Kinetic Study of Transformations of Arsenic Species during Heat Treatment

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The combination of temperatures and pH levels applied in domestic or industrial cooking and in the sterilization of seafood might cause the transformation of certain species of arsenic into other more toxic species, which could pose a risk to the consumer. To clarify the effect of the temperatures traditionally used in cooking or sterilization on the stability of the various species of arsenic, a kinetic study was carried out, using standards of arsenobetaine (AB), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), trimethylarsine oxide (TMAO), tetramethylarsonium ion (TMA^+), and arsenocholine (AC) heated at different temperatures (85−190 °C) and for different treatment times. Various pH levels (4.5, 5.5, 6.5, and 8.0) were applied during the heating process. The results obtained indicated that there were no transformations of arsenic species after temperature treatments up to 120 °C. However, when temperatures between 150 and 190 °C were used, a partial decomposition of AB was achieved, producing TMAO at 150 °C and TMAO and TMA^+ at temperatures of 160 °C or above, in proportions that varied according to the temperature and duration of the heat treatment.

Keywords: *Arsenic; arsenic species; heat treatment; kinetic study*

INTRODUCTION

In recent decades the study of toxic elements has experienced a spectacular advance in terms of analytical techniques and also environmental, physiological, and toxicological aspects. In the case of arsenic, its ubiquity and toxicity have led it to be very widely studied. The different chemical forms of arsenic and their different degrees of toxicity make the determination of arsenic species necessary as a basis on which to establish the possible toxicological implications of the arsenic contents of a product.

Traditionally, speciation studies of As have concentrated on matrices of marine origin because the concentrations of arsenic and the variety of chemical species are much greater in those samples than in organisms that live on the land (*1*). Studies carried out in seafood products have detected the presence of inorganic arsenic [As(III) and As(V)] (*2*); monomethylarsonic acid (MMA), $CH_3AsO_3^{2-}$, and dimethylarsinic acid (DMA), $(CH_3)_2AsO_2^-$ (*3*); trimethylarsine oxide (TMAO), $(CH_3)_3AsO$ (*4*); tetramethylarsonium ion (TMA^+), $(CH_3)_4As^+$ (*5*); arsenocholine (AC), $(CH_3)_3As^+CH_2CH_2OH$ (*5*), and arsenobetaine (AB), $(CH_3)_3As^+CH_2COO^-$ (*6*, *7*). Arsenosugars and arsenolipids are also present. The toxicity of these chemical forms varies, ranging from extremely toxic species to harmless species. Inorganic arsenic [As(III) and As(V)] is the more toxic form. TMA^+, the most highly methylated species of arsenic, presents considerable lethality, being more toxic than MMA and DMA (*8*). The trimethylated compounds (AC, TMAO, and AB) are virtually nontoxic (*8*).

In most cases, seafood products are subjected to heat treatment prior to consumption: cooking (microwave, baking, frying, etc.) or conservation processes (cooking, canning, pasteurizing, sterilizing, etc.). High temperatures are used, either to increase the palatability of the product or to extend its shelf life by conservation processes. During thermal processing many constituents of the product may undergo changes, with the application of heat enhancing loss of water and other soluble constituents such as proteins and vitamins (*9*). However, not only does heat treatment cause solubilization phenomena, but for amines, it is responsible for their transformation into new compounds. It has been seen that the application of heat treatment to fish and shellfish brings about transformation of trimethylamine oxide to trimethylamine and dimethylamine, a transformation that depends on the treatment time and type of organism (*10*). Because the many arsenic species present in seafood products are trimethylated and hence similar in structure to trimethylamine oxide, they might undergo similar transformation processes.

Given the toxicological implications arising from the presence of particular species of arsenic in seafood products, it would be of great interest to obtain information about the possible transformations of arsenic species during the treatment of seafood products prior to consumption (cooking or conservation processes). In this connection, the only prior reference in the literature on the effect of high temperatures on arsenic species is the study carried out by Van Elteren and Šlejkovec (*11*) on standards. The study showed that dry heating (heating in the oven without liquid) at temperatures of 160 °C for long periods brought about the decomposition of certain species of arsenic. Specifically, AB was partially degraded into TMAO and/or TMA^+, DMA into MMA, and MMA into As(V) and As(III). The study in question was very limited, with only two temperatures being used: 100 °C for 100 min and 160 °C for application times of 30 min and 24 h. Moreover, the authors studied

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only the effect of the treatments indicated on AB, DMA, MMA, and TMA^+, without considering other species found in seafood products such as TMAO and AC.

Seafood products consumed by man may have pH levels that differ from those found in the live organism (pH ~7). Consequently, pH is a factor that might affect transformation of arsenic species. During rigor mortis, the pH of the fish decreases to 6.5−5.8 and in some cases may even reach values as low as 5 (*12*). During post-mortem, the decomposition of nitrogenated compounds leads to an increase in pH. This change depends to a large extent on the temperature at which the fish is kept and may reach alkaline values (8−8.5) if it has not been gutted (*13*). On the other hand, when the fish is subjected to processing in the can, the accompanying liquids may give the fish a neutral or slightly acid pH (5.8−7), in the case of oils or natural juices, or an acid pH (4.5−5.8), in the case of some kind of sauce (*14*). These variations in pH might lead to variations in the chemical forms of arsenic.

In the present work, a study was made of the kinetics of the transformation of standards of the organic arsenic species customarily found in seafood products (AB, DMA, MMA, TMAO, TMA^+, and AC) when subjected to temperatures typical of the cooking and industrial processing of these products. The effect of pH was also studied.

MATERIALS AND METHODS

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.

An atomic fluorescence spectrometric (AFS) system (PSA 10.044 Excalibur PS, Analytical) equipped with a boosted-discharge hollow cathode lamp (BDHCL, Photron Super Lamp, Victoria, Australia) was employed for TMA^+, TMAO, and AC quantification. Before this, a PSA 10.004 system (Analytical) with peristaltic pump and gas−liquid separator was used to provide hydride generation. The arsines generated were conveyed to the AFS detector by means of a semipermeable membrane dryer tube (Perma Pure).

A Perkin-Elmer model 5000 atomic absorption spectrometer (AAS) (Perkin-Elmer, PE, Norwalk, CT) was employed for As(III), As(V), MMA, DMA, and AB quantification. The spectrometer was connected to a flow injection system (PE FIAS-400) to provide hydride generation in continuous flow mode. An electrically heated quartz cell was employed. A Hewlett-Packard model 35900 C digital analogical converter with two channels was used to acquire the signals of the AFS and the AAS, which were processed by means of the chromatograph software.

Reagents. Deionized water (18 MΩ cm) was used for preparation of the 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 Hot Chemical Co. (Tokyo, Japan) were used to prepare stock standard solutions of AB, AC, TMAO, and TMA^+.

All glassware was treated with 10% v/v HNO_3 for 24 h and then rinsed three times with deionized water before being used.

Heat Treatment of Standards. Standards of AB, DMA, MMA, TMAO, TMA^+, and AC were subjected to heat treatment in various conditions of temperature, time, and pH. Standards (100 μL) of 10 μg of As mL^{-1} of each arsenic species were placed in ring-marked microhematocrite capillary tubes and inserted in a silicone oil bath. After heat treatment, the capillary tubes were cooled in an ice bath and their contents transferred to chromatograph vials for subsequent analysis. For the study of the effect of pH, the aqueous standards were prepared in citrate buffer of various pH values, prepared from appropiate amounts of citric acid and sodium citrate.

The purity of standard of each arsenical species was verified before it was subjected to heat treatment. This was done by injecting the standard of the arsenical species being studied each time that a heat treatment was applied.

To learn the effect of the combination of time and temperature on the stability of standards of arsenic species, the study was divided into two parts differentiated by the range of temperatures used in each part. In the first stage of the study, temperatures of 85, 90, 95, and 100 °C were applied for different times (15, 22, 30, 37, and 44 min) to simulate industrial and domestic cooking processes. A treatment of 120 °C for 44 min was also assayed, with a view to simulating the sterilization conditions to which canned seafood products are subjected. The effect of pH was studied in this temperature range to simulate, as far as possible, the pH values present in products subjected to heat treatment processes for conservation. Four different pH values (4.5, 5.5, 6.5, and 8.0) were selected, and the most severe heat treatment (120 °C, 44 min) at these four pH levels was employed.

In the second part of the study, the temperatures applied ranged from 150 to 180 °C. In all cases the application time was 44 min.

Determination of Arsenic Species. The arsenic species were quantified using a method developed in our laboratory (*15*). The arsenic species were separated by means of a switching column system connecting two chromatograph columns. The switching valve was initially set whereby the columns were not directly connected, so that they could be conditioned. After conditioning, this position was maintained and sample was injected into the PRP-X200 column. One minute after injection of sample, the valve moved to enable direct connection between the two columns, and this position was maintained until 4 min had elapsed, while the species that eluted in the void volume of the PRP-X200 column were transferred to the PRP-X100 column. The valve was then moved back, so that each column could be eluted with different mobile phases and the species retained in each of them could be separated.

As(III), DMA, MMA, As(V), and AB were separated on the PRP-X100 anionic column, thermo-oxidized, and quantified by HG-AAS. TMA^+, TMAO, and AC were separated on the PRP-X200 cationic column, thermo-oxidized, and quantified by 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.

RESULTS AND DISCUSSION

Effect of Temperature and pH on Standards of Organic Arsenic Species. For temperatures from 85 to 120 °C, transformations of arsenic species were not

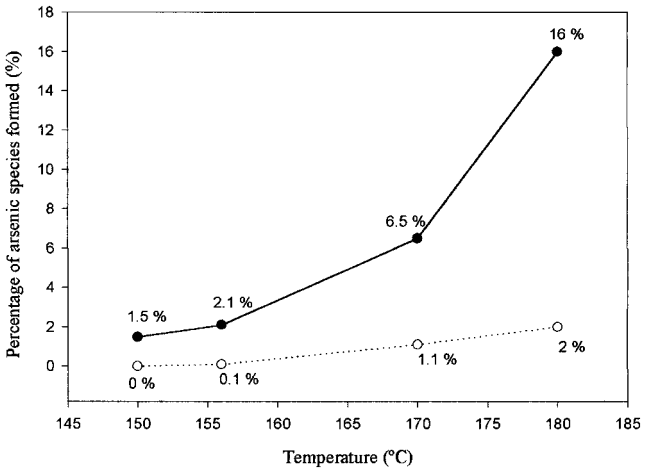

Figure 1. Percentage of transformation of AB into TMAO (●) and TMA^+ (○) at different temperatures. Time of application at each temperature was 44 min.

observed in any of the working conditions described. It is to be expected, therefore, that at the temperatures normally employed in cooking or processing, such as boiling or sterilizing, the arsenic species would remain unaltered. The pH levels tested at a temperature of 120 °C also did not affect the chemical form in which arsenic was present.

At higher temperatures (150−180 °C), AB was transformed at all of the temperatures studied, generating TMAO and/or TMA^+, and the percentage of transformation increased with temperature (Figure 1). These results follow the same pattern of degradation as that observed previously by Van Elteren and Šlejkovec (*11*), who applied temperatures of 160 °C for 30 min and observed degradations of AB into TMAO (10%). When the heating time at 160 °C was extended to 24 h, 80% of the AB was degraded, generating TMAO (11% of the AB treated) and TMA^+ (68% of the AB treated).

There was no transformation of the other species, with the exception of AC. At temperatures of 150−180 °C, there was a slight transformation (<1.1%) of AC into TMAO, whereas a small coversion (0.1−0.2%) to DMA was seen at temperatures of 170 °C or more. For MMA and DMA, the degradations observed by Van Elteren and Šlejkovec (*11*) do not agree with those found in the present study. They observed degradation of MMA to As(III) and DMA to MMA, whereas in our experiments no appreciable transformation of these arsenic species was seen.

Because AB is the only species that undergoes substantial transformations at temperatures >150 °C, the kinetic study concentrated on this species of arsenic. To establish the kinetic model of the transformation, the thermal study was completed by using four temperatures (160, 170, 180, and 190 °C) and four times for each temperature (15, 25, 35, and 44 min).

Table 1 shows the concentrations of TMAO and TMA^+, expressed as arsenic, obtained after the various temperature−time treatments were applied to standards of 10 μg L^{-1} of AB. It can be seen that in all of the treatments AB was transformed into both TMAO and TMA^+, and for all of the combinations tested the concentration of TMAO generated was greater than that of TMA^+. It can also be seen that an increase in temperature leads to an increase in the transformation of AB, and at a given temperature an increase in

Table 1. Concentrations of TMAO and TMA^+, Expressed as Arsenic, Obtained after Applying the Various Temperature−Time Treatments to Standards of 10 μg mL^{-1} of AB

temp (°C)	time (min)	TMAO (μg mL^{-1})	TMA^+ (μg mL^{-1})
160	15	0.10	0.01
	25	0.18	0.02
	35	0.25	0.02
	44	0.30	0.03
170	15	0.18	0.03
	25	0.30	0.05
	35	0.40	0.07
	44	0.65	0.11
180	15	0.51	0.16
	25	0.77	0.23
	35	1.03	0.33
	44	1.18	0.37
190	15	0.68	0.39
	25	1.28	0.70
	35	1.41	0.73
	44	2.02	1.09

treatment time produces an increase in the concentrations of both TMAO and TMA^+.

These experimental results seem to indicate that the thermal transformation takes place as a result of two competing reactions, with AB being the initial substrate for both: a process of decarboxylation generating TMA^+, or cleavage of the $As-CH_2$ bond with subsequent oxidation giving rise to TMAO. Generation of TMA^+ by decarboxylation of AB is a possibility that was indicated by Francesconi et al. (*16*) as a hypothesis to explain the presence of this species in natural seafood products. Consequently, in addition to a mechanism of decarboxylation by natural causes, an alternative thermal mechanism for the generation of this tetramethylated species must be considered. The possibility of a process of transformation of AB in two consecutive stages, with generation of intermediate species, can be dismissed. The treatments applied to TMA^+ showed that there was no thermal degradation of this species. On the other hand, the TMA^+ cannot be produced by transformation of TMAO because methyl group donors do not exist in the aqueous medium. On the basis of these premises, the reactions governing the process of the degradation of AB would be as follows:

$$\mathrm{AB} \xrightarrow{K_1} \mathrm{TMAO}$$

$$\mathrm{AB} \xrightarrow{K_2} \mathrm{TMA}^+$$

The kinetic equation in this case would be given by

$$\ln([\mathrm{AB}]/[\mathrm{AB}]_0) = -(k_1 + k_2)t \qquad (1)$$

where [AB] is the concentration of AB at time t, $[AB]_0$ is the initial concentration of AB, k_1 is the kinetic constant of transformation of AB into TMAO, and k_2 is the kinetic constant of transformation of AB into TMA^+.

Previous studies have stated that AB standards are transformed only in TMAO and TMA^+. For this reason, the concentration of AB at time t is obtained from the difference between the initial concentration of AB and the concentrations of TMAO and TMA^+ formed at time t. The plot of $\ln([AB]/[AB]_0)$ against time was fitted to a straight line for all of the temperatures studied (Figure 2), which shows that the transformation of AB into TMAO and TMA^+ follows a first-order kinetic. For each

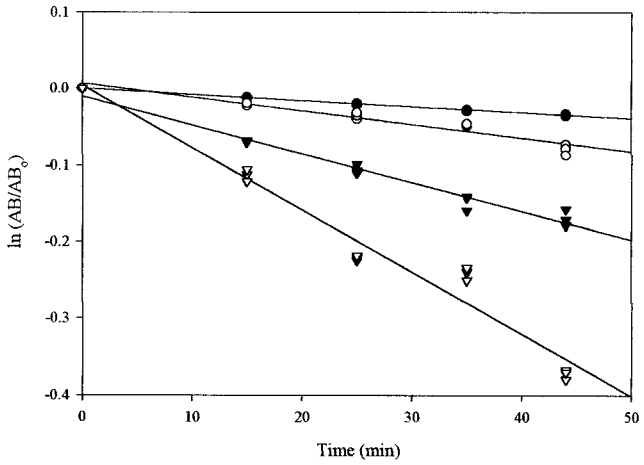

Figure 2. Variation in AB concentration plotted against time at different temperatures [(●) 160 °C; (○) 170 °C; (▼) 180 °C; (▽) 190 °C] and regression lines (—).

Table 2. Kinetic Parameters of Degradation of AB

temp (°C)	k_1 (min^{-1})	k_2 (min^{-1})
160	7.2×10^{-4}	7.6×10^{-5}
170	1.6×10^{-3}	2.8×10^{-4}
180	2.7×10^{-3}	8.3×10^{-4}
190	5.3×10^{-3}	2.9×10^{-3}

temperature the sum of the constants ($k_1 + k_2$), which govern the two reactions of AB transformation, is given by the slope of the corresponding line.

The concentrations of the products, TMAO and TMA^+, with time are expressed by eqs 2 and 3. By division of these equations, a relationship between the two individual constants in the process can be obtained (eq 4)

$$[\text{TMAO}] = (k_1[\text{AB}]_0/k_1 + k_2)(1 - e^{-(k_1+k_2)t}) \quad (2)$$

$$[\text{TMA}^+] = (k_2[\text{AB}]_0/k_1 + k_2)(1 - e^{-(k_1+k_2)t}) \quad (3)$$

$$[\text{TMAO}]/[\text{TMA}^+] = k_1/k_2 \quad (4)$$

where [TMAO] is the concentration of TMAO formed from AB at a given time and [TMA^+] is the concentration of TMA^+ formed from AB at a given time. Using the experimental data that appear in Table 1, for a given temperature it is possible to find a mean value of the quotient of the concentration between the two species of arsenic from the individual values obtained for each time.

From eqs 1 and 4 it is possible to calculate the values of each of the kinetic constants. The values obtained (Table 2) show that at all of the temperatures studied the transformation of AB into TMAO takes place more rapidly than the transformation of AB into TMA^+. At temperatures of 190 °C the difference in the formation of the two products is not as marked as at lower temperatures, at which the difference between the two constants is of an order of magnitude.

For each temperature, time, and initial concentration of AB, the values of the constants obtained experimentally make it possible to establish both the concentration of AB not transformed (eq 1) and the concentrations of TMAO (eq 2) and TMA^+ (eq 3) generated.

The activation energy of the reactions is given by the equation

$$k = A\, e^{-E_a/RT} \quad (5)$$

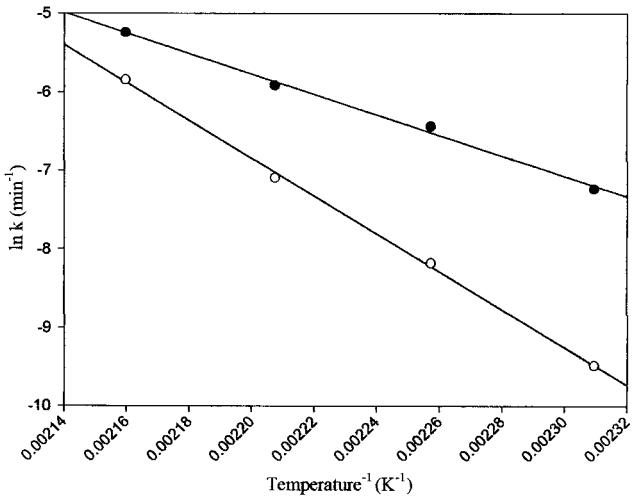

Figure 3. Kinetic constants of transformation of AB plotted against inverse of absolute temperature: (●) reaction 1, AB → TMAO; (○) reaction 2, AB → TMA^+.

where A (pre-exponential factor) and E_a (Arrhenius activation energy) are constants characteristic of each reaction and R is the gas constant (8.3145 J mol^{-1} K^{-1}). By applying logarithms, we have

$$\ln k = \ln A - E_a/RT \quad (6)$$

By plotting ln k against $1/T$ for each arsenic species (Figure 3) we can obtain the activation energy values for the two AB transformation reactions: E_{a1}(TMAO) = 108.6 kJ mol^{-1}; $E_{a2}(TMA^+)$ = 200.2 kJ mol^{-1}. These values show that, for the temperature range studied, the TMAO generation reaction is much faster than the TMA^+ generation reaction.

CONCLUSIONS

The transformations of the various species of arsenic observed in aqueous standards showed the appearance of species formed from AB but having a greater degree of toxicity (TMA^+ and TMAO). A study of the transformations of the aqueous standards made it possible to prove the existence of these processes, characterize them in terms of the kinetic parameters that govern them, and predict the concentrations of TMAO and TMA^+ generated from AB if the time−temperature conditions tested are maintained. Extrapolation of these transformations and their kinetic models to real samples of seafood or any other kind of food should be approached with caution because the temperature distribution in matrices of this complexity is not uniform, and it is also possible that the distribution of the various species of arsenic may not be uniform. There could also be compounds in the matrix that enhance or impede transformations. Further studies are therefore necessary to corroborate whether what was observed in standards also occurs in real samples.

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