The performed research is truly vital, as As (arsenic) concentration in food products is now of great interest. The US ATSDR and EPA enlist As among the most toxic substances which are dangerous for human health.

We suggest a procedure for separate quantitative mass fraction determination for organic (oAs) and non-organic (iAs) arsenic compounds in sea products with solid phase extraction (SPE) application combined with atomic adsorption spectrometry. Samples were prepared according to the following procedure: liquid extraction phase with simultaneous As (III) oxidation into As (IV) with hydrogen peroxide and As (V) extraction into a 0.055 M liquid phase with hydrochloric acid. Arsenic organic and non-organic compounds were separated via solid phase extraction with Strata SAX cartridges (Sorbent Lot Number: S208-0058). To quantitatively assess the obtained samples, we applied atomic-adsorption techniques for As determination with "KVANT-2A-GRG" spectrometer according to the State Standard 51766-2001. We revealed that common As concentration didn’t conform to fixed standards in 8 out of 17 analyzed samples (2 shrimps, 1 crab, 1 fish, and 4 seaweeds). However, iAs concentration was significantly lower than oAs concentration in all the samples. 6 out of 17 analyzed samples didn’t contain any iAs within detection limits (0.1 mg/kg), and apparently all the As concentration occurred due to its organic compounds. The suggested procedure for separate oAs and iAs detection is relatively simple in terms of devices applied in it, and quite cheap, as SPE cartridges needed to perform it can be re-used after re-conditioning. This procedure, after a proper metrological validation, can be implemented in most laboratories which are certified to examine chemical safety of food products.

Key words: arsenic, non-organic form, solid phase extraction, atomic-adsorption spectrometry, risk assessment.

Arsenic (As) is a chemical element of the fourth period 15th group in the periodic system. The electron shell structure: Ar3d104s24p3. According to the materials presented by A. Gomez-Caminero et al. [1], As is a metalloid found in the structure of rocks, soil and groundwater in an average concentration of 2 mg/kg in inorganic and organic forms. The major sources of environment anthropogenic pollution with
As are the extraction and burning of fossil fuels, using arsenic pesticides in agriculture, disposal of chemical weapons stockpiles, and using wood preservatives containing As. In most cases, human exposure to inorganic As (iAs) occurs through contaminated groundwater (drinking water, water used for cooking, and for irrigation of agricultural land). Along with this, people are regularly exposed to As through the consumption of products from aquatic organisms (fish, and shellfish and algae of sea fishery: mollusks, crabs, squid, seaweed, etc.), capable of As bioaccumulation in significant amounts from sea water.

The processes of metabolism and As bioaccumulation in a human body cause active interest in the scientific society [2]. The European Food Safety Agency (EFSA) data show a serious problem caused by As content in food products for public health in the world. The US Agency for Toxic Substances and Disease Registration (ATSDR) and the US Environmental Protection Agency (EPA) enlisted As among the most toxic substances to human health [3].

In toxicological terms, As compounds refer to "thiol poisons" that block sulfhydryl groups of functionally significant proteins, including enzymes. A critical contribution to As toxicity is apparently made by its ability to stimulate reactive oxygen species formation, induce an excessive expression of growth factors, influence the transcription of genes indirectly, induce immunosuppression and apoptosis [4]. iAs compounds are considered as the most dangerous. And trivalent As, as a rule, is more toxic. According to the International Agency for Research on Cancer (IARC), iAs refers to carcinogens of Group 1 (substances with proven carcinogenic activity for humans) [5]. Unlike inorganic forms, the organic derivatives of As (oAs), the most common in seafood and seaweed, are considered low-toxic, according to the world literature, and in the IARC documents referred to as the "non-classified, in terms of human carcinogenicity" (Group 3). In this regard, their toxicological assessment in food products is not carried out [6–10].

In Russian Federation and EAEC countries, the method of atomic absorption spectrometry is currently adopted to assess As content in food products, which makes it possible to quantify the total As content only without fractionation into iAs and oAs. (TR CU 021/2011¹). The permissible levels of total As in food products, according to Technical Regulations "On Food Safety" (TR CU 021/2011) vary from 0.1 mg/kg in raw milk to 5.0 mg/kg in shellfish and algae, and seaweed (in terms of product wet weight). It is these parameters that are used in hygienic assessments, studies, inspections [11–15].

Our research goal of the given study was to develop a procedure for separate determination of iAs and oAs content in seafood (fish, shrimp, squid, mussels, seaweed).

Materials and methods. Fish and non-fish objects of sea fishery (mollusks, crabs, squid, seaweed) purchased in Moscow consumer market were chosen as objects of study.

Reagents used: a standard As sample (a solution with As (III) mass concentration of 0.1 mg/cm³) produced by EcoAnalytical Association "Ecoanalytika", 33% special

purity hydrogen peroxide, according to CU 6-02-570-75, methanol h.p., according to the State Standard GOST 6995-77, hydrochloric acid (HCl), part by weight, mass fraction ≥ 37%, p=1.15 g/cm³, according to GOST 3118-77, ammonium carbonate, mass fraction ≥ 99.999% according to the State Standard GOST 3770-75, acetic acid, h.h. according to the State Standard GOST 61-75, deionized water obtained in "Milli-Q Advantage A10" system, solid-phase extraction cartridges with a strong anion-exchange fixed phase 'Strata SAX' by Phenomenex (sorbent mass 500 mg, volume 6 cm³); special purity acid nitric oxide, concentrated in accordance with the State Standard GOST 11125-84; acid citric monohydrate or anhydrous by the State Standard GOST 3652-69 h.h., carbamide according to the State Standard GOST 6691-77, sodium boron hydride h.ch., sodium hydroxide according to the State Standard GOST 4328-77 h.p.; propan-butane, a mixture in cylinders according to the State Standard GOST 20448-90.

Product samples were scissor-cut into pieces of 1-3 mm in size, weighed to within ± 0.01 g, placed in a Petri dish, frozen and vacuum-dried in LS-500 device (made in Russia). The dried samples were reweighed to determine the moisture content, and then ground into a powder in a


mortar. A homogenized and shredded dry sample of 0.1-0.2 g (taken with an accuracy of ±0.001 g) was introduced into a sectional-view glass tube No.14, of 20 cm³, added with 10 cm³ of extraction solution (3% by mass of hydrogen peroxide in 0.055 M hydrochloric acid on deionized water) and placed in a water bath with a shaker at a temperature of 95°C for 45 minutes. The samples were then cooled to a room temperature, placed in centrifuge tubes, and centrifuged during 10 minutes in an angular rotor centrifuge at 2100 g. The supernatants were transferred to polypropylene tubes and added with deionized water to a volume of 10 cm³; 5 cm³ of each supernatant was selected to determine the total As content using atomic absorption spectrometry; 3 cm³ was taken from the residues for further solid-phase extraction. To test the completeness of the extraction, simultaneously we took 0.1-1.0 g (± 0.001 g) weighted portion of the analyzed dry sample to determine the total As content. iAs was separated from oAs using solid phase extraction on SPE cartridges placed in a vacuum chamber. For completeness of samples solid-phase extraction we adhered to the recommended elution rate: 2 cm³ of liquid/5 min. The cartridges were pre-conditioned with 2 cm³ methanol. The sorbent balancing of cartridges was accomplished with a 2 cm³ solution consisting of equal volumes of 40 mM ammonium carbonate and an extraction solution. Prior to being applied onto a cartridge, supernatant of 3 cm³ was mixed with 3 cm³ 40 mM ammonium carbonate. Using a multi-purpose indicator-paper, we determined the mixture pH, which was to correspond to 6.5 ± 1.0. The buffered solution of the sample was centrifuged for 10 minutes at 4000 rpm, thereupon an aliquot

### Determinations of total arsenic, organic and inorganic arsenic form, in seafood samples

<table>
<thead>
<tr>
<th>No</th>
<th>Sample</th>
<th>Moisture %</th>
<th>Extraction %</th>
<th>As content, mg/kg M±δ</th>
<th>Total As</th>
<th>oAs</th>
<th>iAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Shrimp, cooked-frozen, with head, sample No. 1</td>
<td>72.2</td>
<td>83</td>
<td>3.27±1.14</td>
<td>1.97±0.70</td>
<td>1.30±0.45</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Shrimp, cooked-frozen, with head, sample No. 2</td>
<td>75.3</td>
<td>≥100**</td>
<td>10.6±3.7</td>
<td>7.49±2.62</td>
<td>3.07±1.07</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Whole shrimp, cooked-frozen, sample No. 3</td>
<td>87.5</td>
<td>≥100</td>
<td>6.88±2.41</td>
<td>5.27±1.84</td>
<td>1.60±0.56</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Pacific mussels, peeled</td>
<td>73.5</td>
<td>≥100</td>
<td>0.70±0.24</td>
<td>0.88±0.31</td>
<td>н/о****</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Mussels, cooked-frozen</td>
<td>74.8</td>
<td>≥100</td>
<td>0.81±0.28</td>
<td>0.86±0.30</td>
<td>н/о</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Squid trunk, sample No. 1</td>
<td>84.5</td>
<td>≥100</td>
<td>0.30±0.10</td>
<td>0.34±0.12</td>
<td>н/о</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Squid trunk, sample No. 2</td>
<td>82.9</td>
<td>≥100</td>
<td>0.25±0.09</td>
<td>0.24±0.08</td>
<td>н/о</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Squid No. 3</td>
<td>83.0</td>
<td>≥100</td>
<td>0.04±0.01</td>
<td>0.04±0.01</td>
<td>н/о</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Squid No. 4</td>
<td>83.0</td>
<td>66</td>
<td>0.05±0.02</td>
<td>0.03±0.01</td>
<td>0.02±0.01</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Crab, cooked-frozen</td>
<td>83.2</td>
<td>91</td>
<td>9.90±3.45</td>
<td>6.67±2.33</td>
<td>3.23±1.13</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Halibut</td>
<td>76.6</td>
<td>≥100</td>
<td>2.84±0.99</td>
<td>2.15±0.75</td>
<td>0.69±0.24</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Sea bass</td>
<td>81.4</td>
<td>≥100</td>
<td>0.49±0.17</td>
<td>0.41±0.14</td>
<td>0.08±0.03</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Navaga</td>
<td>81.2</td>
<td>100</td>
<td>28.8±10.1</td>
<td>18.9±6.6</td>
<td>9.86±3.45</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Laminaria, dried</td>
<td>***</td>
<td>≥100</td>
<td>20.3±7.1</td>
<td>20.4±7.1</td>
<td>н/о</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Laminaria, dry, chopped</td>
<td>***</td>
<td>≥100</td>
<td>25.2±8.8</td>
<td>24.5±8.6</td>
<td>0.66±0.23</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Laminaria, frozen, unwashed</td>
<td>***</td>
<td>≥100</td>
<td>17.5±6.1</td>
<td>16.0±5.6</td>
<td>1.54±0.54</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>White Sea fucus, chopped</td>
<td>***</td>
<td>≥100</td>
<td>12.3±3.3</td>
<td>10.2±3.6</td>
<td>2.11±0.74</td>
<td></td>
</tr>
</tbody>
</table>

Note: * – Confidence interval at a significance level of $p=0.95$; ** – Completeness of extraction made 100% within determination error limits.

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of 4 cm$^3$ was applied to the pre-sorbent equilibrated SPE cartridges installed in a vacuum chamber. After the solution passed through cartridge (for 10 minutes), it was washed with 6 cm$^3$ of 0.5 M acetic acid, and then dried under vacuum until complete drying. The combined solutions treated in the cartridge at this stage were an oAs fraction. The cartridge-adsorbed fraction was further eluted with 2.5 cm$^3$ of 0.4 M hydrochloric acid. During an elution, iAs (V) fraction retained on SPE cartridges was collected in polypropylene tubes with further vacuum dehydration for at least 5 minutes. The initial dry product sample, an extract aliquot, oAs and iAs fractions obtained were mineralized, according to the State Standard GOST 26929-94\textsuperscript{12}. After that, the total As content was determined using an atomic-absorption spectrometer "KVANT-2A-GRG" (Russia) with a hydride accessory GGR-107. According to the State Standard GOST P 51766-2001\textsuperscript{13} for this method of analysis, its relative error limit at 2 repetitions and a significance level in confidence interval of 95% is ±35%, the detection threshold is 3 × 10$^{-4}$ μg/cm$^3$.

Results and discussion. The findings upon iAs and oAs separate determination in 17 seafood samples (shrimps, mussels, squid, sea fish and seaweed) are shown in Table.

As follows from the table, in 8 out of 17 samples analyzed (2 samples of shrimp, 1 crab, 1 fish, 4 (all analyzed) samples of algae), total As content does not correspond to the norm. However, in all these cases, iAs content in the samples was significantly lower, comparing to that of oAs. In 6 out of 17 analyzed products, within the range of detection sensitivity (0.1 mg/kg), iAs was not detected, and, apparently, As totally was represented by its organic form. The obtained data confirm the previous assumption that food products risk assessment, in terms of total As content, gives an overestimated result due to the prevalence of a relatively low-toxicity oAs in the whole As pool. As known, products of sea hydrobionts have high nutritional value, being the sources of essential minerals: iodine, selenium, etc., of dietary fiber (algae) and high-grade protein (fish and invertebrates). In view of this, the issue of obtaining scientific data on iAs content in these types of food products is topical. The procedure proposed for separate determination of oAs and iAs has good prospects, since it is relatively simple in hardware, of low cost (SPE cartridges can be reused after reconditioning many times) and, after an appropriate metrological validation, can be implemented in most laboratories certified to examine chemical safety of food products.

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