A new method was developed for total arsenic determination in seafood products such as oysters, mussels, tuna fish, and algae. Matrix decomposition and oxidation to arsenate of all the arsenic compounds in the product were completed in 25 min by using a 3-step program of focused microwaves (40–120 W) with nitric and sulfuric acids. Quantitation was performed by hydride generation–atomic fluorescence detection (HG–AFS). Results of method optimization are presented and discussed. A detection limit <125 \( \mu \text{g/kg} \) arsenic was obtained; the quantitation limit was close to 400 \( \mu \text{g/kg} \), with repeatability and reproducibility <5\% relative standard deviation. Validation was performed by analyzing 4 Reference Materials (arsenic concentration expressed as mg/kg): The National Institute of Standards and Technology SRM 1566a Oyster tissue (14.0 ± 1.2); the Bureau Community of Reference (now Standard Measurements & Testing Program) CRM 278 Mussel tissue (5.9 ± 0.2) and CRM 627 Tuna fish (4.8 ± 0.3); and the International Atomic Energy Agency RM 140 Fucus sample (44.3 ± 2.1).
Reference (BCR) CRM 278 Mussel tissue and CRM 627 Tuna fish; and the International Atomic Energy Agency (IAEA) RM 140 Fucus sample. Finally, we assessed the applicability of the method to fresh oyster samples, without lyophilization, which is of interest in control and routine analysis.

Experimental

Apparatus

(a) **Hydride generator.**—P.S. Analytical (Kent, UK), Model 10.004.

(b) **Atomic fluorescence detector.**—P.S. Analytical Model Excalibur, equipped with a diffusion flame, an arsenic boosted hollow cathode lamp, and a Perma pure drying membrane (Perma Pure Products, Farmingdale, NJ).

(c) **Data acquisition.**—Performed with a microcomputer by using commercial software (Avalon 2.0) from P.S. Analytical.

(d) **Microwave digestor.**—Prolabo A301 (Paris, France) equipped with a TX32 programmer.

(e) **Freeze dryer.**—Telstar (Barcelona, Spain) lyophilization system.

Reagents

(a) **Potassium iodide.**—KI Suprapur (Merck, Darmstadt, Germany).

(b) **Ascorbic acid.**—Merck Pro-analysi l(+)-ascorbic acid.

(c) **Sodium borohydride.**—97% NaBH₄ purum (Fluka, Buchs, Switzerland).

(d) **Sodium hydroxide.**—NaOH Suprapur (Merck).

Solutions

All solutions were prepared with doubly deionized water (USF Purelab Plus, Ransbach Baumbach, Germany) 18.2 MΩ cm resistivity.

(a) **Sodium borohydride 1.5%.**—15 g NaBH₄ dissolved in 1 L 0.4% NaOH; the resulting, slightly turbid mixture, was filtered through a 0.45 μm nylon membrane.

(b) **Hydrochloric acid 2M.**—37% fuming HCl Pro-Analysis (Merck).

(c) **Sample digestion reagents.**—Nitric acid (70% HNO₃), hydrochloric acid (36.5–38.0% HCl), and perchloric acid (69–72% HClO₄) from Baker Instra-Analyzed (J.T. Baker, Deventer, The Netherlands); hydrogen peroxide (31% H₂O₂) VLSI Selectipur, and sulfuric acid (96% H₂SO₄) Suprapur, from Merck.

Table 1. Microwave digestion program for total arsenic determination

<table>
<thead>
<tr>
<th>Step</th>
<th>HNO₃ volume, mL</th>
<th>H₂SO₄ volume, mL</th>
<th>Time, min</th>
<th>Power, W</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>—</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>—</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>5</td>
<td>10</td>
<td>120</td>
</tr>
</tbody>
</table>

Test Sample

**Oysters.**—From Galicia (Northwest Spain) purchased in a local market.

Certified Reference Materials

(a) **SRM 1566a.**—NIST Oyster tissue.

(b) **CRM 278.**—BCR Mussel tissue.

(c) **CRM 627.**—BCR Tuna fish.

(d) **RM 140.**—IAEA Fucus sample.

Standards

(a) **Arsenite.**—1000 mg/L arsenite stock solution prepared from As₂O₅, 99.9926 ± 0.0030 (wt, %), NIST Oxidimetric Primary Standard 83rd, and dissolved in 4 g/L NaOH.

(b) **Arsenobetaine.**—AsB [(CH₃)₃As⁺CH₂COO⁻] supplied by the Service Central d’Analyse, CNRS (Vernaison, France).

Preparation of Oyster Tissue Test Sample

After the shell was removed, the test sample was mechanically crushed to a homogeneous mass. It was then freeze-dried (at −40°C under vacuum pressure, 10.13 Pa) for 48 h, ground to a fine powder, and collected in polyethylene flasks. The final material was stored at −20°C until analysis.

Moisture Determination

A test portion of 500 mg of the test sample was dried in an oven at 70°C for 24 h.

Table 2. Experimental conditions for HG–AFS measurements

|                | Hydride generation |                
|----------------|-------------------|----------------
| Acid           | 2M HCl at 8.0 mL/min |                
| Reductant      | 1.5% (w/v) NaBH₄ in 0.4% (w/v) NaOH at 3.0 mL/min |                
| Sample         | 8.0 mL/min         |                
| Argon flow     | 300 mL/min         |                
| Air flow       | 2.5 L/min          |                
| Injection program |                   |                
| Delay time     | 10 s               |                
| Rise time      | 20 s               |                
| Measure time   | 20 s               |                
| Memory time    | 20 s               |                
| Atomic fluorescence detector | |                
| Lamp primary energy | 27.5 mA |                
| Lamp boost energy | 35.0 mA |                
| Measuring wavelength | 193.7 nm |                
| Signal processing | Peak height |                

Table 1. Microwave digestion program for total arsenic determination
Procedure for Arsenic Determination

A 500 mg test portion of the lyophilized material was placed in an open reflux vessel to which focused microwaves were applied according to the steps shown in Table 1. The digestion program was adjusted by addition of concentrated nitric and sulfuric acids. The resulting solution (ca 5 mL) was diluted to 25 mL with doubly deionized water. A 1 mL volume of this solution was diluted to 25 mL in a solution containing 2M HCl, 1% (w/v) KI, and 0.2% (w/v) ascorbic acid. The solution was maintained at room temperature for 60 min, and arsenic content in the acidic solution was measured by HG–AFS according to conditions listed in Table 2. Quantitation was performed by using external curve derived from arsenite standard solutions, obtained by appropriate dilution with 2M HCl, 1% (w/v) KI, and 0.2% (w/v) ascorbic acid. The final results were reported on dry mass basis. For fresh materials, the same procedure was followed, except 2–3 g of the test portion was used.

Results and Discussion

Optimization of AsB Digestion

A previous study showed that after digestion of mussel tissue with nitric acid solution, the test portion matrix was completely dissolved (16). The same digestion system was applied to oyster tissue, tuna fish tissue, and algae, with total dissolution of all samples. Not only was the matrix completely dissolved, but also total oxidation of arsenic species to arsenate was observed. As mentioned above, AsB is the most resistant compound against oxidizing agents. Therefore, the assessment of total AsB digestion was crucial for establishment of the new methodology.

Several reagents were tested to achieve complete decomposition of AsB to arsenate. A 5 mL amount of aqueous solution containing 1.4 mg/L (expressed as arsenic) of standard of AsB was placed in the digestion vessel and the digestion program shown in Table 3 was applied. Sulfuric acid gave the best recovery of AsB.

Optimization of Microwave Power

Several assays were performed to optimize the power applied in the microwave oven in the third step. A 5 mL volume of a solution containing 1.4 mg/L of arsenic as AsB was assayed in the third step. Powers from 80 to 160 W were assayed in the third step. Results are reported in Table 4. Poor signal was observed when power <120 W was used, whereas quantitative recovery was achieved at higher power. Therefore, 120 W was chosen as the optimal power.

Analytical Performance of the New Method

Quality parameters of the proposed method were calculated after test portions of the lyophilized oyster tissue were analyzed. The linear range, obtained by analyzing several solutions containing arsenic at various concentrations, was wider than 100 mg/kg arsenic.

Detection and quantitation limits were calculated by analyzing in triplicate, several solutions containing arsenic at var-

Table 3. Recovery of AsB after assay of 3-step digestion methods

<table>
<thead>
<tr>
<th>Digestion steps</th>
<th>HNO₃, mL</th>
<th>Reagent, 5 mL</th>
<th>Time, min</th>
<th>Power, W</th>
<th>AsB recovery, % a (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>—</td>
<td>5</td>
<td>40</td>
<td>7.16 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>HCl</td>
<td>10</td>
<td>70</td>
<td>4.35 ± 0.08</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>H₂O₂</td>
<td>10</td>
<td>70</td>
<td>0.72 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>HClO₄</td>
<td>10</td>
<td>100</td>
<td>99.71 ± 0.87</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>H₂SO₄</td>
<td>10</td>
<td>160</td>
<td>99.71 ± 0.87</td>
</tr>
</tbody>
</table>

a Arsenic concentration: 1.4 mg/L as AsB.

Table 4. Optimization of microwave power in step 3 of digestion program (see Table 1)

<table>
<thead>
<tr>
<th>Third step power, W</th>
<th>Arsenic concentration, mg/kg</th>
<th>Recovery, % a</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>0.015</td>
<td>0.013</td>
</tr>
<tr>
<td>100</td>
<td>0.012</td>
<td>0.013</td>
</tr>
<tr>
<td>120</td>
<td>1.45</td>
<td>1.42</td>
</tr>
<tr>
<td>140</td>
<td>1.43</td>
<td>1.44</td>
</tr>
<tr>
<td>160</td>
<td>1.41</td>
<td>1.38</td>
</tr>
</tbody>
</table>

a Arsenic concentration: 1.4 mg/L as AsB.
ious concentrations. The regression line was calculated from the mean values of each working session, were <5% RSD. Results, expressed as the RSD obtained in the analysis of a test portion in 5 nonconsecutive days (3 replicates each day). Results, expressed as the RSD from 10 replicates of the arsenic determination in a test sample of this material (Table 6). Results were in good agreement with values reported for the Certified Reference Materials. Moreover, the standard deviations obtained showed good agreement between the measured values and the certified values was good. More-over, the standard deviations obtained showed good agreement with values reported for the Certified Reference Materials.

**Validation of the Proposed Method**

To validate the method, 4 Certified Reference Materials were analyzed. The determinations were made in triplicate every working session, and on 2 working days, to calculate reproducibility of the method (Table 5). Correlation between the measured values and the certified values was good. Moreover, the standard deviations obtained showed good agreement with values reported for the Certified Reference Materials.

**Application of the Method to Fresh and Dried Oyster Tissues**

Because fresh test samples are the most common materials in the laboratories for control and routine analysis, we studied the applicability of the method to fresh test samples. Several portions of oyster tissue from Northwest Spain were digested and tested after different drying levels: a fresh test sample; a test sample dried at 40°C for 24 h; and a test sample dried at 70°C for 24 h. The results obtained after digestion and quantification were compared with that obtained for a freeze-dried test sample of this material (Table 6). Results were in good agreement. Therefore, the method can be applied directly to samples without freeze-drying, which can be very time consuming and hard to undertake in a control analysis laboratory. The amount of test sample, however, should be increased for fresh test samples, because the moisture, especially in bivalves, can reach very high values (>90%).

### Conclusions

A new method was developed and optimized for determination of total arsenic in seafood products. The microwave-assisted digestion program allows total digestion of the test sample in 25 min, which is faster and cleaner than other processes described in the literature. HG–AFS provides high sensitivity and quick performance, allowing measurements every 70 s. Good results were obtained with fresh oyster test samples, which dramatically shortened the analysis time. Therefore, the method proposed is suitable for control and routine analysis. Moreover, the digestion method can be easily adapted to other spectrometric detection techniques, such as HG–AAS, HG–ICP/ICP/MS.

### Acknowledgments

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### References