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Total Arsenic in Raw and Boiled Portions of Norway Lobster (*Nephrops norvegicus*) from the Central Adriatic Sea

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ABSTRACT: The distribution of total arsenic in different portions of Norway lobster (*Nephrops norvegicus* L., Crustacea) was studied both in fresh samples and after a boiling process. All individuals (n = 80) were selected of medium standard commercial size (13−15 cm). The highest mean concentrations (26.86 ± 1.57 mg/kg wet weight (ww)) were found in the raw brown meat of the crustacean, probably due to its detoxification role, whereas the lowest mean values (15.97 ± 0.85 mg/kg ww) were in the raw exoskeleton. The raw white meat reported mean values of 16.09 ± 0.61 mg/kg ww. The levels of arsenic contamination detected in the boiled portions showed a significant ($p < 0.01$) decrease compared to the raw portions, as a consequence of solubilization phenomena. In fact, a large amount of arsenic from raw lobsters was transferred to the corresponding boiling broth. In the most commonly consumed portion, the white meat, only slight losses (7.22%) in total arsenic content were observed compared to the raw portion.

KEYWORDS: *crustaceans, Adriatic Sea, contamination, human health*

INTRODUCTION

Arsenic (As) is a significant environmental contaminant, which exists mainly in four oxidation states, arsenate (As^{V}), arsenite (As^{III}), arsenic (As^{0}), and arsine (As^{-III}), and its toxicity to organisms depends above all on concentration and speciation. Inorganic forms are much more toxic than organic arsenicals, and As^{III} is usually more toxic than As^{V}.[1] In terrestrial and aqueous environments As is mainly present as inorganic arsenate in aerobic conditions and as arsenite in anoxic conditions.[2] In living organisms organic arsenic compounds generally dominate.[3]

Much of As in the environment occurs naturally. It is found in rocks and soil, water, air, plants, and animals. It can be further released through natural activities such as volcanic action, erosion of rocks, and forest fires. High levels of As are predominant in places with high geothermal activities.[4] Anthropogenic sources of As include industrial emissions, mainly by mineral processing and fossil fuel combustion, and its use in the manufacture of glassware, metal alloys, microelectronics, agricultural pesticides, and wood preservatives.[5]

The accumulation of As in food products may pose a health risk, depending on the quantity and type of As species that are present. A long-term As exposure has been linked to cancers of the bladder and lungs[6] and also of the skin, kidney, liver, and prostate.[7] Inorganic As has also been included in IARC group 1 as carcinogenic to humans,[8] and the European Union, according to WHO guidelines, set a limit of 10 μg/L in drinking water.[9,10] Some other countries, such as Denmark and Australia, considering the health effects of arsenic consumption have adopted stricter arsenic guidelines.[11] The water-soluble As organic species (e.g., arsenobetaine, dimethyl arsinic acid, monomethyl acid) have limited or no toxicity when orally ingested.[3]

The occurrence, distribution, and speciation of As in aquatic systems are particularly important in determining its bioaccumulation and trophic transfer through the food chain. In particular, the dominant inorganic As species are incorporated into microorganisms such as phytoplankton and converted to methylarsenicals and arsenosugars, whereas the organic As species are mineralized to inorganic As and methylarsenicals by bacteria.[12] Saltwater organisms are generally characterized by high total As concentrations, and, in particular, shellfish and crustaceans, present, in addition to the organic arsenic compounds, low concentrations of inorganic As.[3] Although all literature sources agree that inorganic compounds in general comprise only a very small fraction of total As in fish and seafood, these products could contribute to the total As dietary intake carried by other food commodities, such as cereal grains and cereal-based products, in particular, rice grains and rice-based products, drinking water, coffee, beer, and vegetables.[13] Another aspect that should be considered in the risk management is that the food preparation (frying or boiling) could be responsible for the increase/decrease of As content. The risk assessment has long been based on the As levels in raw products, but food is generally consumed after being subjected to processing, which could alter the chemical forms of As. Thermal treatment is probably the process that causes the greatest changes in total As concentrations and As species.[14]

In particular, the authors decided to investigate As levels in Norway lobster (*Nephrops norvegicus* L., Crustacea) because it is an important, valuable commercial species in Italy, mainly eaten uncooked, boiled, or whole as part of a soup.[15] The aims of this

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study were (i) to collect information on total As concentrations in Norway lobster samples caught in a specific fishing area of the central Adriatic Sea, where previous studies[16,17] reported high heavy metals levels, and (ii) to study the effects of a boiling process on total As levels. As concentrations were detected in three different portions of Norway lobster, the white meat and the brown meat (edible portions) and the exoskeleton. This latter is not an edible part per se, but it can be used for chitin and chitosan production, which find a wide variety of applications in many fields such as food and nutrition, biomedicine, biotechnology, agriculture, and environmental protection.[18]

MATERIALS AND METHODS

Sample Preparation and Cooking. Samples of Norway lobsters (n = 80) were fished in the central Adriatic Sea (Italy) in July 2009. All individuals were selected of medium standard commercial size (13−15 cm) and came from the same fishing area that was previously studied.[16,17] The samples were immediately frozen and transported to the laboratory in cold iced boxes. Carapace and total length and weight of each specimen were measured before the analytical procedure. The crustaceans were washed several times with distilled water to remove foreign matter and then divided in 10 groups. Each group was formed by four males and four females (n = 8) to reduce the possible influence of sex on metal concentrations. For each group two lobsters were analyzed as whole, whereas the remaining six lobsters were divided in three portions: (i) exoskeleton, including legs and claws; (ii) white meat from the abdomen excluding the hard upper shell; and (iii) brown meat including cephalothorax content of the digestive system. Eggs were never found in the analyzed samples.

All samples were homogenized. A part (10 g) of the homogenate was analyzed as raw. To process the homogenate by boiling, a ratio of 1:2 (sample/water) was used. The samples were boiled in an analytical glass saucepot filled with ultrapure water for 15 min at 80 °C. Thereafter, part of the boiled homogenate and the corresponding broth were analyzed.

Analysis Procedure. The samples were analyzed in the Environmental Safety Laboratory of Piedmont, Liguria, and Aosta Valley, Italy. Quantitative determination of As was performed by an axial inductively coupled plasma−atomic emission spectrophotometer (ICP-MS Thermo Instruments X Series 2, Thermo Fisher Scientific, Bremen, Germany) equipped with collision cell technology−kinetic energy discrimination (CCT-KED). The internal standard, ^{103}Rh (10 μg/L), was used and the possible formation of polyatomic interferences (such as the polyatomic ion $^{40}Ar^{35}Cl^{+}$) was corrected using the collision cell with He/H_2 mixture. Calibrations curves (1, 5, 20, 50 μg/L) were built using dilutions of commercial standard solutions (AccuStandard, Inc., New Haven, CT, USA). To determine As levels, about 0.3 g of lyophilized sample was weighed and digested in a microwave oven (Ethos One, Milestone, Bergamo, Italy) with 7 mL of 70% nitric acid, 1.5 mL of 30% hydrogen peroxide, and 0.05 mL of 37% fluorhydric acid. The temperature of atomization used for microwave-assisted digestion was 2300 °C. The digested samples were then diluted to a final volume of 25 mL with ultrapure water, and these solutions were analyzed by ICP-MS. The limit of detection (LOD) was 0.001 mg/kg, whereas the limit of quantification (LOQ) was 0.010 mg/kg. The quality control included the analysis of blanks and sample with known metal content; furthermore, the laboratory is accredited under UNI CEI ISO/IEC 17025:2005, and technical competence has been demonstrated by good participation in proficiency tests. Certified Reference Material Lichen BCR-482 (Institute for Reference Materials and Measurements, Geel, Belgium) was utilized, and the recovered concentrations were within 10% of the certified values (Table 1).

Statistical Analysis. Data were assessed for normality by means of the Shapiro−Wilk test. Data were normally distributed; therefore, parametric statistics were applied on them. Analysis of variance (ANOVA) was applied to detect differences in metal concentrations between different matrices in both raw and boiled samples.

Table 1. Results of Quality Assurance Procedure

Certified Reference Material	no. of replicates	certified value (mg/kg)	obtained mean (mg/kg)	standard deviation	recovery (%)
BCR	3	0.85	0.81	0.03	95.27

Furthermore, raw sample data were compared to the respective boiled sample data by means of Student's t test for paired samples. In consideration of the possible bias in normality estimation due to the low numbers of replicates, results of parametric tests were confirmed by means of the equivalent nonparametric test, respectively, Kruskal−Wallis and Wilcoxon tests, both with Monte Carlo exact test extension. The comparison between the levels found in raw and boiled samples was made reporting the values on a dry weight (dw) basis, to exclude the concentration effect due to the boiling process. Broth metal concentrations were also correlated with those of raw and boiled samples by means of the Pearson correlation test. A nonparametric Spearman correlation test was also performed to test the possible confounding effect of outliers and to validate previous results. SPSS 14.0.2 (SPSS Inc., Chicago, IL, USA) was used as the statistical package for means comparison and correlation.

RESULTS AND DISCUSSION

As Content in Raw Samples. In this study total As levels showed a different distribution in Norway lobster body (Figure 1), with the highest mean concentrations (± standard error) in

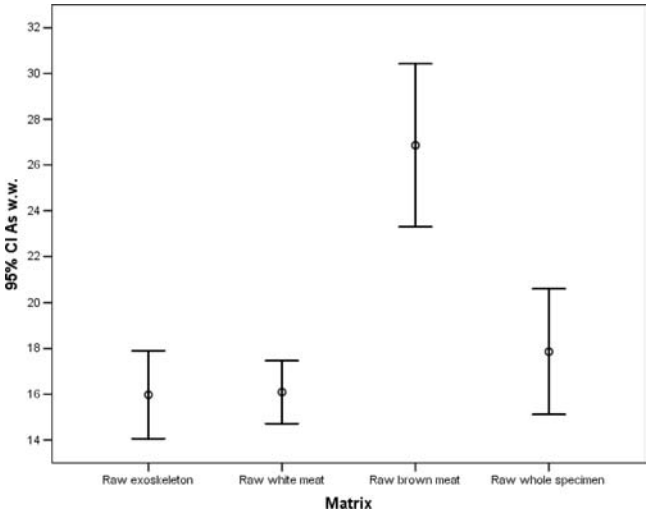

Figure 1. Total As concentrations (mg/kg, mean ± confidence interval) found in the different raw portions of Norway lobster. Data are expressed as mg/kg ww.

the brown meat (26.86 ± 1.57 mg/kg wet weight (ww)). This result could be justified by the mechanism of heavy-metal sequestration and detoxification in crustaceans,[19] which are linked to the hepatopancreas functions (metallothioneins, membrane metal transport proteins, and vacuolar sequestration mechanisms). Indeed, the lowest mean values were detected in the exoskeleton (15.97 ± 0.85 mg/kg ww). This could be due to a supposed mechanism of detoxification and/or elimination through the ecdysis, a crucial step in the life cycle of these invertebrates. The As concentrations found in the brown meat showed a significant statistical difference (p < 0.01) with all other examined portions, whereas those of the exoskeleton showed a significant statistical difference only with the brown meat (p < 0.01).

The total As concentrations (mg/kg ww) in the edible portion of Norway lobster reported in other studies[3,20] were lower if compared to the results of the present study in the white meat (16.09 ± 0.61 mg/kg ww). In particular, Sirot et al.[20] found a total As concentration of 8.75 mg/kg ww, whereas Ruttens et al.[3] detected a mean value of 0.64 mg/kg ww. The accumulation of As in marine organisms varies in relation to many factors such as species, temperature, and salinity of the water as well as weight of the organisms.[21] The diversity of seafood origin can result in variability in the contamination levels, which may explain the differences among the studies. The higher total As content found in this study could be attributed to geographical differences and in particular to the different impacts of the anthropogenic activities along the central Adriatic coasts. Also, Fattorini et al.[22] reported in mussels from the Adriatic Sea higher values of As than those found in specimens collected from French coasts and the Tyrrhenian Sea.

In addition, the present study confirmed that As concentrations in Norway lobster had different patterns of distribution among the examined portions. It has been reported that arsenical compounds are not uniformly distributed in tissues of marine species.[23]

As Content in Boiled Samples. With regard to the distribution of total As in boiled samples, the white meat and the whole specimen showed similar concentrations (6.54 ± 0.56 and 6.58 ± 0.58 mg/kg ww, respectively), and only the lowest values found in the boiled exoskeleton showed a significant difference ($p < 0.01$) with all other portions (Figure 2). Also, for the broths (Figure 3), the lowest values (5.23 ± 0.50 mg/kg ww) were obtained from the boiling of the exoskeleton, showing a significant difference ($p < 0.01$) from those of the white meat (8.39 ± 0.78 mg/kg ww) and the whole specimen (8.28 ± 0.48 mg/kg ww).

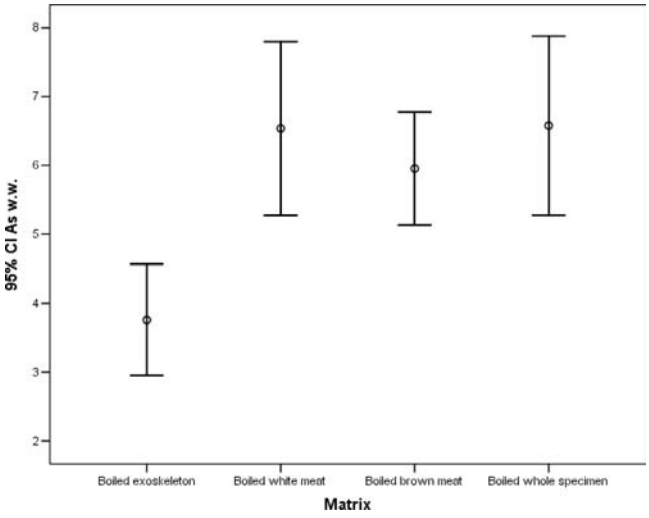

Figure 2. Total As concentrations (mg/kg, mean ± confidence interval) found in the different boiled portions of Norway lobster. Data are expressed as mg/kg ww.

Boiling Effects. It is known that some modifications in total As content and in As species may take place during the preparation of food for human consumption.[14] The various processes may cause a considerable increase or decrease in As concentrations in food commodities and thus in the dietary exposure.[13] In this study all of the examined portions showed a significant decrease ($p < 0.01$) after the boiling process (Figure 4).

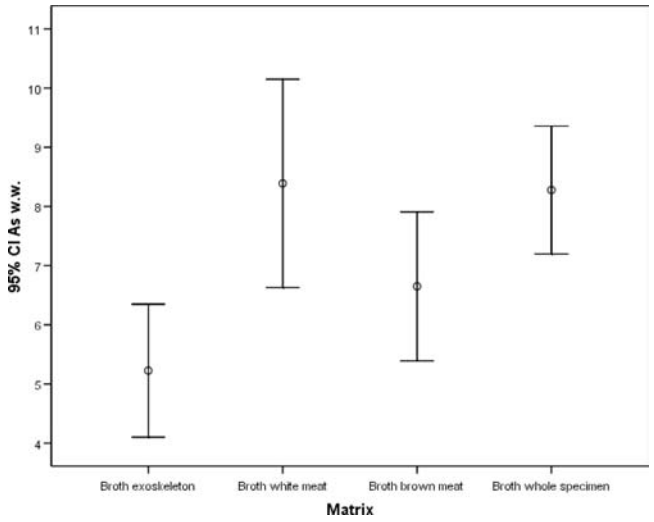

Figure 3. Total As concentrations (mg/kg, mean ± confidence interval) found in broth derived from the different boiled portions of Norway lobster. Data are expressed as mg/kg ww.

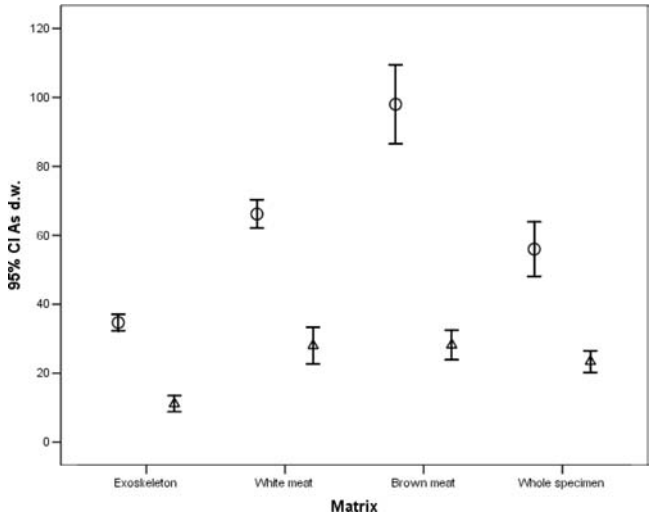

Figure 4. Comparison between total As concentrations (mean ± confidence interval) detected in raw (○) and boiled (△) portions of Norway lobster. Data are expressed as mg/kg dw.

An extensive survey on Canadian foods reported the increase of As concentrations after cooking, probably due to the weight loss of the product.[24] Similarly, Ersoy et al.[25] observed that As concentration in fresh sea bass fillets increased considerably when cooked in a microwave oven or fried and suggested that the increase could be related to changes in the moisture content. In an extensive study involving 11 types of seafood products, Devesa et al.[26] reported both decreases and increases in total As content after the cooking process. They argued that the final increase in total As levels was due to its concentration resulting from loss of water and other soluble compounds during cooking, whereas decreases were the result of As losses because of solubilization or volatilization. Reports of As species solubilization have been cited in the literature. In the above-mentioned study, Devesa et al.[26] observed the presence of arsenobetaine in the liquid resulting from cooking crustaceans.

In our study, the comparison between the raw and boiled samples was made reporting the values on a dry weight basis (Figure 4), so that the concentration effect due to the boiling process could be excluded, but only the total As was investigated and therefore the possible changes of As forms could not be evaluated. In particular, there was not a notable loss of As (7.22%) in the white meat (Table 2), whereas higher losses were observed for the exoskeleton (43.82%) and the brown meat (53.10%). It is not clear whether the decrease of total As observed in the boiled samples compared to the raw portions derived from the volatilization of some components or from the chemical modifications of arsenical compounds not detectable by the applied analytical method. The difference among the examined portions could be attributed to a higher content of lipid-soluble arsenicals, which remained bound to the matrix during boiling, in the white meat than in the other two portions. Despite this, until now, only a few lipid-soluble arsenicals have been identified, and knowledge concerning the abundance, identity, and toxicity of these compounds is limited compared to the water-soluble arsenic compounds.[27] The category of seafood poses a particular problem in trying to calculate the inorganic component, because it is reported that most of the As is in the organic form. A conversion factor was applied to inorganic As in seafood. On that occasion the percentage of inorganic As was assumed to be between 5 and 10% of total As, values that were later shown to be gross overestimates.[28] A problem with this sort of approach is that the relative proportion of inorganic As in fish and seafood is small and tends to decrease as the total As content increases, and the ratio may vary depending on the seafood type.[13] A more practical approach[13] may be to assume a constant contribution of inorganic As from fish (0.03 or 0.015 mg/kg fresh mass) and from seafood (0.10 or 0.05 mg/kg fresh mass). It has been reported that mussels and crustaceans have higher levels of inorganic As than fish.[29]

Table 2. Percentages of Total As Loss in the Examined Portions

	% As in raw portions	% As in boiled portions	% As in broth	% As in boiled + broth	% As loss
exoskeleton	100	23.52	32.66	56.18	43.82
white meat	100	40.64	52.14	92.78	7.22
brown meat	100	22.15	24.75	46.9	53.10
whole	100	36.84	46.30	83.14	16.86

Also, As content in the water used for the cooking process is considered of special importance because it determines whether the concentrations in the prepared food may be considerably higher or lower compared to the raw product. In this research the boiling of samples was made with ultrapure water to exclude the possible amount of inorganic As derived from water. With regard to this last source, it is known that drinking water, and then also the water used in cooking practice, can be contaminated by As and a long-term intake can cause the development of arsenicosis.[30]

Consumers who are most exposed to arsenic are those with a high consumption of seafood or people from areas where the drinking water is high in arsenic. This study provides new data about total As concentrations in Norway lobster, a seafood well appreciated and used in many special meals both at home and in restaurants. In the marine food chain, As concentrations decrease with the increase of trophic level and vary widely on a geographical basis.[31] The analyzed samples were fished in a well-known polluted area, and their high values of total As could reflect the sediment's contamination. Considering the total As distribution, the raw brown meat was more greatly contaminated than the white meat. Even if As speciation was not performed, the results showed that the total As distribution in Norway lobster differed according to the tissue portions and the cooking treatment. The raw portions reported higher values than the corresponding boiled portions, but it is important to highlight that a part of total As was solubilized into the broth. Therefore, the intake of As from seafood can differ according to which particular tissues are consumed, and it should always be evaluated on the basis of the consumer's eating habits. Improved knowledge concerning chemical structures, levels, bioavailability, and toxicity of the arsenical compounds is essential for a more comprehensive risk assessment of As compounds present in seafood and, in particular, in edible crustaceans. Moreover, special attention should be paid to the risk posed by food processing by As-contaminated water for people living in those areas. These pieces of information will also be of importance for the ongoing discussion on the regulation of As and As compounds in feed and food.

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Notes

The authors declare no competing financial interest.

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