Effect of Cooking Temperatures on Chemical Changes in Species of Organic Arsenic in Seafood

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The concentrations of arsenobetaine (AB), tetramethylarsonium ion (TMA^+), and trimethylarsine oxide (TMAO) were determined in samples of sole, dory, hake, and sardine, raw and after being subjected to cooking processes—baking, frying, and grilling—at various temperatures. In all cases, the temperature attained inside the product during the cooking process was measured. The arsenic species extracted from the samples with methanol/water were separated by means of a column switching technique between a PRP-X100 column and a PRP-X200 column. AB was detected by hydride generation atomic absorption spectrometry, whereas TMA^+ and TMAO were detected by hydride generation atomic fluorescence spectrometry. The results obtained showed that, in all of the types of seafood studied, TMA^+ appeared after cooking, possibly because heating facilitates decarboxylation of AB to TMA^+.

Keywords: *Arsenic; organoarsenical species; seafood; cooking; chemical changes*

INTRODUCTION

Seafood products are an important source of proteins, polyunsaturated fatty acids, and a wide range of vitamins (B, D, and A) and minerals (calcium, phosphorus, iron, etc.) (*1*). At the same time, however, they are a source of various microelements that, in certain quantities, may be toxic for human beings (*2*). One of these elements is arsenic, the toxic potential of which is unquestionable. This toxicity depends on the chemical form in which the arsenic is present. Inorganic arsenic [As(III) and As(V)] is the more toxic form. Tetramethylarsonium ion (TMA^+), the most highly methylated species of arsenic, presents considerable lethality, being more toxic than monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (*3*). The trimethylated compounds [arsenocholine (AC), trimethylarsine oxide (TMAO), and arsenobetaine (AB)] are virtually nontoxic (*3*).

Seafood products contain a wide variety of these chemical forms. In most cases, AB is the main species present, although speciation studies in seafood samples have shown the presence of other species of organic arsenic such as DMA, TMA^+, AC, and MMA, in proportions that vary with the type of fish (*4*, *5*). Inorganic arsenic has also been found, although in low quantities (*6*). Studies with molluscs have detected certain kinds of arsenosugars (*7*), the toxicity of which has not been evaluated. The only relevant information available concerns the hydroxylated form of dimethylarsinylribosides (glycerol-ribose), which present a cytotoxicity 0.04% of that of sodium arsenite (*8*).

A very substantial part (72%) of world production of seafood is destined for human consumption (*9*). A considerable proportion of the part destined for consumption is subjected to some kind of cooking treatment. Cooking is a process that, because of the temperatures employed, leads to degradation of nutrients, oxidation of vitamins and lipids, and solubilization of vitamins, minerals, and proteins (*10*). Any compound present in the product, whether it is a macronutrient, a micronutrient, or a contaminant, may be affected by the heat applied, and arsenic is no exception.

The chemical changes in arsenic resulting from high temperatures have not been sufficiently studied. 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 (*11*) observed the transformation of AB into TMAO and TMA^+, DMA into MMA, and MMA into As(III) and As(V). Previous studies carried out in our laboratory (*12*), using a wider range of temperatures (85−190 °C) and various times (15−44 min), revealed the transformation of AB into TMAO at temperatures of 150 °C or above and the transformation of AB into TMA^+ at temperatures of 160 °C or above, following first-order kinetics. Van Elteren and Šlejkovec (*11*) concluded that the transformations they observed could not take place in real samples of seafood because the water content would prevent them from reaching the temperatures required for the transformations to occur. This statement, however, has not been corroborated. Studies are needed, therefore, to confirm whether what was observed in standards also occurs in real samples.

The aim of the present work is to study the chemical changes in species of organic arsenic in seafood products during the heating employed in cooking.

MATERIALS AND METHODS

Reagents. Deionized water (18 MΩ cm) was used for the preparation of reagents and standards. All chemicals were of *pro analysi* quality or better. A commercial standard solution of As(V) (1000 mg L^{-1}) was used (Merck). The stock standard solutions of MMA and DMA (1000 mg L^{-1}) were prepared by dissolving appropriate amounts of commercially available salts in water: MMA (Carlo Erba, Italy) and DMA (Fluka Chemika Biochemika, Madrid, Spain). Similarly, standards supplied by

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10.1021/jf0013297 CCC: $20.00
Published on Web 04/12/2001

Table 1. Contents of Total Arsenic (As), AB, and TMA^+ and Percentages of TMA^+ with Respect to Total Arsenic, in Cooked Products [Contents Expressed as As on Dry Basis (db)]

fish	type of cooking	temp[a] (°C)	cooking time (min)	total As (μg/g, db)	AB (μg/g, db)	TMA^+ (μg/g, db)	TMA^+ (%)
sole	frying	110	16	17.5 ± 0.1	15.4 ± 0.4	1.79 ± 0.02	10.2
dory	frying	100	11	12.5 ± 0.5	12.58 ± 0.02	0.71 ± 0.01	5.7
		110	20	11.97 ± 0.01	10.6 ± 0.2	1.27 ± 0.06	10.6
	grilling	99	5	8.8 ± 0.1	9.4 ± 0.3	0.25 ± 0.01	2.8
		106	8	11.6 ± 0.3	11.0 ± 0.4	0.91 ± 0.03	7.8
		108	7	10.4 ± 0.3	10.2 ± 0.2	0.37 ± 0.01	3.6
	baking	100	12	13.2 ± 0.1	13.6 ± 0.6	0.047 ± 0.002	0.4
		160	25	17.2 ± 0.2	17.4 ± 0.5	0.44 ± 0.02	2.6
hake	baking	94	25	8.8 ± 0.1	8.6 ± 0.2	0.42 ± 0.02	4.8
		100	20	7.73 ± 0.01	7.9 ± 0.1	0.67 ± 0.02	8.7
		120	17	8.3 ± 0.2	7.6 ± 0.3	0.93 ± 0.01	11.2
	grilling	100	12	4.2 ± 0.1	3.97 ± 0.02	0.21 ± 0.01	5.0
		116	12	4.0 ± 0.1	3.82 ± 0.03	0.267 ± 0.003	6.8
		131	12	4.1 ± 0.1	3.5 ± 0.1	0.12 ± 0.01	2.9
sardine	grilling	90	5	10.21 ± 0.01	6.5 ± 0.5	0.470 ± 0.001	4.6
		90	5	8.1 ± 0.1	6.0 ± 0.2	0.377± 0.004	4.7
		120	5	14.6 ± 1.0	13.6 ± 0.4	1.28 ± 0.02	8.8

[a] Maximum temperature attained inside the product.

Hot Chemical Co. (Tokyo, Japan) were used to prepare stock standard solutions of AB, AC, TMAO, and TMA^+.

As prereducing solution for the samples before quantification of total arsenic, a mixture containing 5% m/v KI and 5% m/v ascorbic acid was used. The $NaBH_4$ reducing solution for hydride generation was prepared daily and filtered through Whatman No. 42 paper. All glassware was treated with 10% v/v HNO_3 for 24 h and then rinsed three times with deionized water before being used.

Sample Preparation and Cooking. Samples of various types of seafood were analyzed (dory, hake, sole, and sardine), all purchased in public markets. Once the inedible parts had been removed, the samples were analyzed raw and after being subjected to various kinds of cooking processes. Subsequently, each sample was frozen at −20 °C and freeze-dried 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 mill. The resulting powder was stored in previously decontaminated twist-off flasks and kept in the freezer until analysis.

The cooking treatments selected were frying, baking, and grilling because the temperature applied to the seafood product in these processes generally exceeds 150 °C. The study was carried out with a semi-industrial cooker. Two kinds of oven were used, one heated electrically for cooking the dory and the other heated by gas for cooking the samples of hake. The grill consisted of a (stainless steel) metal surface ∼1 cm thick, heated directly by a gas flame. The frying pan was a stainless steel container ∼5 mm thick, heated directly over a gas flame. The basic differences between the grill and the frying pan were the lesser thickness and smaller surface of the latter. A Lutron model DM-6025C digital clamp meter was used to monitor the temperature during the process. The clamp meter was kept in the piece that was being cooked throughout the cooking process. The temperature inside the ovens and on the surface of the grill and frying pan was also measured.

The treatments employed for each type of seafood (type of cooking, maximum temperature attained by the product, and cooking time) are shown in Table 1 and were those generally used for the consumption of each type of seafood. For dory the same specimen was used for determination of species of arsenic in raw product and after one treatment of cooking. For each treatment a different specimen of dory was used. The size of the specimens of hake made it possible to use the same piece for the determination of the species of arsenic in the raw product and after all cooking treatments. For sole and sardine it was not possible to use the same piece for analysis of the product before and after cooking. For these products, different specimens were used, purchased at the same source and in the same batch to avoid very marked differences between individual specimens as far as possible. In no case were additional ingredients employed. The treatment time varied between the minimum time required to ensure that the product was not uncooked and the maximum time that provided a product suitable for consumption.

Determination of Total Arsenic and Arsenic Species. Total arsenic was quantified in the lyophilized samples by flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS) after a dry-ashing step with a $Mg(NO_3)_2$/MgO mixture (*13*).

The arsenic species were quantified using the method developed by Súñer et al. (*14*). The arsenic species were separated by means of a switching column system connecting two chromatograph columns. As(III), DMA, MMA, As(V), and AB were separated in the PRP-X100 anionic column, thermooxidized, and quantified by HG-AAS. TMA^+, TMAO, and AC were separated in the PRP-X200 cationic column, thermooxidized, and quantified by hydride generation atomic fluorescence spectrometric (HG-AFS). The mobile phase used for elution of the species retained in the PRP-X200 cation-exchange column was 100 mmol L^{-1} ammonium dihydrogen phosphate (Merck), adjusted to pH 4.5 with 100 mmol L^{-1} disodium hydrogen phosphate anhydrous (Merck). For elution of the species retained in the PRP-X100 anion-exchange column, a gradient of 1−20 mmol L^{-1} ammonium dihydrogen phosphate (Merck) of pH 9.3 was used.

Instrumentation. For high-performance liquid chromatographic (HPLC) separation of arsenic species a switching column valve (Rheodyne six-port automated) was used between two columns: a Hamilton PRP-X200 (cation-exchange column, Teknokroma, Barcelona, Spain) and a Hamilton PRP-X100 (anion-exchange column, Teknokroma). Operation of the switching column valve was controlled by the chromatograph software. The two columns were located in a thermostated column compartment in a Hewlett-Packard (HP) model 1100 chromatograph (Hewlett-Packard, Barcelona, Spain), which also comprised the following modules: quaternary pump, on-line degassing system, and automatic injector. The mobile phase for the PRP-X100 column was degassed with the on-line degassing system of an HP model 1050 chromatograph and was unpulsed using the quaternary pump (HP model 79852A) belonging to the chromatograph. A guard cartridge (Hamilton) was used, filled with the same stationary phase as the PRP-X200 column and placed before the PRP-X200 column.

Two detectors were used for quantification; an AFS system (PSA 10.044 Excalibur PS, Analytical) coupled to a PSA 10.004 system (Analytical) to provide hydride generation; and an

atomic absorption spectrometer (AAS) (Perkin-Elmer model 5000, Perkin-Elmer, PE, Norwalk, CT) connected to a flow injection system (PE FIAS-400) to provide hydride generation in continuous flow mode.

For quantification of total arsenic in previously dry-ashed samples, an AAS PE 3300 equipped with a PE AS-90 autosampler and a PE FIAS-400 flow injection system was employed. Other equipment used include a lyophilizer equipped with a microprocessor controlling the lyophilization process (FTS Systems, New York); 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); an Eppendorf 5810 centrifuge (Merck); a Heraeus Biofuge Pico centrifuge (Merck); a mechanical shaker (Rotabit, Selecta, Spain); a Julabo model HC heated bath (Merck); and a model DM-6025C digital clamp meter (Lutron).

RESULTS AND DISCUSSION

The total arsenic contents in the raw products varied with the type of seafood. Sole presented the highest value [47.6 μg g^{-1}, dry basis (db)], followed by dory (9.0−14.1 μg g^{-1} db) and sardine (9.8−13.9 μg g^{-1} db), and finally hake (4.0−9.2 μg g^{-1} db). The AB values represented practically the total quantity of arsenic (sole, 46.3 μg g^{-1} db; dory, 8.8−15.6 μg g^{-1} db; sardine, 6.9−14.1 μg g^{-1} db; hake, 3.6−10.2 μg g^{-1} db).

In the cooked product (Table 1), once again the highest total arsenic content was found in the sample of sole (17.5 g g^{-1} db), although, unlike what happened in the raw product, this value was similar to those presented by the other types of seafood studied (dory, 8.8−17.2 μg g^{-1} db; sardine, 8.1−14.6 μg g^{-1} db; hake, 4.0−8.8 μg g^{-1} db). AB continued to be the major species in the cooked product. In the sample of sole it was 15.4 μg g^{-1} db, in dory it varied between 9.4 and 17.4 μg g^{-1} db, in sardine between 6.0 and 13.6 μg g^{-1} db, and in hake between 3.5 and 8.6 μg g^{-1} db.

Comparison of the total arsenic and AB contents in the raw and cooked products showed that the values were similar except for sole, for which the raw product presented a higher content. This is not surprising if we bear in mind that, as mentioned earlier, the samples of sole analyzed raw and after cooking corresponded to different specimens, subject to variability between individuals, which was very notable in the case of white fish (16.4−196.1 μg g^{-1} db; *15*).

The presence of TMA^+ was not observed in any of the raw seafood samples. However, the analysis of arsenic species present in the cooked products (Table 1) showed the presence of TMA^+ in all of the samples. The results obtained from this study of real samples do not agree completely with what was observed in aqueous solutions of standards (*12*). Transformation of AB standards into TMA^+ was not observed at temperatures below 160 °C; in the present study, however, although the temperatures attained in the samples of seafood were generally much lower (90−130 °C), reaching only 160 °C in one particular case (Table 1), transformations of AB into TMA^+ were observed after the cooking process. A possible explanation is that, although the inside of the fish does not reach this temperature, the part of the surface that is in contact with the heat source may reach much higher temperatures. It must be remembered that the grill, frying pan, and oven were all at temperatures close to 250 °C.

TMAO was not detected in any of the samples, although its presence was to be expected in view of the results obtained with standards, when heating of AB generated both TMAO and TMA^+. Probably the higher temperature of the surface of the product would explain the nonappearance of TMAO after the cooking process. It has been seen that, as the temperature increases, the constant of formation of TMA^+ from AB increases at a greater rate than the constant of formation of TMAO (*12*). It may be that at temperatures close to 250 °C the reaction that predominates is the formation of TMA^+. Moreover, Van Elteren and Šlejkovec (*11*) observed that very long treatments at a particular temperature led to a greater formation of TMA^+ than of TMAO. Probably, the more drastic treatment favors the formation of TMA^+ rather than TMAO.

Table 2. Weight Loss Factor (*F*) Due to the Different Cooking Treatments and Theoretical TMA^+ Concentrations in the Raw Products Due to Weight Losses (TMA^+ Results Expressed as Micrograms per Gram of Arsenic, Wet Basis)

fish	type of cooking	temp (°C)	cooking time (min)	F^a	TMA^+ raw^b
sole	frying	110	16	0.55	0.36
dory	frying	100	11	0.76	0.14
		110	20	0.55	0.34
	grilling	99	5	0.81	0.03
		106	8	0.75	0.18
		108	7	0.77	0.07
	baking	100	12	0.74	0.01
		160	25	0.50	0.10
hake	baking	94	25	0.73	0.09
		100	20	0.70	0.11
		120	17	0.63	0.17
	grilling	100	12	0.80	0.07
		116	12	0.80	0.05
		131	12	0.79	0.02
sardine	grilling	90	5	0.76	0.11
		90	5	0.79	0.13
		120	5	0.75	0.30

[a] *F*, weight loss factor. Ratio of cooked weight (g) to raw weight. [b] The theoretical TMA^+ concentration in the raw product was calculated by multiplying the *F* factor by the TMA^+ content existing in the cooked product.

The results obtained with real samples show that there is no relationship with the kinetic model developed for standards (*12*). Consequently, it is not possible to predict the TMA^+ and TMAO contents produced from AB by applying the kinetic model to real samples of seafood. This lack of correlation may be due to the complexity of the matrix studied, leading a heterogeneous temperature distribution in the product to be homogeneous during the cooking time.

When the changes observed in the cooking process are evaluated, three important phenomena which occur during that process must be borne in mind. Cooking leads to loss of water and soluble and volatile compounds, with consequent loss of weight, concentration of compounds because of this weight loss, and possibly chemical transformation of certain compounds as a result of the heat employed. In view of this combination of factors, TMA^+ contents detected after cooking of the sample might be explained by concentration of the TMA^+ present in the raw product as a result of weight loss. However, weight losses observed in this study after cooking (19−50%) are not sufficient to produce the increases in TMA^+ observed. Table 2 shows the theoretical TMA^+ contents that would be required in the raw product if the TMA^+ observed in the cooked product were due solely to concentration as a result of weight

loss. The theoretical TMA^+ value for the raw product was calculated by applying the weight loss factor (F) to the TMA^+ content (Table 1) present in the corresponding cooked product. It can be seen that in all cases the theoretical TMA^+ in the raw product is above the limit of detection of the methodology [0.0007 $\mu g\ g^{-1}$ wet basis (wb); *14*]. Therefore, if TMA^+ had been present in the raw product, it would have been detected. Because this was not the case and TMA^+ was not detected in any of the raw samples, we can dismiss the possibility that the presence of TMA^+ in the cooked product might be due to concentration of the contents present in the raw product.

The other possible explanation for the appearance of TMA^+ would be generation of this species from AB. However, the phenomena reported before that take place during cooking make it very difficult to balance the masses of the various arsenic species before and after heating, and therefore it is not possible to establish a correlation between variation in AB and increase in TMA^+. It is possible that decarboxylation of AB is the path for the formation of TMA^+ during the cooking process, as has been observed in standards (*12*).

The TMA^+ contents that appeared after the heat treatment applied to all of the real samples of seafood range from 0.05 to 1.79 $\mu g\ g^{-1}$ db, expressed as arsenic. The sample of grilled sole provided the highest TMA^+ content in the entire study (1.79 $\mu g\ g^{-1}$ db), representing 10.2% of the total As quantified in the cooked product.

The dory was cooked by frying, grilling, and baking in an electric oven. The TMA^+ contents in the cooked dory ranged from 0.05 to 1.27 $\mu g\ g^{-1}$ db, expressed as arsenic. Expressed as a percentage of total arsenic in the cooked product, the TMA^+ represents 0.4−10.6%. It can be seen that, for each type of cooking treatment, the percentage of TMA^+ with respect to total arsenic increases with time. For times that are close, the effects of grilling and frying on generation of TMA^+ are similar, whereas baking produces lower levels of TMA^+. In the sample of dory baked for 25 min, with the temperature inside the fish reaching 160 °C, the quantity of TMA^+ generated was 0.44 $\mu g\ g^{-1}$ db. However, in the sample that was fried for a shorter time (20 min), with the temperature inside it reaching 110 °C, the TMA^+ generated was much greater (1.27 $\mu g\ g^{-1}$ db).

In the samples of hake, which were baked in a gas oven and grilled, the TMA^+ contents observed in the samples that were baked (0.42−0.93 $\mu g\ g^{-1}$ db) were higher than those of the samples that were grilled (0.12−0.27 $\mu g\ g^{-1}$ db). The TMA^+ contents, expressed as a percentage of total arsenic, gave values of 4.8−11.2% in baked hake and 2.9−6.8% in grilled hake. However, it must be pointed out that the concentrations of AB in the raw samples that were baked were twice as high as the concentrations of AB in the raw samples that were grilled. Therefore, the levels of AB in the raw product have a considerable effect on the level of the TMA^+ contents quantified in the cooked product.

Baking produced higher concentrations of TMA^+ in the samples of hake (0.42−0.93 $\mu g\ g^{-1}$ db) than in the samples of dory (0.05−0.44 $\mu g\ g^{-1}$ db). These differences cannot be attributed to the type of sample, given that the samples of hake were thicker than the samples of dory, which is a flatfish, so that it seems reasonable to suppose that there would be a less uniform distribution of heat in the hake and therefore less transformation of AB into TMA^+. Therefore, a difference in the heating capabilities of the two ovens employed, gas and electric, for cooking the hake and dory, respectively, must be considered. Inside the oven, transmission of heat to the product takes place by conduction, due to contact between the product and the baking pan, and by convection as the heated air circulates within the oven (*10*). The samples of hake baked in the gas oven were placed on the baking pan, whereas the samples of dory baked in the electric oven were placed on a grid, which, unlike the baking pan, was a noncontinuous surface. The significance of the conductive process in the surface on which the sample was placed in each oven might be different. The baking pan in the gas oven used for the hake may have reached a higher temperature, and the heat may have been transmitted to a greater surface area of the seafood than in the case of the grid on which the dory was placed in the electric oven. The higher temperature possibly attained on the surface of the hake samples may have caused a greater transformation of AB into TMA^+ in the gas oven.

Finally, with the sardines only one cooking process, grilling, was applied during 5 min. The temperatures reached inside the products were 90 and 120 °C. The TMA^+ contents ranged from 0.38 to 1.28 $\mu g\ g^{-1}$ db, representing 4.6% (90 °C) to 8.8% (120 °C) of the total arsenic in the cooked product.

In view of these results we can conclude that various factors affect the quantity of TMA^+ formed during cooking: the type of seafood, the time employed, the kind of cooking treatment, and the original AB content. In the present work, it was not possible to establish whether one of these factors had a greater effect than the others. That will require a more extensive study.

It is also important to emphasize that the cooking treatments were performed without the addition of any other ingredient. In domestic or industrial cooking, additional ingredients such as oil or various types of sauce or broth are frequently used. The transformations observed in this study might vary in the presence of such additional ingredients, and therefore, a study of this aspect would be of very great interest.

CONCLUSIONS

The fact that AB may undergo changes during heating, being transformed into another, more toxic species of arsenic (TMA^+), reinforces the need to carry out studies on products cooked in the same conditions as those applied when they are consumed in order to evaluate the intake of different species of arsenic and the toxicological implications.

ACKNOWLEDGMENT

We are grateful to Sergio Algora, Victoria Benito, María Jesús Clemente, and Esther López for assistance in the performance of analytical work.

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Received for review November 7, 2000. Revised manuscript received February 16, 2001. Accepted February 16, 2001. V.D. and M.A.S. received Spanish Research Personnel Training Grants from the Generalitat Valenciana (Consellería de Cultura, Educació i Ciència) and the Ministerio de Educación y Cultura, respectively.

JF0013297