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Arsenic in Bosnia and Herzegovina market seafood: Effects of matrix modifiers on measured concentration

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Abstract: Arsenic concentration in seafood could potentially reach very high levels and represent a significant health risk for humans. In this study, the concentration of arsenic in various seafood: *crabs* (shrimp, prawns), *molluscs* (mussels), and *cephalopods* (squid) available both fresh on the market and frozen in supermarkets in Sarajevo, Bosnia and Herzegovina were determined by the electrothermal atomic absorption spectrometry (ETAAS). The results obtained using different matrix modifiers: $Mg(NO_3)_2$, $Ni(NO_3)_2$, $Pd(NO_3)_2$, and mixture $Pd(NO_3)_2$ + $Mg(NO_3)_2$ were compared. The best recovery rate of 98.4 % arsenic for the reference material ERM-CE278k, was achieved after the addition of the mixture $Pd(NO_3)_2$ + $Mg(NO_3)_2$. The mean arsenic concentrations were 1.551 ± 0.836 mg kg^{-1} 1.298 ± 0.410 mg kg^{-1}, and 2.794 ± 0.958 mg kg^{-1} for crustaceans, molluscs and cephalopods, respectively, by using mixture $Pd(NO_3)_2$ + $Mg(NO_3)_2$ as matrix modifier. Arsenic concentrations in the same sample measured using different matrix modifiers varied widely, even above 70 %. With the current consumption rate of seafood products, both cancerogenic and non-cancerogenic risks associated with exposure to arsenic through seafood are very low for the residents of Bosnia and Herzegovina.

Keywords: ETAAS; matrix modifier; target hazard quotient; cancer risk.

INTRODUCTION

Seafood is a well-known source of numerous nutrients. Many countries have issued national guidelines that support the regular intake of seafood. According to the U.S. Department of Agriculture and U.S. Department of Health and Human

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Services recommendations, an optimal seafood intake should be an app. 225 grams per week.[1] The main reasons that support these claims are based on the content of high-quality proteins found in the seafood. Those proteins are easily digestible because levels of connective tissue in seafood are significantly lower compared to red meat or chicken. In addition, seafood contains a particular type of polyunsaturated fatty acids (PUFAs) including omega-3 fatty acids that positively impact human health.[2] Consumption of seafood 1-2 times a week is desirable for adults, while consumption of 3-4 times a week during pregnancy is associated with better functional outcomes of neurodevelopment in children.[3] Seafood may or may not be contaminated with toxic elements. The accumulation of this elements in marine organisms, including seafood, depends primarily on their habitat and species and internal factors such as size, weight, age, sex, sexual maturity, and stress.[4]

Lethal doses of arsenic for adults are between 100 and 300 mg.[5] Numerous epidemiological studies have reported a strong association between exposure to arsenic and systemic health effects.[6] Long-term exposure to arsenic can lead to neurological and cognitive dysfunction in children and adults.[7] Knowing that most of As ingested with food are very fast excreted from the body,[8] adverse effects for human health can arise only after excessive exposure.[9] International Agency for Research on Cancer (IARC) classifies inorganic arsenic compounds as cancerogenic for humans. On the other hand, organic arsenic compounds are potentially cancerogenic.[10] However, cancer risk in humans due to exposure to As through seafood consumption is considered low.[11]

Arsenic toxicity in humans is mainly related to exposure to inorganic arsenic, which through binding to thiol or sulfhydryl groups on proteins can inactivate over 200 enzymes.[12] Most likely, this is the mechanism of action underlying the effects of arsenic on various organic systems. At the same time, inorganic arsenic has been shown to inhibit mitochondrial respiration, which may cause DNA mutation and the development of cancer.[13] The percent of inorganic arsenic in seafood is, according to different literature sources, less than 2-3 %[14-18] up to 10-13 %[16, 19, 20]. It is generally accepted that inorganic arsenic is critical for human health risk assessment.[21] However, some findings indicate that organic arsenic compounds undergo biotransformation, leading to trivalent toxic arsenic intermediates or some end products that are more toxic than the parent arsenic.[13]

The use of precise and reliable analytical techniques to determine arsenic at 0.01 mg kg^{-1} levels has become a standard requirement. Inductively coupled plasma mass spectrometry (ICP-MS) and electrothermal atomic absorption spectrometry (ETAAS) are detection techniques that meet this requirement.[22] Better control of chemical processes and reduction of interference of the present components of the matrix are the most important goals for analysts who deal with these techniques. They can be achieved by adding certain chemical compounds,

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known as matrix modifiers that reduce interference by separating the test compound from the matrix.[23]

Matrix modifiers achieve their effect by binding to the analyte element, thus preventing its loss at high pyrolysis temperatures, which are sufficient to remove most of the matrix. In general, this can be achieved by two main mechanisms: decreasing the analyte's volatility or increasing the matrix's volatility. Arsenic compounds are highly volatile; therefore, samples can lose arsenic during the preparation phase.[24] Test method for ETAAS issued by U.S. EPA[24] recommend adding nickel nitrate or palladium nitrate prior to analysis to minimize volatilization losses of arsenic during drying and ashing. Adding nickel enables char temperatures up to 1500 °C can be achieved without an arsenic loss.[25] Achieving the highest pyrolysis temperature is not the only prerequisite for the optimal effect of the matrix modifier.[26] Therefore, some authors prefer palladium which allows pyrolysis temperatures up to 1300 °C with high sensitivity.[27] Palladium nitrate belongs to the platinum group matrix modifiers that catalyse analyte reduction under high pyrolysis temperatures. It forms low volatility intermetallic compounds with the analyte.[26] Palladium nitrate is often used to determine arsenic in seafood.[28] Given that the classes above of matrix modifiers have different modes of action, an attempt was made to combine modifiers from both groups. For this purpose, the mixture of palladium nitrate and magnesium nitrate is most often used, which is also considered a universal matrix modifier.[27] Although some research has been conducted to examine exposure to heavy metals through fish and seafood in the adult population of Bosnia and Herzegovina,[29] there is still insufficient information on arsenic concentrations in seafood and related health risks.

This study aimed to determine the concentration of arsenic in various seafood: crabs (shrimp, prawns), molluscs (mussels), and cephalopods (squid) available both fresh on the market and frozen in supermarkets in Sarajevo, Bosnia and Herzegovina. In addition, the effect of the use of different matrix modifiers on As concentration was investigated. Finally, the risk to human health from arsenic intake through seafood was assessed.

EXPERIMENTAL

Chemicals

Standard arsenic solution (1g L^{-1}) in HNO_3 (0.5 mol L^{-1}), CertiPUR®, was obtained from Merck, Germany. Matrix modifiers stock solution: magnesium nitrate [$Mg(NO_3)_2$, 10 g L^{-1}], palladium nitrate [$Pd(NO_3)_2$, 2 g L^{-1}], and nickel nitrate [$Ni(NO_3)_2$, 10 g L^{-1}], trace element analyses purity, were obtained from Carlo Erba Reagents, Italy. Nitric acid, HNO_3 (65 %), Suprapur™, was purchased from Thermo Fisher Scientific, USA. Ultrapure water was obtained from Mili-Q-Direct 8, Millipore, USA. Certified reference material ERM-CE278k (mussel tissue) was purchased from Sigma-Aldrich, UK.

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Instruments

Microwave digestion system producer Berghof MWS-3+, Germany; Electrothermal atomic absorption spectrometer - ETAAS - PinAAcle 900T AAS THGA, producer Perkin Elmer - supported by the software WinLab 32.

Samples

Analysis has been performed on a total of 61 samples of three different types of seafood: *crustaceans* (n = 29), the *molluscs* (n = 17), and the *cephalopods* (n = 15). More than half of the samples (59 %) were procured fresh on the fish market and stored in the fridge at 4 °C. The other samples (41 %) were procured frozen in the supermarket and kept in the freezer. All samples were collected between June and the end of September 2019. This study did not take into account seasonal variations and different suppliers in the market. Before the analysis, all the samples were removed from the fridge and freezer to reach room temperature, after which they were prepared by separating the edible portion from the inedible (guts, scales, heads, and bones). The animal's commonly edible part (tissue) was selected for analysis and were homogenised by grinding in stainless steell chopper. Arsenic concentration was determined in each sample using all investigated matrix modifiers.

Sample preparation

All samples were treated following the applicable standard methods EN 13804:2015 and EN 13805:2015. The frozen samples were thawed at room temperature. About 0.5 g of comminuted and homogenized sample was taken for microwave digestion in PTFE vessels. After that, 5 ml HNO_3 were added, and the samples were placed on microwave digestion. The digested samples were transferred into polypropylene volumetric flasks, diluted to 25 ml with ultrapure water, and used for total As determination.

Electrothermal atomic absorption spectrometry

Determination of arsenic was performed by ETAAS, supported by the software WinLab 32 and following the standard EN 14332:2004. The operating parameters of the ETAAS used during the analysis are shown in Table I. program given by producer of ETAAS for determination arsenic. The injection temperature was 20 °C, and the samples were analysed in duplicate. The injected volume of the sample was 20 µl, while the injected volume of matrix modifiers was 5 µl. Concentrations of all matrix modifiers were 1 µg μL^{-1}.

TABLE I. ETAAS operating parameters

Step		Temperature (°C)	Ramp time (min)	Hold time (min)	Flow of argon (ml/min)
1a.	Drying	110	1	30	250
1b.		130	15	30	250
2.	Pyrolysis	1200	10	20	250
3.	Atomization	2000	0	3	0
4.	Cleaning	2450	1	3	250

Calibration

Standard arsenic solution (1 g L^{-1}), was diluted to get a calibration curve. Calibration was done with 5 different concentrations (0-1-5-10-15-20 µg L^{-1}) and blank. Calibration were linear, with correlation coefficients above 0.991. Real samples were analysed without and with adding

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matrix modifiers. Stock solution matrix modifiers were diluted and used in amount of: 5 µg $Mg(NO_3)_2$, 5 µg $Pd(NO_3)_2$, 8 µg mix of [5µg $Pd(NO_3)_2$ + 3µg $Mg(NO_3)_2$], and 5µg $Ni(NO_3)_2$.

Quality control

Certified reference material ERM-CE278k was analysed in each sample series, to perform quality control. Certified As value for ERM-CE278k, given by producer is 6.7 ± 0.4 mg kg^{-1}. Also, solvents and blank were included in each series of digestion and analysis. LOQ value of methods is 0,01 mg kg^{-1}. Certified and analysed As values and trueness (recoveries) with different matrix modifiers were presented in Table II. Recoveries were calculated by using following equation (1):

$$\text{`Trueness (Recovery), \%} = (\text{Analysed As value} / \text{Certified As value}) \times 100 \quad (1)$$

TABLE II. Analysed As values and trueness (recoveries) ERM-CE278k obtained without and with different matrix modifiers

Element	Matrix modifier (MM)	Analyzed value*, mg/kg	Recovery, %
As	without MM	2.29 ± 0.1	34.2
	$Mg(NO_3)_2$	1.28 ± 0.1	19.1
	$Pd(NO_3)_2$	7.09 ± 0.2	105.8
	$Ni(NO_3)_2$	3.32 ± 0.2	49.5
	$Pd(NO_3)_2$ + $Mg(NO_3)_2$	6.50 ± 0.1	98.4

*The data are presented as means ± standard deviation.

Risk assessment

Target Hazard Quotient (THQ) and Cancer Risk (CR) have been determined for assessing non-cancerogenic and cancerogenic risk. THQ and CR were calculated by using the following formulas:[30]

$$THQ = E_{fr} \times ED \times FIR \times C / R\,fD \times BW \times TA \times 10^{-3} \quad (2)$$

$$CR = E_{fr} \times ED \times FIR \times C \times CSF / BW \times TA \times 10^{-3} \quad (3)$$

In formulas (2) and (3), E_{fr} refers to exposure frequency (365 days/year), and ED is exposure duration (years), where the average human lifetime of 70 years was used. FIR stands for fish (food) ingestion rate (g per day). Since there is no available official data regarding seafood consumption in Bosnia and Herzegovina, FIR was estimated by using FAOSTAT food supply quality data for 2018, the latest year with available records. According to this source, 0.04, 0.05, and 0.2 kg per capita per year was the consumption for *crabs*, *molluscs*, and *cephalopods*, respectively.[31] The C is the element concentration in the sample (mg kg^{-1}). Concentration for inorganic arsenic, which represents the most toxic arsenic form, was calculated as 3 % of the mean for total arsenic concentration, in line with the approach from other authors from Bosnia and Herzegovina.[17] BW stands for average body weight (kg), where an average adult weight of 70 kg was used, and TA refers to exposure time for non-carcinogens (365 days per year · ED). R_fD refers to oral reference dose mg kg^{-1} per day), and CSF is a cancerogenic slope factor. According to United States Environmental Protection Agency the R_fD and CSF for arsenic are 0.0003 and 1.5 mg kg^{-1} per day respectively.[32]

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This research gives a snapshot of the current arsenic levels in seafood available to the citizens of Bosnia and Herzegovina. Performed risk assessment based on the collected data provides an initial understanding of the situation. To accurately assess the population's exposure to arsenic through this diet, several years of monitoring are needed.

RESULTS AND DISCUSSION

The results of quality control (Table II) showed that recoveries were between 19.1 % (the lowest) with $Mg(NO_3)_2$ and 105.8 % (the highest) with $Pd(NO_3)_2$ as the matrix modifier (Table II). The recovery obtained with a mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$] as matrix modifier was 98.4 %, and this result was considered representative since arsenic concentration was closest to the certified value. Seafood samples were analysed first without matrix modifiers and then with various matrix modifiers as a CRM material. The results are presented in Table III. Arsenic concentrations are shown as mean values with standard deviation, and range for crustaceans (n = 29), molluscs (n = 17), and cephalopods (n = 15). Based on the results obtained by measuring recovery of the reference material, which we consider representative, the As results obtained with the mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$] were compared with the results obtained by measuring with the other matrix modifiers. None of the samples, regardless of the matrix modifier used during the analysis, showed an arsenic concentration higher than 15 mg kg^{-1}, defined as the upper limit by state regulation on maximum levels of specific contaminants in food.[33]

A risk assessment analysis has been performed based on the gained arsenic concentrations to estimate the probability of adverse health effects potentially caused by exposure to arsenic-containing seafood. THQ and CR were calculated using the methodology initially developed to determine concentrations of toxic substances ingested through the consumption of edible fish.[30] However, this methodology has been used by many authors for assessing the risk from the consumption of seafood as well.[15, 34, 35] For the THQ values < 1, adverse health effects are not expected. If the THQ value is > 1, non-cancerogenic adverse health effects could be experienced. On the other hand, according to U.S. EPA,[36] carcinogenesis is a phenomenon for which risk evaluation based on the presumption of a threshold is inappropriate. Therefore, CR results are evaluated upon acceptable lifetime risk level (ARL) equal to $1 \cdot 10^{-5}$ as defined by U.S. EPA,[37] representing a risk for developing cancer over a human lifetime of 1 in 100,000.

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TABLE III. Total arsenic concentration in seafood measured with different matrix modifiers and risk assessment results

Samples	Arsenic, mg/kg	No matrix modifier	$Mg(NO_3)_2$	$Pd(NO_3)_2$	$Ni(NO_3)_2$	$Pd(NO_3)_2$ + $Mg(NO_3)_2$
Crustaceans N = 29	Mean ± SD	1.281 ± 0.908	0.773 ± 0.458	1.601 ± 0.908	0.795 ± 0.340	1.551 ± 0.836
	Range	0.010 – 3.570	0.020 – 1.976	0.225 – 4.144	0.086 – 1.740	0.252 – 3.741
	p value*	1.000	< 0.003	0.173	< 0.001	N/A
	THQ (10^{-4})	2.0	1.2	2.5	1.2	2.4
	CR (10^{-7})	0.9	0.5	1.1	0.6	1.1
Cephalopods N = 15	Mean ± SD	1.225 ± 1.023	0.902 ± 0.311	1.346 ± 0.453	0.582 ± 0.242	1.298 ± 0.410
	Range	0.020 – 2.752	0.325 – 1.487	0.490 – 1.818	0.135 – 0.852	0.405 – 1.706
	p value*	0.108	< 0.001	0.663	< 0.001	N/A
	THQ (10^{-4})	9.6	7.1	10.5	4.6	10.2
	CR (10^{-7})	4.3	3.2	4.7	2.1	4.6
Molluscs N = 17	Mean ± SD	1.367 ± 0.740	0.774 ± 0.316	2.537 ± 0.793	1.042 ± 0.219	2.794 ± 0.958
	Range	0.218 – 2.710	0.163 – 1.288	1.070 – 3.851	0.686 – 1.363	1.106 – 4.301
	p value*	< 0.001	< 0.001	0.061	< 0.001	N/A
	THQ (10^{-4})	2.7	1.5	5.0	2.0	5.5
	CR (10^{-7})	1.2	0.7	2.2	0.9	2.5

THQ - Target Hazard Quotient; CR – Cancer Risk; * comparison with mixture $Pd(NO_3)_2$ and $Mg(NO_3)_2$

Crustaceans

It is not surprising that a high concentration of this heavy metal can be found in seafood shrimps.[15] However, that was not the case in this research, where gained arsenic concentration for *crustaceans* was in the range 0.010-4.144 mg kg^{-1} (wet weight) with a mean concentration of 1.551 ± 0.836 mg kg^{-1} observed for the samples analysed with the mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$] as a matrix modifier. The highest observed mean arsenic concentration was 1.601 ± 0.908 mg kg^{-1} with $Pd(NO_3)_2$. In contrast, the lowest one was 0.773 ± 0.458 mg kg^{-1} determined with the $Mg(NO_3)_2$. It can be noticed that the mean As concentrations obtained with $Pd(NO_3)_2$ and with a mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$] are very similar (1.601 ± 0.908 mg kg^{-1} and 1.551 ± 0.836 mg kg^{-1}, respectively). This finding indicates that the effect of $Pd(NO_3)_2$ on reducing the interference of the present matrix components is crucial. Similar mean As values were also obtained with $Mg(NO_3)_2$ and $Ni(NO_3)_2$ (0.773 ± 0.458 mg kg^{-1} and 0.795 ± 0.340 mg kg^{-1}, respectively), indicating that these two modifiers work similarly. The mean As concentration obtained without matrix modifier was 1.281 ± 0.908 mg kg^{-1} and was between the values obtained with Pd $(NO_3)_2$/[$Pd(NO_3)_2$ + $Mg(NO_3)_2$], and $Mg(NO_3)_2$/$Ni(NO_3)_2$. Based on these findings, it can be concluded that $Mg(NO_3)_2$ and $Ni(NO_3)_2$ interfere with the determination of arsenic and should not be used alone for these purposes.

The most extensive range of concentrations was found for the samples analysed without matrix modifiers, while the smallest was in the group with

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$Ni(NO_3)_2$ (Fig. 1). For individual samples, the highest arsenic concentration of 4.144 mg kg^{-1} was recorded using $Pd(NO_3)_2$. The lowest concentration was 0.010 mg kg^{-1} in the sample without a matrix modifier. Several literature sources reported mean arsenic values for shrimp in a similar range of 0.27-2.18 mg kg^{-1}.[38-39] In Olmedo *et al.* work,[38] fresh samples had the higher mean As concentrations of 0.739 mg kg^{-1} than 0.509 mg kg^{-1} recorded in frozen samples. Other studies have reported much higher arsenic values for shrimp in the range of 19.13-51.18 mg kg^{-1}.[15]

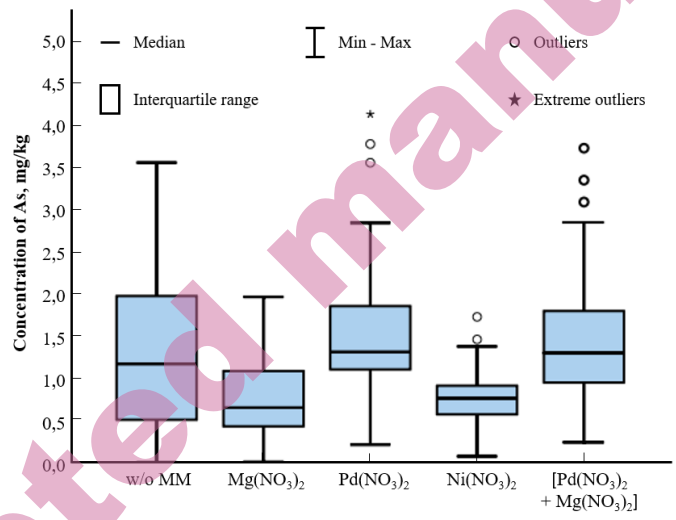

Fig. 1. Arsenic concentration (mg kg^{-1}, wet weight) for *crustaceans*, measured with different matrix modifiers

In comparison with the mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$], the results with $Pd(NO_3)_2$ and without matrix modifier did not show a significant difference (p = 0.173, and p = 1.000, respectively). The mean arsenic concentrations were 3.22 % higher and 17.41 % lower, respectively, compared with the values obtained with the mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$]. In contrast, the differences between As concentrations obtained with the mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$] and concentrations obtained with $Mg(NO_3)_2$ and $Ni(NO_3)_2$ modifiers have shown statistical significance ($p < 0.05$). The mean arsenic concentration measured with the mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$] was 50.17 % higher than the concentration observed with $Mg(NO_3)_2$ and 48.74 % higher than the value noticed with $Ni(NO_3)_2$.

Estimates for THQ and CR have shown that consumption of *crustaceans* does not pose significant non-carcinogenic or carcinogenic health risks. The calculated THQ results are far below the limit of 1 regardless of the matrix modifier used and range between $1.2 \cdot 10^{-4}$ and $2.5 \cdot 10^{-4}$. These values are significantly lower than those observed by the Bonsignore *et al.* study, ranging from 0.200 to 0.140.[15] In

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addition to the lower levels of arsenic reported in this study, a significantly lower ingestion rate strongly contributed to the observed difference. These two factors notably influenced CR, calculated as $0.5 \cdot 10^{-7} - 41.1 \cdot 10^{-7}$. Once again, these levels are much lower than those reported by Bonsignore *et al.*[15]

Cephalopods

Results of arsenic concentration in *cephalopods* are shown in Fig. 2. The mean arsenic concentration was 1.298 ± 0.410 mg kg^{-1} for samples treated with the mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$], which was the lowest value compared to *crustaceans* and *molluscs* (Table 3). Observed mean As concentrations ranged from 0.582 ± 0.242 mg kg^{-1} for $Ni(NO_3)_2$ to 1.346 ± 0.453 mg kg^{-1} for $Pd(NO_3)_2$ modifier. In contrast, the lowest and the highest single sample arsenic concentrations were 0.020 and 2.752 mg kg^{-1}, respectively, observed in samples not treated with matrix modifiers (Fig. 3). Pronounced diversity can also be found in the values of As concentration published in the literature. Some authors have reported relatively low arsenic levels below 5 mg kg^{-1},[16] while the others have reported a high concentration of 36.63 mg kg^{-1}.[40] Several authors have reported arsenic levels in broad ranges, including the low and high boundaries mentioned above.[14,18]

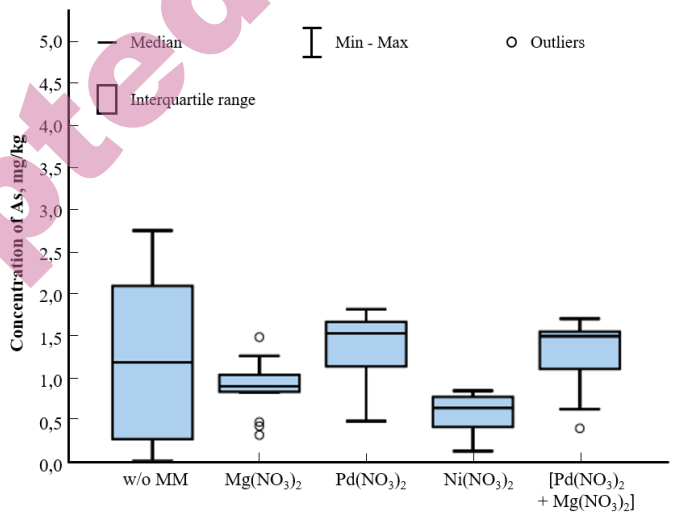

Fig. 2. Arsenic concentration (mg kg^{-1}, wet weight) for *cephalopods*, measured with different matrix modifiers

In the same way as for *crustaceans*, a significant difference ($p < 0.05$) between the mean arsenic concentration measured with the mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$], and both $Mg(NO_3)_2$ and $Ni(NO_3)_2$ modifiers was observed. The mean As value obtained with the mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$] was higher for 30.51

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% and 44.84 % compared with those measured separately with $Mg(NO_3)_2$ and $Ni(NO_3)_2$ modifiers. As expected, no significant difference has been determined when the mean As concentration obtained with $Pd(NO_3)_2$ was compared to the concentration obtained with [$Pd(NO_3)_2$ + $Mg(NO_3)_2$] mixture (p = 0.663). The mean As value with $Pd(NO_3)_2$ was just 3.70 % higher than the value with the modifiers mixture. Similarly, the result without the matrix modifier does not show statistical significance (p = 0.108). Still, the mean As concentration obtained with the modifiers mixture was higher by 5.62 % compared with concentration without matrix modifier.

Although the values of arsenic were the lowest in *cephalopods*, both carcinogenic and non-carcinogenic risks are higher compering to *crustaceans* and *molluscs* due to the highest consumption. THQ scoRes. ranged from $1.5 \cdot 10^{-4}$ to $5.5 \cdot 10^{-4}$, which is still well below the cut-off value of 1. The results are much lower than those reported by Bonsignore *et al.*, which have ranged from 0.15 to 2.00.[15] A considerable difference was also observed in the results of CR scores. Calculated values for inorganic arsenic in this research were in the range $2.1 \cdot 10^{-7} – 4.7 \cdot 10^{-7}$, whereas the CR score for cephalopods reported in the literature was considerably higher, amounted $2.6 \cdot 10^{-4} – 8.1 \cdot 10^{-4}$.[15]

Molluscs

Results of arsenic concentration in *molluscs* are shown in Fig. 3. Obtained concentration ranged 0.163 – 4.301 mg kg^{-1}. The highest mean concentration of 2.794 ± 0.958 mg kg^{-1} was observed in the group of results obtained with the mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$] as a matrix modifier. The lowest one of 0.774 ± 0.316 mg kg^{-1} was obtained in the case of $Mg(NO_3)_2$ as a matrix modifier. The single sample's highest and lowest arsenic concentrations were also found in those two groups (4.301 and 0.163 mg kg^{-1}, respectively).

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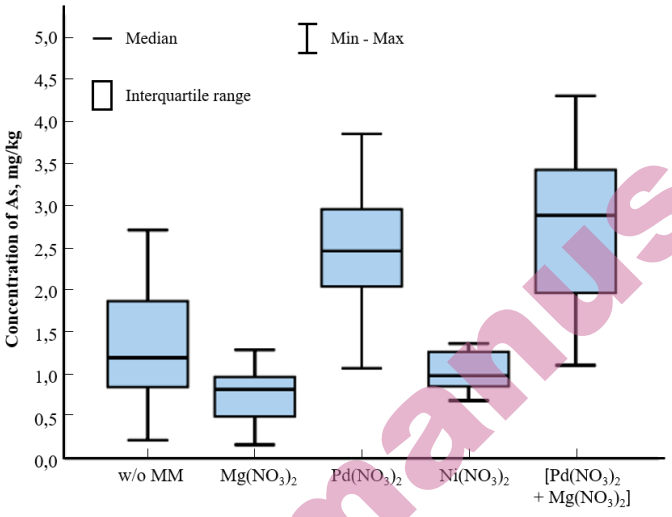

Fig. 3. Arsenic concentration (mg kg^{-1}, wet weight) for *molluscs*, measured with different matrix modifiers

Similar to the results for *Crustaceans*, it was again observed that the mean concentrations of As obtained with $Pd(NO_3)_2$ and a mixture of [$Pd(NO_3)_2$ + $Mg(NO_3)_2$], were quite close (2.537 ± 0.793 mg kg^{-1} and 2.794 ± 0.958 mg kg^{-1}, respectively). Relatively close mean concentrations of As were also obtained when $Mg(NO_3)_2$ and $Ni(NO_3)_2$ were used (0.774 ± 0.316 mg kg^{-1} and 1.042 ± 0.219 mg kg^{-1}, respectively). This finding again indicates that the mechanism of action of these modifiers, $Mg(NO_3)_2$ and $Ni(NO_3)_2$, which primarily eliminate interference from inorganic chlorides, in our research was not helpful. Indeed, according to Welz *et al.* sodium chloride caused very few problems in determining arsenic.[27]

Most available literature sources have reported arsenic concentrations in *molluscs* with a similar range as found in our research. Several locations of the Adriatic Sea are the main source of fresh seafood products available at markets in Bosnia and Herzegovina. Mussels collected along the Croatian coast contained total arsenic in 2.33-2.56 mg kg^{-1},[28,40] while mussels at the cost of Montenegro contained 1.73–2.41 mg kg^{-1}.[42] Recent research has shown that the main arsenic concentration in fresh and frozen mussels marketed in Serbia was 3.97 mg/kg and 1.56 mg kg^{-1}, respectively.[43] Differences in arsenic concentration between fresh and frozen products are also described by Olmedo *et al.*, reporting lower As levels in both publications.[38]

No statistically significant difference was observed between results with the mixture [$Pd(NO_3)_2$ + $Mg(NO_3)_2$] in comparison to results with $Pd(NO_3)_2$ modifier (p = 0.061). The mean As concentration obtained with [$Pd(NO_3)_2$ + $Mg(NO_3)_2$] mixture was slightly higher (9.20 %). Compared to other results, the mean

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concentration of As obtained with the mixture [($Pd(NO_3)_2$ + $Mg(NO_3)_2$] was significantly higher: 51.07 % than the mean concentration without matrix modifier, 72.30 % than with $Mg(NO_3)_2$ and 62.71 % than with $Ni(NO_3)_2$ modifier. All differences were statistically significant ($p < 0.05$). The low range with low mean values observed for the results with $Mg(NO_3)_2$ and $Ni(NO_3)_2$ clearly shows that these modifiers are not suitable for determining As.

Compared to *crustaceans* and *cephalopods*, *molluscs* had the highest observed As concentrations (Table 3). Nevertheless, those concentrations are still well below the upper limit of 15 mg kg^{-1} defined by the local Bosnia and Herzegovina regulation.[33] A low consumption rate of only 0.05 kg per capita per year[31] resulted in very low THQ and CR scores. Ferrante *et al.* have determined a strong impact of the level of exposure to As through *molluscs* consumption on both non-cancerogenic and cancerogenic risks.[34] Calculated THQ scoRes. from this work were in the range of $1.5 \cdot 10^{-4} – 5.5 \cdot 10^{-4}$, which was much lower than 0.331-2.320 reported by Ferrante *et al.*[34] and also than 0.209-0.262 reported by Conte *et al.*[19] Similarly, calculated CR results in the range of $0.7 \cdot 10^{-7} – 2.5 \cdot 10^{-7}$ are still much lower than $1.49 \cdot 10^{-4}$, which was found to be the lowest level of exposure in Conte *et al.*[19]

CONCLUSIONS

This research has provided arsenic concentration in seafood available on markets in Sarajevo, the capital of Bosnia and Herzegovina. Obtained results have shown low levels of arsenic, toxic element associated accordingly with low health risks arising from seafood consumption. Since the portion of inorganic arsenic, which has a critical role in the impairment of human health, is minor in seafood, only excessive exposure could increase health risks. With the current very low consumption rate of seafood products, both cancerogenic and non-cancerogenic risks associated with exposure to arsenic through seafood are very low for the residents of Bosnia and Herzegovina. Under the described conditions, different matrix modifiers did not show a more significant impact on the health risk assessment. However, it is essential to emphasize that some differences in As concentrations of the same samples measured using different matrix modifiers were more than 70 %. Additional research is needed to determine why some modifiers had better efficacy than others and to explain the intensive, beneficial response of seafood samples on $Pd(NO_3)_2$ matrix addition.

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ИЗВОД

АРСЕН НА ТРЖИШТУ МОРСКИХ ПЛОДОВА У БОСНИ И ХЕРЦЕГОВИНИ: ЕФЕКТИ МОДИФИКАТОРА МАТРИЦЕ НА ИЗМЕРЕНУ КОНЦЕНТРАЦИЈУ

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Концентрација арсена у морским плодовима може потенцијално достићи веома високе вредности и представљати значајан здравствени ризик за људе. У овој студији, концентрација арсена у разним морским плодовима, као што су: ракови (шкампи, козице), мекушци (дагње) и главоношци (лигње), доступним у свежем и смрзнутом стању на тржишту у Сарајеву, Босна и Херцеговина, одређена је електротермалном атомском апсорпционом спектрометријом (ETAAC). Упоређени су резултати добијени коришћењем различитих модификатора матрице: $Mg(NO_3)_2$, $Ni(NO_3)_2$, $Pd(NO_3)_2$, и смеше [$Pd(NO_3)_2$ + $Mg(NO_3)_2$]. Најбоља стопа опоравка од 98,4 % арсена за референтни материјал ERM-CE278k, постигнута је након додавања смеше [$Pd(NO_3)_2$ + $Mg(NO_3)_2$]. Просечне концентрације арсена биле су 1,551 ± 0,836 mg kg^{-1}, 1,298 ± 0,410 mg kg^{-1}, односно 2,794 ± 0,958 mg kg^{-1}, за ракове, мекушце и главоношце, уз примену смеше [$Pd(NO_3)_2$ + $Mg(NO_3)_2$] као модификатора матрице. Концентрације арсена у истом узорку мерене коришћењем различитих модификатора матрице су се веома разликовале, чак и изнад 70%. Уз тренутну стопу потрошње производа од морских плодова, канцерогени и неканцерогени ризици повезани са изложеношћу арсену кроз морске плодове су веома ниски за становнике Босне и Херцеговине.

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