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Organoarsenical Species Contents in Cooked Seafood

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The organoarsenical species arsenobetaine (AB), arsenocholine (AC), tetramethylarsonium ion (TMA^+), dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA) were determined in 64 cooked seafood products (fish, bivalves, squid, crustaceans) included in a Total Diet Study carried out in the Basque Country (Spain). For cooking, various treatments were employed (grilling, roasting, baking, stewing, boiling, steaming, microwaving). The results obtained show that in cooked seafood AB is the major species, followed by DMA and TMA^+. AC and MMA are minor species. The results in cooked seafood were compared with the arsenic species contents obtained for the same product raw. After cooking there was an increase in DMA for sardines and bivalves and an increase or appearance of TMA^+ for meagrim, anchovy, Atlantic horse mackerel, and sardine. The data provided add to the very scant information available about organoarsenical species contents in cooked seafood.

KEYWORDS: arsenic; organoarsenical species; seafood; cooking

INTRODUCTION

The total arsenic intake in a given population is largely determined by the amount of seafood consumed (*1*, *2*). The presence of arsenic in marine organisms was detected by Bertrand in the early 1900s (*3*), and in 1973 Lunde showed that the element was distributed in the form of both inorganic and organic species (*4*). Since then great attention has been paid to elucidating the cycling of arsenic in marine ecosystems, developing methodologies for arsenic speciation, and discovering the presence of new species.

Inorganic arsenic species [As(III) and As(V)], the most toxic species found in foods, are present in fish and shellfish samples (*5*). Organic species such as TMA^+ (tetramethylarsonium ion), MMA (monomethylarsonic acid), and DMA (dimethylarsinic acid), which are less acutely toxic than inorganic arsenic, and AB (arsenobetaine), AC (arsenocholine), and TMAO (trimethylarsine oxide), which are considered nontoxic (*6*), are also found in fish and fish products (*7*−*11*). Last, arsenosugars, mainly dimethylarsinoylribosides, found only in shellfish such as bivalves and gastropods (*11*, *12*), are species whose toxicity requires further study. Given the great difference in the toxicity of the various species of arsenic, better knowledge of the arsenical composition of seafood might contribute to a better understanding of the human health risks associated with consumption of these products.

A recent critical review by Francesconi and Kuehnelt (*13*) showed that more than 400 research articles have been published from 2000 to the end of 2003 reporting the development or application of arsenic speciation analysis. Despite this, results for food samples, and especially for market foods, are rarely available in the literature, so that in environmental, biological, or toxicological fields conclusions are often drawn without a proper scientific basis (*13*, *14*).

A document recently published by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (*15*) evaluated statements on arsenic in food. In their conclusions, they note that the general assumption that organic arsenic is less toxic than inorganic arsenic is based on an extremely limited database and that the available data are not sufficient for setting a Provisional Tolerable Weekly Intake (PTWI) for organic arsenic. However, there is no evidence that exposure to organic arsenic through high levels of fish consumption has resulted in harmful effects (*15*). The existing uncertainty makes it necessary to research organic arsenic both at toxicological level and at the level of risk assessment. In this connection, the provision of data with which to estimate intakes of organoarsenical species is a pending task.

Most studies concerning organic arsenic species have been carried out on raw samples. For raw fish and shellfish, the scant data in the literature show that, whereas AB appears to be found in all seafood products, the other organic species present a very irregular distribution (*7*−*11*). This information is a great contribution from the viewpoint of the arsenic cycle in the marine medium, but it gives a biased view of the toxicity of the product ingested by the consumer. The transformation of organoarsenical species during cooking has been shown in experiments with fish samples (*16*, *17*). These transformations may involve a change in the toxicity of seafood, and therefore,

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the risk assessment process should consider the organoarsenical species in the most important cooked seafood components of a diet.

The aim of the present study is to determine the concentrations of AB, DMA, TMA^+, MMA, and AC in individual samples of cooked seafood products which form part of a Total Diet Study carried out by the Basque Government's Health Department as part of the Food Chemical Safety Surveillance Program. As the organoarsenical species contents in the raw food items had been determined previously (*10*), in the present study it was possible to evaluate the effect of cooking on concentrations of these organoarsenical species.

EXPERIMENTAL PROCEDURES

Equipment. The high-performance liquid chromatography (HPLC) system employed consisted of a Hewlett-Packard (HP) model 1100 (Hewlett-Packard, Barcelona, Spain) equipped with a quaternary pump, online degassing system, and automatic injector. A switching column valve (Rheodyne 6-port automated) was used between two columns: cation exchange column PRP-X200 (Hamilton 10 μm, 250 mm $\times$ 4.1 mm inside diameter (i.d.); Teknokroma, Barcelona, Spain) and anion exchange column PRP-X100 (Hamilton 10 μm, 250 mm $\times$ 4.1 mm id, Teknokroma). Operation of the switching column valve was controlled by the chromatograph software.

For arsenic detection, two detectors were employed. The atomic fluorescence spectrometer (AFS) (PSA 10.044 Excalibur PS, Analytical, UK) was equipped with a boosted-discharge hollow cathode lamp (BDHCL, Photron, Super Lamp, Victoria, Australia) and was coupled to a hydride generation (HG) system (PSA 10.004, Analytical, UK). The atomic absorption spectrometer (AAS) (Perkin-Elmer model 5000, USA) was equipped with a flow-injection (FI) system (Perkin-Elmer, FIAS-400) in order to provide HG in continuous flow mode. A Hewlett-Packard Model 35900 C digital analogical converter with two channels was used to acquire the AFS and AAS signals, which were processed by means of the chromatograph software.

Other equipment used included a lyophilizer equipped with a microprocessor controlling the lyophilization process (FTS Systems, New York, USA) and connected to a computer, a PL 5125 sand bath (Raypa, Scharlau S. L., Barcelona, Spain), a K1253 muffle furnace equipped with a Eurotherm Controls 902 control program (Heraeus S. A., Madrid, Spain), a KS 125 Basic mechanical shaker (IKA Labortechnik, Merck, Barcelona, Spain), a mechanical shaker (Rotabit, Selecta, Spain), an Eppendorf 5810 centrifuge (Merck), a Heraeus Biofuge Pico centrifuge (Merck), and a Julabo model HC heated bath (Merck).

Reagents. Deionized water (18.2 MWΩ cm) was used for the preparation of reagents and standards. All chemicals were of pro analysi quality or better. All glassware was treated with 10% (v/v) HNO_3 for 24 h and then rinsed three times with deionized water before being used. The stock standard solutions of MMA (1000 mg L^{-1}, Carlo Erba, Italy) and DMA (1000 mg L^{-1}, Fluka Chemika Biochemika, Spain) were prepared by dissolving appropriate amounts of commercially available salts in water. Standards supplied by Hot Chemical Co. (Tokyo, Japan) were used to prepare aqueous stock standard solutions of AB, AC, and TMA^+.

Sample Collection and Preparation. Samples were purchased from retail outlets throughout the Basque Country and collected from August 1997 to June 1998 in different locations. The samples used for this study were selected on the basis of their high domestic consumption in the Basque Country (*18*). In total, 64 samples of seafood products classified into 10 different categories were analyzed. Eight of them consisted of a single seafood product: meagrim, hake, small hake, anchovy, Atlantic horse mackerel, sardine, squid, and salted cod. The remaining two categories included various similar types of seafood products with a low individual contribution to the total diet: bivalves (clam and mussel) and crustaceans (scampi, shrimp, and prawn).

Each sample consisted of one or more specimens of a particular kind of seafood (depending on their size) purchased in the same place and month. Immediately after being caught, each sample was prepared and cooked according to the most common domestic practice. Before cooking, inedible parts of fish (head, tail, and digestive tract) and squid samples (cartilaginous skeleton) were removed. In bivalve samples the shell and in crustaceans the exoskeleton were eliminated after cooking.

The cooking processes (grilling, roasting, baking, steaming, stewing, boiling, or microwaving) were applied without using fat or other additional ingredients (only distilled water when necessary). The 10 different types of seafood were not all submitted to the same cooking treatment. Each sample was cooked using only one particular treatment selected at random from those used for that particular seafood 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. The individual cooked samples were then homogenized and frozen at -20 °C. Subsequently, they were lyophilized and ground in a domestic apparatus. The resulting powder was stored in previously decontaminated twist-off flasks and kept at 4 °C. The analysis of arsenic and arsenic species was done within six months of the lyophilization of the samples.

Extraction of Arsenic Compounds. Lyophilized sample (1.00 g) was extracted three times (20 mL $\times$ 3) with methanol/water (1 + 1 v/v). The extracts were combined and evaporated to dryness. The dry residue was dissolved in 3 mL of water and centrifuged, and the supernatant was filtered through a Whatman 0.45-μm nylon membrane prior to injection into the chromatographic column (*19*). The validity of this method for the extraction of the arsenic species of interest was verified in a previous study (*19*).

Determination of Organoarsenical Species. The species were separated by HPLC (Hewlett-Packard model 1100) using a column switching system between a PRP-X100 anionic exchange column and a PRP-X200 cationic exchange column. The program employed for the operation of the switching system valve, described in detail in Súñer et al. (*19*), permits separation of DMA, MMA, and AB in the PRP-X100 column and of TMA^+ and AC in the PRP-X200 column. After chromatographic separation, the outlet of the PRP-X100 column was thermo-oxidized, cooled in an ice bath, and quantified by HG-AAS. The outlet of the PRP-X200 column was thermo-oxidized, cooled in an ice bath, and quantified by HG-AFS. Arsenic compounds were identified by matching the retention times of the peaks in the sample with those obtained from standards and were quantified using the calibration curves of the corresponding standards.

Triplicate analyses were performed for each sample. The suitability of the analytical method employed for organoarsenical species determination has been checked previously by evaluating its analytical characteristics (*19*). The limit of detection varies between 0.91 ng g^{-1} dw for AB and 0.23 ng g^{-1} ww for DMA. The precision ranges from 1 to 12%, and the recovery is >95% for all species. Throughout the experiment, the accuracy of measurement was checked by analyzing BCR-627 tuna fish (Institute for Reference Materials and Measurements, IRMM, Brussels, Belgium), a commercially available reference material in which AB and DMA contents are certified. In each analytical batch, samples, blanks, and BCR-627 were analyzed.

RESULTS

For the presentation of the results obtained, the seafood products were arranged in four groups: lean fish with a fat content ~1% (hake, meagrim, and small hake); fatty fish with a fat content >1% (anchovy, Atlantic horse mackerel, and sardine); shellfish (bivalves, squid, and crustaceans); and preserved fish (salted cod).

Tables 1−4 show the concentrations of AB, DMA, MMA, TMA^+, AC, and total As (μg g^{-1} as arsenic, wet weight, ww), the ranges in which they lie, the percentages of moisture, and the type of cooking to which each sample was subjected. Data in the literature on arsenic species contents in cooked seafood products are extremely sparse (*16*, *17*), making it difficult to compare the results obtained in this study with previous data.

Organoarsenical Species Contents. Of the arsenic species analyzed, the highest concentration was that of AB and this was the only species detected in all the samples. The same

Table 1. Organoarsenical Species, Total As, and Moisture Contents in Lean Fish; Results Expressed in μg g^{-1} ww as As

treatment	AB	DMA	TMA$^+$	MMA	AC	total As[a]	moisture (%)
			Meagrim				
grilling	6.2	0.024	0.226	<LOD[b]	<LOD	5.9	72
grilling	2.9	0.007	0.124	<LOD	<LOD	2.6	77
grilling	8.2	0.063	0.036	<LOD	<LOD	9.2	71
roasting	5.9	0.033	0.262	<LOD	<LOD	5.9	72
roasting	0.93	0.027	<LOD	<LOD	0.009	1.2	73
baking	3.8	0.044	0.020	<LOD	<LOD	4.0	70
c	0.93–8.2	0.007–0.063	<LOD–0.262	<LOD	<LOD–0.009	1.2–9.2	
			Hake				
microwaving	5.9	<LOD	<LOD	<LOD	<LOD	5.9	78
microwaving	1.3	0.049	<LOD	<LOD	0.004	1.3	75
stewing	1.3	0.036	<LOD	<LOD	0.012	1.7	78
boiling	0.67	0.026	<LOD	<LOD	<LOD	0.76	75
boiling	0.60	0.047	<LOD	<LOD	<LOD	0.88	77
baking	3.5	0.047	<LOD	<LOD	0.005	3.8	74
c	0.60–5.9	<LOD–0.049	<LOD	<LOD	<LOD–0.012	0.76–5.9	
			Small Hake				
stewing	0.93	0.034	<LOD	<LOD	0.010	1.1	77
stewing	4.0	0.047	<LOD	<LOD	0.010	3.8	78
Stewing	2.6	0.043	<LOD	<LOD	0.008	2.3	77
stewing	1.3	0.038	0.009	<LOD	0.009	1.5	76
stewing	1.3	0.088	<LOD	<LOD	0.009	1.5	78
microwaving	4.1	0.054	<LOD	<LOD	0.021	4.2	77
microwaving	2.3	0.024	<LOD	<LOD	0.009	2.3	77
microwaving	2.7	0.059	<LOD	<LOD	0.013	2.6	79
baking	2.4	0.089	<LOD	<LOD	0.012	2.3	73
baking	2.5	0.044	<LOD	<LOD	0.011	2.1	75
c	0.93–4.1	0.024–0.089	<LOD–0.009	<LOD	0.008–0.021	1.1–4.2	

[a] Results of total arsenic were published previously (*20*). [b] LOD, limit of detection (ng g^{-1} wet weight): AB, 0.91; DMA, 0.23; MMA, 0.83, TMA$^+$, 0.68; AC, 0.6. [c] Ranges of levels found in the samples analyzed.

Table 2. Organoarsenical Species, Total As, and Moisture Contents in Fatty Fish; Results Expressed in μg g^{-1} ww as As

treatment	AB	DMA	TMA$^+$	MMA	AC	total As[a]	moisture (%)
			Anchovy				
grilling	0.56	0.025	0.020	<LOD[b]	<LOD	0.84	67
grilling	3.4	0.040	<LOD	<LOD	<LOD	3.3	69
grilling	1.25	0.30	0.055	0.007	<LOD	1.68	64
grilling	9.9	0.031	0.571	<LOD	<LOD	9.3	67
grilling	7.3	0.024	0.248	<LOD	<LOD	7.9	64
grilling	3.3	0.075	0.179	<LOD	<LOD	3.9	62
c	0.56–9.9	0.024–0.30	<LOD–0.571	<LOD–0.007	<LOD	0.84–9.3	
			Atlantic Horse Mackerel				
grilling	0.67	0.158	0.018	0.003	<LOD	1.4	65
grilling	0.24	0.142	0.008	0.003	<LOD	0.85	69
baking	0.40	0.106	<LOD	0.001	<LOD	0.87	74
baking	0.52	0.104	<LOD	0.002	<LOD	0.88	69
baking	0.70	0.040	<LOD	<LOD	<LOD	1.2	71
baking	0.93	0.144	<LOD	0.003	<LOD	1.5	71
c	0.24–0.93	0.040–0.158	<LOD–0.018	<LOD–0.003	<LOD	0.85–1.5	
			Sardine				
roasting	3.2	0.43	0.117	0.010	<LOD	4.1	57
roasting	7.0	0.49	0.224	0.001	<LOD	7.2	61
roasting	5.4	0.280	0.053	0.007	<LOD	6.5	67
grilling	4.4	0.47	0.021	0.014	<LOD	5.4	60
grilling	0.78	0.279	<LOD	0.004	<LOD	1.4	76
baking	0.47	0.206	<LOD	0.005	<LOD	1.5	57
c	0.47–7.0	0.206–0.49	<LOD–0.224	0.001–0.014	<LOD	1.4-7.2	

[a] Results of total arsenic were published previously (*20*). [b] LOD, limit of detection (ng g^{-1} wet weight): AB, 0.91; DMA, 0.23; MMA, 0.83, TMA$^+$, 0.68; AC, 0.6. [c] Ranges of levels found in the samples analyzed.

extensive distribution of AB was observed in the corresponding raw samples (*10*). The concentration of AB varied between 0.110 and 24 μg g^{-1} (ww), with the highest value appearing in a sample of shrimp.

DMA was not detected in any samples of squid or salted cod. In the remaining samples the concentrations ranged from 0.007 to 0.49 μg g^{-1}, ww. The contents were greater in fatty fish (0.024−0.49 μg g^{-1}, ww) and bivalves (0.157−0.277 μg g^{-1}, ww) than in lean fish (0.007−0.089 μg g^{-1}, ww) and crustaceans (values below 0.018 μg g^{-1}, ww). TMA$^+$ was not detected in all the samples, although it was the second most abundant species in terms of concentration, varying between 0.008 and 0.571 μg g^{-1}, ww. It appeared in some samples of meagrim (0.020−0.262 μg g^{-1}, ww) and in all the fatty fish

Table 3. Organoarsenical Species, Total As, and Moisture Contents in Shellfish; Results Expressed in μg g^{-1} ww as As

treatment	AB	DMA	TMA$^+$	MMA	AC	total As[a]	moisture (%)
			Bivalves				
steaming[b]	0.91	0.157	na[d]	0.010	na	2.9	73
steaming[b]	1.12	0.198	na	0.007	na	2.3	75
steaming[b]	0.87	0.243	na	0.015	na	2.1	76
steaming[c]	0.91	0.277	na	0.016	na	5.7	75
steaming[c]	0.54	0.158	na	0.004	na	3.4	76
Squid							
boiling	0.32	<LOD[e]	<LOD	<LOD	<LOD	0.84	73
boiling	0.58	<LOD	<LOD	<LOD	<LOD	1.0	71
boiling	1.95	<LOD	<LOD	<LOD	<LOD	2.5	73
grilling	0.18	<LOD	<LOD	<LOD	<LOD	0.87	68
stewing	0.91	<LOD	<LOD	<LOD	<LOD	1.1	67
roasting	0.14	<LOD	<LOD	<LOD	<LOD	0.27	60
f	0.14–1.95	<LOD	<LOD	<LOD	<LOD	0.27–2.5	

[a] Results of total arsenic were published previously (*20*). [b] Mussel. [c] Clam. [d] na: not analyzed. [e] LOD, limit of detection (ng g^{-1} wet weight): AB, 0.91; DMA, 0.23; MMA, 0.83, TMA$^+$, 0.68; AC, 0.6. [f] Ranges of levels found in the samples analyzed.

Table 4. Organoarsenical Species, Total As, and Moisture Contents in Preserved Fish; Results Expressed in μg g^{-1} ww as As

treatment	AB	DMA	TMA$^+$	MMA	AC	total As[a]	moisture (%)
			Salted Cod				
grilling	0.51	<LOD[b]	<LOD	<LOD	<LOD	0.61	66
grilling	0.37	<LOD	<LOD	<LOD	<LOD	0.45	70
grilling	0.62	<LOD	<LOD	<LOD	<LOD	0.94	69
stewing	0.28	<LOD	<LOD	<LOD	<LOD	0.56	65
stewing	0.72	<LOD	<LOD	<LOD	<LOD	0.89	71
microwaving	0.92	<LOD	<LOD	<LOD	<LOD	1.1	68
microwaving	0.75	<LOD	<LOD	<LOD	<LOD	0.67	71
microwaving	0.11	<LOD	<LOD	<LOD	<LOD	0.31	74
c	0.11–0.92	<LOD	<LOD	<LOD	<LOD	0.31–1.1	

[a] Results of total arsenic were published previously (*20*). [b] LOD, limit of detection (ng g^{-1} wet weight): AB, 0.91; DMA, 0.23; MMA, 0.83, TMA$^+$, 0.68; AC, 0.6. [c] Ranges of levels found in the samples analyzed.

(0.008−0.571 μg g^{-1}, ww) and crustaceans (0.011−0.149 μg g^{-1}, ww). TMA$^+$ could not be analyzed in bivalves because of the complexity of the chromatogram obtained, in which we observed chromatographic signals possibly attributable to arsenosugars, species whose chromatographic separation was not optimized or considered in the present work. MMA was only found in fatty fish (0.001−0.014 μg g^{-1}, ww) and bivalves (0.004−0.016 μg g^{-1}, ww), being a very minor species in both cases. Last, AC, which was also a minor species, as well as in the corresponding raw products (*10*), was only found in lean fish (all the samples of small hake, three samples of hake, and one sample of meagrim), with concentrations varying between 0.004 and 0.021 μg g^{-1}, ww. In the bivalves it was not possible to analyze AC because of the complexity of the chromatogram.

In Atlantic horse mackerel (**Table 1**) and bivalves (**Table 3**), the sum of the As from the various species quantified was less than the total arsenic of the product (Atlantic horse mackerel 50−76%; bivalves 27−66%). In the bivalves, this might be attributable to the presence of arsenosugars, species not quantified in this study but frequent in these products (*21*). However, in Atlantic horse mackerel, the low percentage might be attributable to the presence in this fatty fish of a significant proportion of arsenic bound to lipids, as postulated by Branch et al. (*9*) for samples of mackerel. In these samples, the presence of other species different than those analyzed in this paper has not been described in the literature.

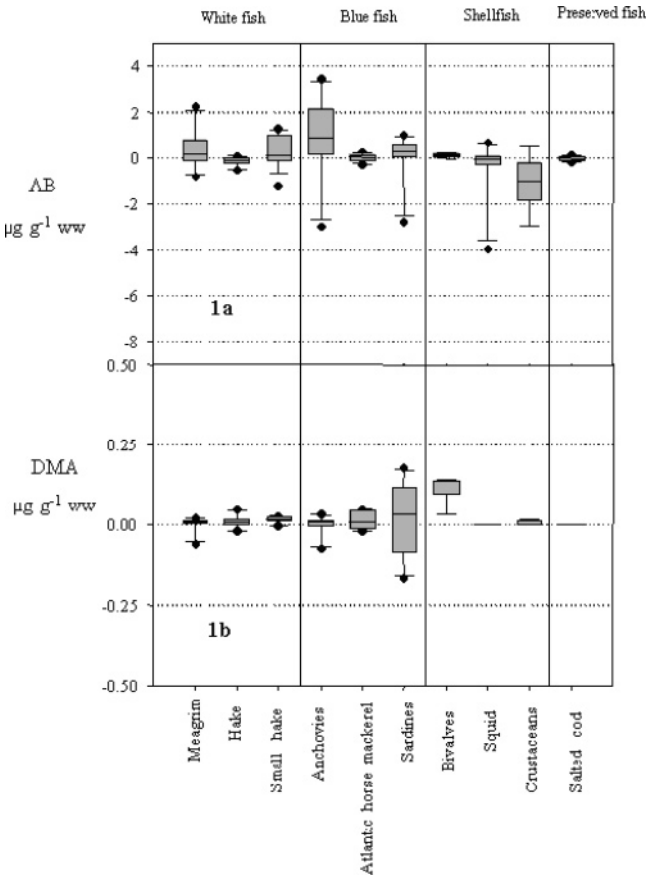

Figure 1. Differences in the concentration of (a) AB and (b) DMA in cooked and raw seafood products, expressed as μg g^{-1} ww (as As). The vertical lines that divide the graph separate the seafood products into the four groups analyzed. The box represents the differences in the population found between percentiles 25 and 75. The line dividing the 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.

Effect of Cooking on Organoarsenical Species Contents. The concentrations of the arsenic species obtained in the cooked products were compared with those obtained previously by Súñer et al. (*10*) in the same raw products. The differences in concentration (cooked-raw) of each arsenic species are presented in **Figures 1a**, **1b**, **2a**, **2b**, and **3** for the different seafood categories, using the box plot graph.

The factors which might alter the concentration of a species of arsenic present in the raw product as a result of cooking follow two different tendencies. On one hand, losses of water and other soluble compounds take place during cooking, producing a weight loss with respect to the raw product of up to 20% in the samples analyzed in this study. In this case the loss does not affect the absolute contents of the organoarsenical species, but it does lead to an increase in their concentrations although there are no transformations between species. In the literature there are reports that during cooking some arsenic species may also be generated from others, with a consequent increase in their concentration. This has been shown by the generation of TMA$^+$ from AB (*16*, *17*). On the other hand, there may be a loss of organoarsenical species from the raw product to the cooking liquid. In our laboratory this process has been verified in earlier studies performed with samples that do not belong to the range of samples collected for the Total Diet Study in the Basque Country described in the present work. The data obtained (data not shown) reveal solubilization to the cooking

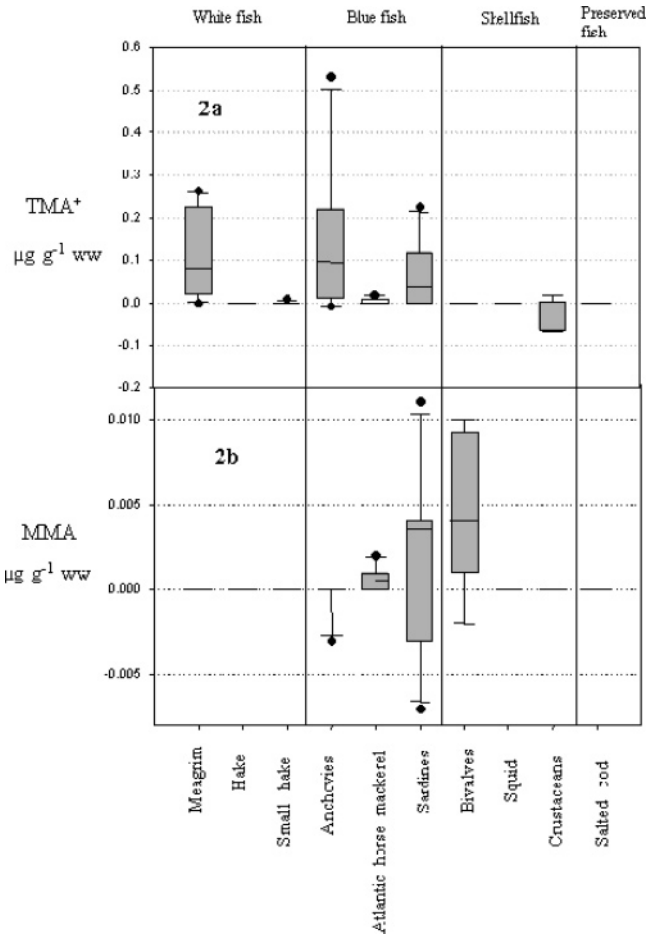

Figure 2. Differences in the concentration of (a) TMA^+ and (b) MMA in cooked and raw seafood products, expressed as μg g^{-1} ww (as As). The vertical lines that divide the graph separate the seafood products into the four groups analyzed. The box represents the concentrations in the population found between percentiles 25 and 75. The line dividing the 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.

liquid of AB in fish, AB and DMA in mussel and peppery furrow, and AB and TMA^+ in clams. Consequently, depending on which factors have the stronger effect, a gain or loss of arsenic species might be observed after cooking.

Figure 1a shows that in most of the seafoods analyzed there is an increase in AB after cooking (median value of the differences above zero). Only in hake and crustaceans are there losses of AB as a result of cooking, as shown by the median of the distribution (mean losses of 0.11 μg g^{-1} ww in hake and 1.24 μg g^{-1} ww in crustaceans; equivalent to mean losses of 3% and 14% with respect to the AB content in the raw product). In hake, Atlantic horse mackerel, bivalves, and salted cod the behavior of the samples is very homogeneous, while in other categories there is greater dispersion, with differences in concentrations of up to 3.5 μg g^{-1} ww, as in the case of anchovies.

For DMA (**Figure 1b**), in all the types of seafood analyzed, the distribution of the differences shows medians greater than zero. The greatest loss of DMA takes place in a sample of sardine (0.167 μg g^{-1}, ww; 37% with respect to the DMA content in the raw sample), and the greatest increase in DMA after cooking (0.177 μg g^{-1}, ww; 71% with respect to the DMA content in the raw sample) appears in another sardine sample.

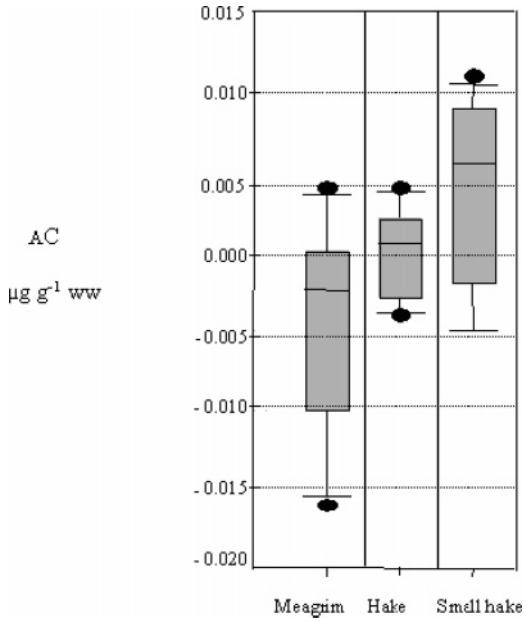

Figure 3. Differences in the concentration of AC in cooked and raw lean fish, expressed as μg g^{-1} ww (as As). The vertical lines that divide the graph separate the seafood products into the four groups analyzed. The box represents the concentrations in the population found between percentiles 25 and 75. The line dividing the 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.

Bivalves also show a pronounced increase in DMA after cooking (a mean increase of 0.115 μg g^{-1}, ww).

TMA^+ (**Figure 2a**) differs from the other species in its behavior, appearing in some types of cooked seafood for which it was absent in the raw samples. The samples of meagrim, Atlantic horse mackerel, and sardine that show an increase in the concentration of TMA^+ after cooking did not have concentrations above the limit of detection of the methodology employed in the raw state. The increase for these samples varies between 0.008 and 0.264 μg g^{-1}, ww. This species was present in all the anchovy samples in the raw state, and after cooking it increased between 0.01 and 0.532 μg g^{-1}, ww. Losses of TMA^+ were only found in the samples of crustaceans (0.002−0.069 μg g^{-1}, ww) occurring in most of the samples.

For MMA (**Figure 2b**), which is only detected in raw and cooked samples of Atlantic horse mackerel, sardines, and bivalves, there is an increase of contents after cooking (0.001−0.011 μg g^{-1} ww, multiplying the MMA content in the raw sample by up to 4.8). Finally, AC (**Figure 3**), which was only present in lean fish, experienced both maximum losses of 0.016 μg g^{-1}, ww, in a sample of meagrim, a loss representing 95% of the AC present in the raw meagrim, and maximum gains after cooking of 0.011 μg g^{-1}, ww, in a sample of small hake, an increase equivalent to multiplying the AC present in the raw sample by 18.

The results show that the most notable effects of the cooking process are those that affect DMA and TMA^+. For DMA, the increase described in the bivalves might be a result of the transformation of arsenic species of greater complexity, perhaps the arsenosugars present in this seafood (*21*, *22*). The references in the literature on this point are contradictory. McSheehy et al. (*23*) indicated that in acid conditions there may be a breaking of the bond in dimethylated arsenosugars, generating DMA. However, Gamble et al. (*24*) subsequently showed that

Table 5. TMA^+ Contents (expressed in $\mu g\ g^{-1}$ ww as As) in Raw and Cooked Seafood Products in Samples with an Increase in TMA^+ after Cooking

		TMA^+	
treatment	type of seafood	raw[a]	cooked
grilling	meagrim	<LOD[b]	0.226
	meagrim	<LOD	0.124
	meagrim	<LOD	0.036
	anchovy	0.010	0.020
	anchovy	0.035	0.055
	anchovy	0.039	0.571
	anchovy	0.028	0.248
	Atlantic horse mackerel	<LOD	0.018
	Atlantic horse mackerel	<LOD	0.008
	sardine	<LOD	0.021
roasting	meagrim	<LOD	0.262
	anchovy	0.011	0.179
	sardine	<LOD	0.117
	sardine	<LOD	0.224
	sardine	<LOD	0.053
baking	meagrim	<LOD	0.020

[a] Values obtained in a previous study (*10*). [b] LOD, limit of detection of TMA^+: 0.68 ng g^{-1} wet weight.

arsenosugar standards that undergo acid hydrolysis (pH 1.1) do not generate DMA but another arsenosugar. As the steaming conditions employed for cooking the bivalves analyzed in this study (distillated water at 100 °C) did not involve an acid attack on the matrix, the increase in DMA might be attributable to a concentration of the analyte. The concentration of DMA after cooking would also explain the contents detected in sardines, in which, although the presence of arsenosugars has not been reported so far, an increase in DMA was observed after roasting.

The increase in or appearance of TMA^+ observed after the cooking of certain samples might be due to decarboxylation of AB. This mechanism for producing TMA^+ from AB was first proposed by Francesconi et al. (*25*) to explain the presence of TMA^+ in marine organisms. This suggestion was subsequently confirmed experimentally in aqueous standards of AB subjected to temperatures higher than 150 °C by Van Elteren and _Slejkovec (*26*) and Devesa et al. (*27*). In samples of sole, dory, hake, and sardine fried, grilled, or baked, a recent study carried out by Devesa et al. (*16*) showed the appearance of TMA^+, which had not been detected in the raw product. The authors cited stated that, although the temperature inside the products did not reach 150 °C during cooking, the surface on which the product was placed for cooking reached far higher temperatures (approximately 250 °C). This might cause, the surface layer of the fish in direct contact with the heating surface, an increase in temperatures high enough to cause thermal decarboxylation of AB. In parallel with this study, Hanaoka et al. (*17*) detected TMA^+ generated from AB in roasted shark and crayfish.

The samples in which an increase in TMA^+ was observed after cooking are presented in **Table 5**. This table shows the contents of TMA^+ in the raw product, obtained in a previous study (*10*), together with the contents of TMA^+ in the same product after cooking (obtained in the present study). It can be seen that all the samples that presented an increase in TMA^+ after cooking were roasted, grilled or, in one case, baked. This corroborates the results obtained by Devesa et al. (*16*) mentioned earlier, and once again indicates that the type of heat treatment seems to have an effect on the decarboxylation of AB. Treatments in which high temperatures may be attained, such as roasting, baking, or grilling, favor the process of decarboxylation. In contrast, those types of cooking in which water is used as a cooking liquid (stewing, boiling, or steaming) do not attain temperatures above 100 °C, which seem to be necessary for the generation of TMA^+ from AB. In microwave cooking, also, the temperature reached by the food product does not normally exceed 100 °C (*26*), and the use of a rotating plate prevents overheating of specific parts of the food.

The increases in TMA^+ after cooking were also not always of the same order. For example, in grilled anchovy there were very small increases in two samples (0.010 and 0.020 $\mu g\ g^{-1}$, ww), whereas in two other samples the increases were over 0.200 $\mu g\ g^{-1}$, ww. This might indicate that the samples did not all reach the same surface temperature, possibly resulting from the application of different cooking times in specific cases, since cooking time was not strictly controlled.

In conclusion, the results obtained show that AB is the major species in cooked seafood, followed by DMA and TMA^+. The remaining species analyzed, AC and MMA, are very minor. The heat treatments to which the seafoods analyzed were subjected during cooking only produced increases in DMA and an increase in or appearance of TMA^+. This study helps to make up for the lack of knowledge about organoarsenical species contents in seafood products, highlighted by the UK's Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment.

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