered in the present study, while the other 3 correspond to the following categories: other white fish, other blue fish, and canned fish. The intake of total arsenic calculated by means of the TDS for the average consumer in the Basque Country during the period July 1997−June 1998 was 287 μg/day (data to be published shortly). Apart from analyzing the total diet "fish group" as such, the contents of total and inorganic arsenic were also analyzed in the 10 main food items included in this group, both raw (*11*) and cooked. By combining the data for the concentrations of total arsenic in each of the 10 food items after cooking with the corresponding daily consumption of each item by the Basque population (*31*), the intake of total arsenic was 210 μg/day. This value accounted for 73% of the intake obtained on the basis of the analysis of the total diet "fish group" (287 μg/day).

In Spain, the total diet study is conducted only in the Basque Country, therefore, to calculate the mean intake of total and inorganic arsenic for the Spanish population, the data for the concentrations of each food item (Tables 1−4) were combined with the corresponding consumption of each item by the Spanish population

Table 8. Intake of Total and Inorganic Arsenic for the Spanish Population

food item	consumption (g/day)	intake (μg/day) total arsenic	intake (μg/day) inorganic arsenic
hake	3.2	6.3	0.02
meagrim	5.5	27.8	0.07
small hake	20.7	48.7	0.14
anchovy	4.2	23.2	0.21
Atlantic horse mackerel	2.9	4.3	0.10
sardine	4.7	22.3	0.34
bivalves	3.5	11.4	0.47
crustaceans	3.7	19.0	0.20
squid	7.1	13.8	0.08
salted cod	2.7	1.9	0.03
sum of intakes (10 food items)		178.7	1.66
total intake calculated		**245**	**2.3**

(*32*). The result obtained (178.7 μg/day) was corrected, assuming that in this case also the value thus calculated only accounted for 73% of the actual intake (Table 8). After cooking, the final value for mean intake of total arsenic is 245 μg/day, one of the highest reported in the literature and only exceeded by the 345 μg/day determined for the Japanese population (*3*).

The same approximation was used to estimate the intake of inorganic arsenic by the Spanish population. The value obtained, 2.3 μg of inorganic arsenic per person per day, represents as expected, less than 1% of the intake of total arsenic, because the proportion of inorganic arsenic to total arsenic in seafood is very small. Bearing in mind that the PTWI for inorganic arsenic is 15 μg/kg body weight, and assuming an average body weight of 65 kg, the intake of inorganic arsenic represents only 1.7% of this reference value. Consequently, there is an ample safety margin for the intake of inorganic arsenic resulting from the consumption of seafood, even in populations where the consumption is very high (mean intake of seafood for the Spanish population: 77 g/day).

CONCLUSIONS

Statistical comparison of the contents of total and inorganic arsenic in cooked and raw products revealed a significant increase in the concentration of total arsenic after cooking for bivalves and salted cod, and in the concentration of inorganic arsenic for bivalves and squid.

As a result of the high consumption of seafood, the mean intake of total arsenic calculated for the Spanish population is one of the highest of those reported in the literature (245 μg/day). However, the mean intake of inorganic arsenic is less than 1% of the intake of total arsenic and represents only 1.7% of the PTWI. Consequently, there is an ample safety margin for the intake of inorganic arsenic, even in populations whose consumption of seafood is very high.

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