Arsenic release from foodstuffs upon food preparation

Karlien Cheyns[1], Nadia Waegeneers[1], Tom Van de Wiele[2], Ann Ruttens*[1]

[1]Veterinary and Agrochemical Research Centre (CODA-CERVA), Leuvensesteenweg 17, 3080 Tervuren, Belgium

[2]Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, 9000 Ghent, Belgium

*Corresponding author: Ann.Ruttens@coda-cerva.be +32 27692234

Abstract

In this study the concentration of total arsenic (As) and arsenic species (inorganic As, arsenobetaine, dimethylarsinate and methylarsonate) was monitored in different foodstuff (rice, vegetables, algae, fish, crustacean, molluscs) before and after preparation using common kitchen practices. By measuring the water content of the foodstuff and by reporting arsenic concentrations on a dry weight base we were able to distinguish between As release effects due to food preparation and As decrease due to changes in moisture content upon food preparation. Arsenic (species) were released towards the broth during boiling, steaming, frying or soaking of the food. Concentrations declined with a maximum of 57 % for total arsenic, 65 % for inorganic As and 32 % for arsenobetaine. Based on a combination of our own results and literature data we conclude that the extent of this release of arsenic species is species specific with inorganic arsenic species being released most easily, followed by the small organic As species and the large organic As species.

Keywords

Arsenic; food; inorganic arsenic; speciation; preparation

Introduction

Arsenic (As) concentration in food was not regulated for a long time due to its high complexity and species dependent toxicity [1]. Unlike other elements (cadmium, lead, mercury), it is not the total concentration of As (As_{tot}) that determines the toxicity in food but the species distribution and concentration of the element [2]. This awareness and the growing analytical possibilities during the last ten years resulted in the collection of As speciation data in food and the appearance of the first European legislation for inorganic As (As_i) in rice and some rice products [3].

Both the total As concentration and the As speciation depend on the food matrix and cultivation conditions (soil type, water, pesticide use, cultivation practices, …). Relatively low concentrations of As are measured in plant-based food of terrestrial origin. Plants can take up As_i or methylated species (methylarsonate (MA), dimethylarsinate (DMA)) and accumulate this As in the leaves, roots, shoots or grains. Arsenic in plants is predominantly As_i, DMA and to a lesser amount MA or the tetramethylarsonium ion (TETRA) [4–6]. The percentage of methylated species depends on the ability of the soil microbial community to methylate the As_i present in soil [7]. Arsenic concentrations in rice are often a factor 10 higher compared to other grains (e.g. wheat and barley) because of a higher accumulation efficiency from the soil and the anaerobic cultivation conditions [8–10], with values of market rice typically ranging between 0.08 mg kg^{-1} and 0.47 mg kg^{-1} WW (as sold; i.e. air dry) [11–13]. Less data are available for vegetables but the reported concentrations are generally low (< 0.0099 mg kg^{-1} WW; as sold i.e. fresh) [14]. Among all terrestrial food, the highest As concentrations (DW base) are likely found in mushrooms (up to 14 mg kg^{-1} DW). In this matrix, As can be present as DMA, MA, As_i and the non-toxic arsenobetaine (AB)[15,16].

Vegetable food of marine origin (algae) behaves differently and As can accumulate with concentrations up to 150 mg kg^{-1} in the product as sold (i.e. air dry) [17–20]. Studies suggest that algae take up arsenate from the water and are able to detoxify this by reduction, oxidative methylation and adenosylation with formation of methylated intermediate compounds and arsenosugars[21]. Other authors suggest that AB is formed to act as osmolyte by mimicking its nitrogen-analogue glycine betaine[22]. However, there is no complete evidence that AB formation is performed by the macro-algae themselves, the AB detected in the macro-algae could be associated with epiphytes as these micro-organisms could not be removed in former studies[22]. When exposure to As_i is high, a biotransformation limit can be reached and As_i is not further transformed but accumulates in the organism[21]. Especially in the hijiki algae (*Hijikia fusiforme*) high concentrations of As_i are reported, ranging 30-117 mg kg^{-1} DW, as sold [17,20,23,24]. Concentrations of As_i are mostly < 0.8 mg kg^{-1} DW in other algae (e.g. nori, wakame,…)[17,24].

Concentrations of As in marine animals can rise up to 100 mg kg^{-1} WW (i.e. fresh) because of their high bio-accumulation capacities [25–27]. These marine animals accumulate As mainly from their feed. Hence, mostly organic forms of As are found in these animals, with the non-toxic AB predominating [26,28–30]. Other organic species detected in marine animals are MA, DMA, trimethylarsine oxide (TMAO) or more complex components such as arsenocholine (AC) and arsenosugars [31]. The inorganic species are rarely detected, or present at very low concentrations. Sloth et al. (2005)[27] analysed 29 marine samples from Norway and reported concentrations of As_i from <0.0006 mg kg^{-1} up to 0.020 mg kg^{-1}. In a former study, we measured As_i concentrations in scampi, shrimps and mussels between <0.003 mg kg^{-1} and 0.022 mg kg^{-1} [26]. Like in marine algae a possible biotransformation limit was seen in blue

mussels, where exceptionally high As_i concentrations (up to 5.8 mg kg^{-1} As_i) were detected [32].

In comparison with marine animals, lower total As concentrations are reported in freshwater animals [33]. In addition, the species composition varies more in these animals. While some studies find AB as main species [33,34], others report other dominant species (e.g. DMA, arsenosugars) [35,36]. In general, total As concentrations are higher in marine animals than in freshwater animals. Schoof et al. (1999)[14] reported in a basket study an average concentration of As in freshwater fish of 0.160 mg kg^{-1} and 2.36 mg kg^{-1} in sea fish. Likewise, Ruttens et al. (2012)[26] measured lower As concentrations in freshwater organisms (median of 0.03-0.70 mg kg^{-1}) compared to marine animals (median 0.23-16.2 mg kg^{-1}).

Despite the high number of studies reporting As speciation data in various unprocessed food items, the number of studies on the impact of food preparation on As release is limited, especially when it concerns speciation data. One of the most documented matrices in this regard is rice. Decrease in rice As content upon washing has been reported to be dependent of the rice type and washing procedure [37–40]. Release of As towards the excess (uncontaminated) cooking water is observed by several authors. When the cooking water is discarded, an average decrease in As concentrations with 33-35% has been reported [37,39]. Rice cooking to dryness did not reduce As concentration [38,39]. Similar reductions of As were found in pasta and vegetables after cooking in excess of water [41,42]. Less explicit data are available in case of cooking marine matrices. Release of some As species from marine organisms is described by Devesa et al. (2001)[43]. Soaking and boiling of hijiki contribute to As release [20,23,44].

While food preparation can have an obvious impact on As content in the prepared food, speciation changes have only rarely been studied, yet they are not unlikely. Arsenic species transformation during processing of marine food has been reported in only a few cases. Devesa et al. (2005)[45] described an increase of DMA in molluscs upon cooking. The authors suggested that this species may have been formed by a transformation of arsenosugars. A relative increase of TETRA was observed in some marine samples after processing [45,46]. Decarboxylation of AB at high (>120°C) temperatures with the formation of TETRA was observed earlier [47].

It is clear that there is a knowledge gap in how food preparation can impact As content in the foodstuff and whether speciation changes occur. Rice is a matrix that is already intensively documented but the effect of food processing varies with food item and cooking procedure. The effect of moisture loss or gain in the food items is often not separated from the true As (species) release. Therefore, this study comprises a broad spectrum of foodstuffs and a particular effort is made to separate the effect due to changes in water content from other effects like species release and transformation.

Materials and Methods

Materials

Chemicals

Water used for chemical analysis was either home produced doubly distilled water (for the determination of total As with ICP-MS; inductively coupled plasma mass spectrometry), or ultra pure water generated by purifying distilled water with the Milli-Q integral 3 system

combined to an Elix 3 pre-system (Merk Millipore, Billerica, USA) (for the determination of As species with HPLC-ICP-MS; High Performance Liquid Chromatography coupled to ICP-MS).

Nitric acid (Suprapur, SpA 67-69 %) was purchased from Romil (Cambridge, UK), and ammoniumcarbonate (pro analyse) from Merk (Darmstadt, Germany).

Stock solutions of the individual As species, with an As concentration of 1000 mg L^{-1}, were prepared from the following reagents: arsenic (III) oxide solution (standard for ICP, Fluka, Buchs, Switzerland), arsenic (V) oxide hydrate (> 99.99%) (Sigma-Aldrich, St. Louis, USA), arsenobetaine (> 95.0%) (Fluka, Buchs, Switzerland), dimethylarsinic acid (99.5%) and monosodium acid methane arsonate (99.0%) (Chemservice, West Chester, USA), trimethylarsine oxide and tetramethyl arsonium iodide (Tri Chemical laboratories, Yamanashi, Japan). Multispecies stock solutions of 250 µg L^{-1} were prepared from these stocks by appropriate dilutions. Final calibration standards of 0, 2, 5, 10 and 20 µg L^{-1} were prepared daily from the multispecies stock solution.

A multi-elemental Varian tuning solution (10 µg L^{-1}) (Spectropure, Arlington, USA) was used to prepare tuning solutions for ICP-MS in 4% (v/v) nitric acid.

Food sample collection and food preparation

Different food samples, belonging to various food groups, were bought in Belgian supermarkets. The food matrices were: two types of rice (white and brown), three different vegetables (carrots, leeks, and onions), one type of mushroom (*Agaricus bisporus)*, one type of potato, one alcoholic drink (red wine), two types of fish (cod and trout), one crustacean (scampi), two types of molluscs (mussels and scallops) and two types of edible seaweeds (nori-*Porphyra sp.* and hijiki-*Hizikia fusiformis*). Three independent batches were sampled for

each food matrix, with the exception of hijiki were only one brand was available and hence only one batch was analysed.

All samples were analysed as sold and after preparation, using some commonly applied kitchen practices. Each preparation was done in triplicate for all batches. The preparation methods included boiling, steaming, microwave preparation and pan frying. An overview of the treatments per matrix is presented in Table 1. Tap water was used for washing and boiling (As=0.15 ±0.03 µg L^{-1}).

The rice batches (1 kg in total for each rice type) were homogenized manually before further treatment. Prior to cooking, sub batches were made of 125 g each. The following rice/water ratios (by weight) were used: 10/32 w/w (excess water was evaporated; rice boiled to dryness) and 5/32 w/w (excess water was collected). Cooking time was as indicated on the packaging (10-20 minutes).

Vegetables (1 kg in total for each vegetable) were pealed if relevant, rinsed twice, and chopped in pieces of about 1 cm^3. Sub batches of ±300 g each were subjected to the various treatments. Carrots and leeks were boiled in stainless steel cooking wear for 10 minutes with a vegetable/water ratio of 12/23 w/w. Additional sub batches were steamed for 15 minutes using a kitchen steamer (Multi Gourmet, Braun).

Onions and mushrooms were fried in vegetable butter for 8 minutes in aluminum-teflon frying pans.

Fish, scallops and scampi were cleaned, with removal of non-edible parts, and sub batches (±300 g) were fried for 5 minutes in aluminum-teflon frying pans without the addition of any fat. In addition sub batches (±300 g) of the fishes were prepared in the microwave (5

158 minutes 750 W) and sub batches of mussels were steamed in their own broth for 5 minutes
159 in a stainless steel cooking pot.

160 Nori seaweeds (±20 g/sub batch) were boiled for 3 minutes with a seaweed/water ratio of
161 0.5/33 w/w. The soup was mixed prior to analysis.

162 For Hijiki, only one batch was sampled which was divided in sub batches for different
163 treatments. The first preparation technique was soaking, i.e. the sub batches were immersed
164 in water for 15 minutes where after the water was removed. Other sub batches of Hijiki
165 were boiled in water for 10 minutes with a seaweed/water ratio of 0.5/33 w/w. For one
166 treatment the water was removed from the matrix and for another treatment the seaweed
167 was mixed with the water as a soup.

168 All preparations (except steaming) were done on individual electric cookers (Domo
169 DO309KP). Water was always brought to boiling temperature before the foodstuff was
170 added. The differences in water/solid ratios among foodstuffs are due to the difference in
171 water absorbing capacities of the matrices, which asks for differences in optimal water
172 quantities. During preparation the temperature of all foodstuffs was logged with a
173 temperature probe connected with a Picolog® data registration system, temperature in the
174 food matrices never exceeded 100°C.

175 ## Methods

176 *Moisture content*

177 Moisture content of all samples was determined by oven drying sub samples of ± 20 g each
178 (48h at 50°C; Memmert, Germany) and weighing them before and after drying. Arsenic

concentrations determined on 'whole weight' base (mg kg^{-1} WW) could therefore be recalculated to 'dry weight' base (mg kg^{-1} DW).

Sample preparation

MINERALISATION OF TOTAL AS

Homogenized samples were acid digested in teflon vessels (PTFE, polytetrafluoroethene) in a microwave oven (CEM MARS XPress, Matthews, USA), according to a validated and accredited 'in house' method. Samples were weighed (typical weights are presented in Table 2) in duplicate in the microwave vessels. After addition of 4 ml nitric acid and 4 ml bidistilled water, the vessels were closed and placed into the microwave system. The samples were heated to 180°C in 15 min and maintained at that temperature during 30 minutes. After cooling, samples were diluted on a weight basis (1 g sample solution + 9 g bidistilled water) in analytical 15 ml tubes.

EXTRACTION OF AS SPECIES

Homogenized samples (± 0.25 g to 1 g, depending on the matrix, see Table 2) were weighted in PFA microwave tubes with magnetic stirrers. After addition of 9 mL 0.07 M HNO_3 and 1 mL H_2O_2 (30%) for vegetable matrices and algae, or 10 mL H_2O for wine, fish, crustacean and molluscs, the samples were extracted for 30 minutes in the microwave with activated stirring function (CEM, Mars XPRess). Extracts were centrifuged (10 minutes, 12500 g) and filtrated (0.45 µm, Millipore) before analysis. Extraction of As_i from marine animals is, up to now, associated with the presence of analytical difficulties. Recent proficiency tests for As_i in dogfish liver were found to be spread over a wide range and no certified reference material for As_i in marine animals exists yet [48]. However, Pétursdóttir et al, 2014[49] showed that

extraction of As_i from seafood is fairly robust when H_2O or diluted acid is used as extractant. Wine cannot be measured as such by HPLC-ICP-MS, dilution with H_2O is recommended[50]. In plants, As is apparent less soluble, which can be explained by the formation of As-phytochelatins complexes sequestered in the vacuoles[51]. We preferred to use diluted acid in case of vegetal matrices to assure sufficient extraction efficiency in these more resistant matrices[52]. In combination with the H_2O_2 added, As^{III} oxidizes to As^{V} and only the total inorganic As can be quantified. This method is very similar to CEN method EN16802:2016 [53]. Consequently, acid extraction was used for vegetables and algae, and water extraction was used for seafood and wine, the latter allowing a separate quantification of As^{III} and As^{V} [54]. After analysis, both inorganic species can be summed to calculate the total As_i concentration.

Sample analysis

TOTAL AS ANALYSIS

Determination of total As was performed by ICP-MS (VARIAN 820; Varian, Melbourne, Australia), with H_2 as reaction gas. Quantification of As in the digests was performed by external calibration (calibration range: 1 µg L^{-1} -10 µg L^{-1}) using acidified dilutions (4% nitric acid) of a multi-element stock solution from Analytika (Prague, Czech Republic). The matrix relevant certified reference material (CRM) IRMM 804 (rice flour; As_{tot} = 0.049 ± 0.004 mg kg^{-1}, IRMM, Belgium), NMIJ 7405 (Hijiki seaweed As_{tot}= 35.8 ± 0.9 mg kg^{-1}, National Metrology Institute of Japan) or BCR-627 (tuna fish; As_{tot} = 4.8 ± 0.3 mg kg^{-1}; IRMM, Belgium) was added to each sample batch. Although a good agreement was observed between the certified and the measured values (80-120%), sample results were corrected for the

deviation of the measured values from the certified value, to allow an optimal comparison of results across different sample batches. The limit of quantification (LOQ) of the method for total As determination, calculated as 10 times the standard deviation of 20 blank samples, corresponds to 0.010 µg L^{-1} in solution. The LOQ in the matrix depends on the dilution factor applied (Table 2).

As SPECIES ANALYSIS

The analysis of As species was performed using HPLC-ICP-MS (high performance liquid chromatography – inductively coupled plasma – mass spectrometry; Varian, Mulgrave, Austalia). The considered species in this study are: As_i (=As^{III}+ As^{V}), MA, DMA and AB.

The chromatographic separation of the As species was performed on anion exchange columns. Two different chromatographic methods were used, depending on the expected species in the matrix. Combined with the different extraction solutions, this resulted in three different analytical methods, elucidated in Table 3. The separation of AB on an anion exchange column is generally poor because AB does not interact with the column and tends to co-elute with other uncharged or cationic species. Their separation can be improved by using an ion pair reagent (benzenedisulfonic acid) in the mobile phase which creates differences in elution behavior [31]. In fish, molluscs and crustacean the latter separation method was preferred, because of the potential presence of this type of As species. In the other matrices a sufficient separation of the As species present can be obtained by using an ammoniumcarbonate solution [53] (Table 3). However, algae and mushrooms might contain a complex mixture of As species, and it was beyond the scope of this study to analyse all of them, only As_i, MA and DMA were quantified in these matrices. The calibration curve of AB was further used to quantify all cationic and uncharged species in these matrices. The

246 chromatographic software (GALAXY) of the ICP-MS instrument was used for quantification of
247 the peak area. A five point external calibration (0-5 µg L^{-1}) with the respective standard
248 compounds was carried.

249 The matrix relevant CRM NMIJ 7503a (rice flour; As_i = 0.0841 ± 0.0030 mg kg^{-1}, DMA= 0.0133
250 ± 0.0009 mg kg^{-1}; National Metrology Institute of Japan), NMIJ 7405 (hijiki seaweed; As_i =
251 10.1 ± 0.5 mg kg^{-1}; National Metrology Institute of Japan) or BCR-627 (tuna fish; AB = 3.897 ±
252 0.225 mg kg^{-1}; DMA = 0.145 ± 0.022 mg kg^{-1}; IRMM, Belgium) was added to each sample
253 batch. Although a good agreement was observed between the certified and the measured
254 values (80-120%), sample results were corrected for the deviation of the CRM values, in
255 order to reduce potential differences between the various analytical batches. The limit of
256 quantification (LOQ) of the method for total As determination, calculated as 10 times the
257 standard deviation of 20 blank samples, corresponds to 0.010 µg L^{-1} in solution. The LOQ in
258 the matrix depends on the dilution factor applied to the sample (Table 2).

259 ## Statistics

260 A 2-factor ANOVA, followed by a multiple comparison test using a Bonferroni correction
261 (Software: Graphpad prism 6.0) was used for statistical analysis of the results. The nominal
262 factors were the processing treatments on the one hand and the 3 lots on the other hand for
263 all matrices except for hijiki (in the latter case only 1 lot was studied and the treatment
264 effect was the only factor considered). When the effect of the processing treatment was not
265 the same for all lots (i.e. a significant interaction effect was observed between the 2 factors)
266 then the effects of the processing treatments were analysed separately for each lot. When
267 no significant interaction effect was observed main effects were interpreted.

Results

As (species) concentrations in the raw matrices

Concentrations of total As and As species for all raw matrices are summarized in Table 4. Although rice has the reputation to show higher As_i concentrations compared to other terrestrial plant species, the results in Table 4 indicate that when concentrations are recalculated to dry weight base, total As and As_i overlap in vegetables and rice (0.02-0.18 mg As_{tot} kg^{-1} DW in vegetables and 0.16-0.39 mg As_{tot} kg^{-1} DW in rice; 0.01- 0.15 mg As_i kg^{-1} DW in vegetables and 0.12-0.17 mg As_i kg^{-1} DW in rice). This illustrates that the high moisture content of fresh vegetables tends to mask their sometimes relatively high As_i concentrations. Inorganic arsenic concentrations are low in mushrooms (0.03-0.04 mg As_i kg^{-1} DW), but can rise up to 90.8 mg As_i kg^{-1} DW in hijiki algae. In the marine organisms, the main As species was AB, with exception of mussels, in which large amounts of other species could be detected, including As_i (up to 0.13 mg As_i kg^{-1} DW).

Effect of food preparation on moisture content

Food preparation influences the moisture content of the food matrices. This can hence impact data interpretation when results are expressed on fresh weight basis. We therefore recalculated As release values to a DW basis. This allows distinguishing between As release from the foodstuff attributed to changes in water content and As release caused by the food preparation. The % DW before and after treatment are presented in Table 4 and Table 5.

Boiling rice evidently led to an increase of the moisture content of the grains (% DW decreases from 89-90% to 37-52 %). The % DW dropped upon boiling and increased upon steaming in leeks and carrots. The % DW in onion increased after frying, indicating that water was lost out of the onions during the treatment. The values of the % DW after frying

of the mushrooms, show that more water was lost in the second batch (% DW increase from 6.2 to 19%) compared to the other two batches (increase from 6.7 to 11% and from 6.3 to 11%). Preparing seaweed samples, which were bought as dried leaves, included hydratation during soaking and/or boiling; consequently, the % DW drastically decreased (from 83-90 % to minimum 6.2% in hydrated seaweed, or to 2 % in soup). The % DW consistently increased for all treatments of fish and crustacean (with maximum 6 %), which indicated a loss of water. Mussels, steamed in their own broth, showed an increase in % DW (from 8.5%-12% to 28%-31%).

Effect of food preparation on As (species) concentrations

The % change in concentration after different treatments are given in Table 5, overall, no quantitative data on MA were obtained as MA concentrations were lower than LOQ before or after preparation.

A significant decrease in concentration of As_{tot}, Asi and DMA was seen in all three of the lots after boiling white rice in an excess of water. However, the extent of the effect was not the same for all lots (significant interaction effect). No effects on As species concentrations were observed when the same rice was cooked until dryness, except for one lot, where the DMA concentration was reduced. An unidentified peak with a short retention time (3.2 minutes) was observed in the latter case. Significant decreases of $As_{tot,}$ As_i and DMA were observed in all lots of brown rice when boiling in an excess of water.

Boiling and steaming carrots also reduced the average concentrations of As and As_i in all lots, but was only significant in lot 3. Arsenic was detected in the broth of all treatments (results not shown). When boiling or steaming leek, a main treatment effect indicated also a decrease in As (species) concentrations. Arsenic was detected in the broth of all treatments

314 (results not shown). Frying onion did not significantly affect As or As_i concentrations. Heating
315 wine did not affect the As_{tot} or As_i concentration.

316 Frying mushrooms induced different effects on the As (species) concentration in the distinct
317 lots (significant interaction effect). A clear and significant decrease of all As species was
318 observed in the lot 2 (the lot where more moisture was lost), while this was not observed in
319 the other batches.

320 Making soup of nori or hijiki did not induce effects on As_{tot} concentrations, because the
321 boiling liquid was included in the analysis. Soaking or boiling hijiki (with removal of the
322 excess water) led to significant decreases of $As_{tot,}$ As_i and DMA. The concentration of As_i was
323 significantly more reduced when hijiki was boiled (-65%) compared with the soaking process
324 only (-40%). No significant difference in DMA concentration was observed between soaking
325 and boiling. Remarkably, the concentration of cationic and uncharged species was not
326 significantly reduced by the different treatments.

327 Preparation of fish and crustacean did not induce effects on As_{tot} and AB concentrations.
328 Frying scallops, caused a slight (<15%) but significant increase in the concentration of As_{tot}
329 and AB for lot 3. This is likely a contingency because there are no factors that might create
330 As during preparation and such increase was not noted in the other lots.

331 Steaming mussels decreased significantly the concentration of As_{tot}. Concentrations of DMA
332 decreased to values lower than the quantification limit in lot 2 and 3, in lot 1 a statistically
333 significant decrease could be shown. Also the concentrations of the other identified species
334 (AB and As_i) were significantly reduced by the steaming process. Remarkably, the decrease
335 of the As species was larger than the percentage decrease in As_{tot}.

Discussion

Food preparation significantly decreased levels of As_{tot} and several As species in foodstuffs. This is primarily caused by the release of As during the preparation process, with removal of the broth. However, preparations where the broth was evaporated during food preparation (e.g. rice boiling till dryness) did not result in changes in the As content. Our observations are confirmed by previous reports (Table 6) : our study demonstrates the As release process to be widely distributed across a broad spectrum of foodstuffs with different structures. . More research is, however, needed to determine whether there is a link between the amount of As release and the structure of the foodstuff.

High temperatures, increased contact times and/or water movement enhance the As release. Arsenic in hijiki is released more extensively after boiling (50% decrease) compared to soaking only (28% decrease) (Table 5). Arsenic was released as As_i and DMA after soaking of hijiki (no decrease of the cationic and uncharged species) and the extra decrease after cooking was only in the form of As_i, (no extra decrease of DMA). Likewise, in former studies (Table 6), washing of rice did not reduce As to the same extent as cooking did, and soaking of seaweed did not reduce As to the same extent as cooking did. Heat treatment improves solubility and accelerates the breaking of bonds between the arsenic and the food matrix and facilitates its solubilization[55].

Interestingly, our data also confirm earlier observations of the species-dependent specificity of the As release process[38,39,56]. For instance, As_i is released more easily from rice compared to DMA. Steaming of mussels caused a release of all quantified species to the broth, including organic and inorganic species (AB, As_i and DMA). The decrease of these species was larger than As_{tot} decrease, which suggests that unidentified species were released to a

lower extent (Table 5). This is also illustrated by differences in relative peak areas observed when chromatograms of the mussel extracts before and after treatment (Figure 1) are compared. A possible explanation for the difference in release behavior between As_i, DMA and AB may be found in the different acid dissociation constants of these species. For example, As^{III} (pK_{a1}=9.2) is uncharged at neutral pH and is more mobile than DMA (pK_{a1}=6.1) and AB (pK_{a1}=2.9) and therefore more likely to migrate from the food matrix[57]. In addition to the differences in ionic behavior an explanation could be present in differences in lipophilicity of the molecules. Estimated partition coefficients (P) generally increase with the length of the hydrocarbon chain (e.g. logP As^{III}=-8.6; logP MA=-1.1 - -0.9 ; logP DMA=-0.33 - -0.07 ; logP AB=0.94-1.84 [58]). This can be linked with the observed decreasing release behaviour, but more research is needed to get insights in the true physico-chemical processes which occur during preparation processes.

Based on a combination of our own results and literature data (Table 6) one could conclude that release of As is species specific. Generally, As_i is released most easily, followed by organic species, with the small organic molecules being released more easily than the large ones.

Extrapolation of these results to extractability of different As species during laboratory extraction supports the current advice against a correction for extraction efficiency (recovery) when performing As speciation analysis in rice [53].

No transformation of As species were observed upon the food preparation techniques in this study, except for one lot of white rice were a new As peak was observed upon boiling the rice to dryness. This coincided with a decrease in the DMA concentration. Other authors found indications of As species transformations in specific marine species. A relative increase

382 in DMA concentration after cooking was observed in sardines and bivalves in a study of
383 Devesa et al., 2005 [45]. The authors suggested that the DMA was formed after transformation
384 of arsenosugars. Also, increase of TETRA have been reported upon cooking of meagrim,
385 anchovy, Atlantic horse mackerel, or sardine [45,46]. This TETRA might be formed by
386 decarboxylation of AB at high temperatures [47]. However, this was only observed at
387 temperatures higher than 120°C. The absence of transformation of As species in the current
388 study might be explained by the low temperatures during food preparation (max 100°C;
389 results not shown).

390 This study clearly indicates that consumers can lower their As intake by boiling rice and
391 vegetables in an excess of water and discarding the broth which contains the released As.
392 Inorganic arsenic, in particular, the most toxic As species present in food, is being released
393 easily. Decreases ranging 53-66 % in boiled rice and 12-43% in boiled vegetables were
394 observed in our study. Taking into account the fact that estimated background intake of As_i
395 in European countries is in the range of $BMDL_{01}$-values (Bench mark dose lower confidence
396 limit) proposed by EFSA [59,60], all efforts able to reduce dietary exposure to As_i can help to
397 protect consumers' health.

398 However, it must be kept in mind that the same release effect can also reduce
399 concentrations of some vitamins, essential trace elements or other nutrients, as was shown
400 by Gray et al., 2015 [61] (when cooking rice in an excess of water). A sound variation in food
401 consumed and cooking methods applied may therefore guarantee the best contribution to a
402 long and healthy life.

403 ## Nomenclature

Acronyms and chemical formulae (fully deprotonated form) of the arsenic compound included in the present study.

Acronym	Arsenic compound	Formula
As^{V}	Arsenate	$AsO(O^-)_3$
As^{III}	Arsenite	$As(O^-)_3$
As_i	*Inorganic arsenic*	
As_{tot}	*Total arsenic*	
MA	Methylarsonate	$CH_3AsO(O^-)_2$
DMA	Dimethylarsinate	$(CH_3)_2AsO(O^-)$
TETRA	Tetramethylarsonium ion	$(CH_3)_4As^+$
TMAO	Trimethylarsine oxide	$(CH_3)_3AsO$
AB	Arsenobetaine	$(CH_3)_3As^+CHCOO^-$
AC	Arsenocholine	$(CH_3)_3As^+CH_2CH_2O^-$

Abbreviations Used

BMDL Bench Mark Dose lower confidence Limit
CRM Certified Reference Material
DW Dry Weight
HPLC High Pressure Liquid Chromatoography
ICP Inductively Coupled Plasma
LOQ Limit of Quantification
MS Mass Spectrometry
WW Wet Weight

Aknowledgement

The authors would like to thank Ann-Catherine Blanpain, Krissy Brouwers, Ronny Machiels and Fréderic Van Steen for their technical assistance. This research was funded by the Federal Public Service (FPS) of Health, Food Chain Safety and Environment-Contract RF 11/6247 'BIOTRAs'.

References

(1) Petursdottir, A. H.; Sloth, J. J.; Feldmann, J. Introduction of regulations for arsenic in feed and food with emphasis on inorganic arsenic, and implications for analytical chemistry. *Anal. Bioanal. Chem.* **2015**, 8385–8396.

(2) Francesconi, K. A. Toxic metal species and food regulations--making a healthy choice. *Analyst* **2007**, *132* (i), 17–20.

(3) European Commission. Commission Regulation (EU) 2015/1006 of 25 June 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs. *Off. J. Eur. Union* **2015**, *L161* (14), 1993–1995.

(4) Hansen, H. R.; Raab, A.; Price, A. H.; Duan, G.; Zhu, Y.; Norton, G. J.; Feldmann, J.; Meharg, A. A. Identification of tetramethylarsonium in rice grains with elevated arsenic content. *J. Environ. Monit.* **2011**, *13*, 32–34.

(5) Cubadda, F.; Ciardullo, S.; D'Amato, M.; Raggi, A.; Aureli, F.; Carcea, M. Arsenic contamination of the environment-food chain: A survey on wheat as a test plant to investigate phytoavailable arsenic in italian agricultural soils and as a source of inorganic arsenic in the diet. *J. Agric. Food Chem.* **2010**, *58*, 10176–10183.

(6) Zhao, F.-J.; Stroud, J. L.; Eagling, T.; Dunham, S. J.; McGrath, S. P.; Shewry, P. R. Accumulation, distribution, and speciation of arsenic in wheat grain. *Environ. Sci. Technol.* **2010**, *44* (14), 5464–5468.

(7) Lomax, C.; Liu, W.; Wu, L.; Xue, K.; Xiong, J.; Zhou, J.; Steve, P. Methylated arsenic species in plants originate from soil microorganisms. **2012**, No. 193, 665–672.

(8) Williams, P. N.; Villada, A.; Deacon, C.; Raab, A.; Figuerola, J.; Green, A. J.; Feldmann, J.; Meharg, A. a. Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. *Environ. Sci. Technol.* **2007**, *41* (19), 6854–6859.

(9) Zhao, F. J.; Zhu, Y. G.; Meharg, A. A. Methylated arsenic species in rice: Geographical variation, origin, and uptake mechanisms. *Environ. Sci. Technol.* **2013**, *47*, 3957–3966.

(10) Li, F.; Zheng, Y. M.; He, J. Z. Microbes influence the fractionation of arsenic in paddy soils with different fertilization regimes. *Sci. Total Environ.* **2009**, *407* (8), 2631–2640.

(11) Heitkemper, D. T.; Vela, N. P.; Stewart, K. R.; Westphal, C. S. Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* **2001**, *16*, 299–306.

(12) D'Ilio, S.; Alessandrelli, M.; Cresti, R.; Forte, G.; Caroli, S. Arsenic content of various types of rice as determined by plasma-based techniques. *Microchem. J.* **2002**, *73*, 195–201.

(13) Meharg, A. A.; Lombi, E.; Williams, P. N.; Scheckel, K. G.; Feldmann, J.; Raab, A.; Zhu, Y. G.; Islam, R. Speciation and distribution of arsenic and localization of nutrients in rice grains. *New Phytol.* **2008**, *42*, 1051–1057.

(14) Schoof, R. A.; Yost, L. J.; Eickhoff, J.; Crecelius, E. A.; Cragin, D. W.; Meacher, D. M.; Menzel, D. B. A market basket survey of inorganic arsenic in food. *Food Chem. Toxicol.* **1999**, *37*, 839–846.

(15) Nearing, M. M.; Koch, I.; Reimer, K. J. Arsenic Speciation in Edible Mushrooms. *Environ. Sci. Technol.* **2014**, *48*, 142013–14210.

(16) Falandysz, J.; Borovička, J. Macro and trace mineral constituents and radionuclides in mushrooms: Health benefits and risks. *Appl. Microbiol. Biotechnol.* **2013**, *97* (2), 477–501.

(17) Almela, C.; Jesús Clemente, M.; Vélez, D.; Montoro, R. Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain. *Food Chem. Toxicol.* **2006**, *44*, 1901–1908.

(18) García-Salgado, S.; Quijano, M. A.; Bonilla, M. M. Arsenic speciation in edible alga samples by microwave-assisted extraction and high performance liquid chromatography coupled to atomic fluorescence spectrometry. *Anal. Chim. Acta* **2012**, *714*, 38–46.

(19) García-Sartal, C.; Romarís-Hortas, V.; Barciela-Alonso, M.; Moreda-Piñeiro, A.; Dominguez-Gonzalez, R.; Bermejo-Barrera, P. Use of an in vitro digestion method to evaluate the bioaccessibility of arsenic in edible seaweed by inductively coupled plasma-mass spectrometry. *Microchem. J.* **2011**, *98* (1), 91–96.

(20) Ichikawa, S.; Kamoshida, M.; Hanaoka, K.; Hamano, M.; Toshikazu, K.; Tamio Maitani. Decrease of arsenic in edible brown algae Hijikia fusiforme by the cooking process. *Environ. Biol. Toxicol.* **2006**, *20*, 585–590.

(21) Geiszinger, A.; Goessler, W.; Pedersen, S. N.; Francesconi, K. A. Arsenic biotransformation by the brown macroalga, Fucus serratus. *Environ. Toxicol. Chem.* **2001**, *20* (10), 2255–2262.

(22) Popowich, A.; Zhang, Q.; Le, X. C. Arsenobetaine: The Ongoing Mystery. *Natl. Sci. Rev.* **2016**, DOI 10.1093/nsr/nww061.

(23) Hamano-Nagaoka, M.; Hanaoka, K.; Usui, M.; Nishimura, T.; Maitani, T. Nitric acid-based partial-digestion method for selective determination of inorganic arsenic in hijiki and application to soaked hijiki. *J. Food Hyg. Soc. Japan* **2008**, *49* (2), 88–94.

(24) Rose, M.; Lewis, J.; Langford, N.; Baxter, M.; Origgi, S.; Barber, M.; MacBain, H.; Thomas, K. Arsenic in seaweed-Forms, concentration and dietary exposure. *Food Chem. Toxicol.* **2007**, *45*, 1263–1267.

(25) Edmonds, J. S.; Francesconi, K. A. Arsenic in seafoods: Human health aspects and regulations. *Mar. Pollut. Bull.* **1993**, *26* (12), 665–674.

(26) Ruttens, A.; Blanpain, A. C.; De Temmerman, L.; Waegeneers, N. Arsenic speciation in food in Belgium. Part 1: Fish, molluscs and crustaceans. *J. Geochemical Explor.* **2012**, *121*, 55–61.

(27) Sloth, J. J.; Larsen, E. H.; Julshamn, K. Survey of inorganic arsenic in marine animals and marine certified reference materials by anion exchange high-performance liquid chromatography-inductively coupled plasma mass spectrometry. *J. Agric. Food Chem.* **2005**, *53* (15), 6011–6018.

(28) Borak, J.; Hosgood, H. D. Seafood arsenic: Implications for human risk assessment. *Regul. Toxicol. Pharmacol.* **2007**, *47*, 204–212.

(29) Li, W.; Wei, C.; Zhang, C.; Van Hulle, M.; Cornelis, R.; Zhang, X. A survey of arsenic species in Chinese seafood. *Food Chem. Toxicol.* **2003**, *41*, 1103–1110.

(30) Sirot, V.; Guérin, T.; Volatier, J. L.; Leblanc, J. C. Dietary exposure and biomarkers of arsenic in consumers of fish and shellfish from France. *Sci. Total Environ.* **2009**, *407* (6), 1875–1885.

(31) Kohlmeyer, U.; Kuballa, J.; Jantzen, E. Simultaneous separation of 17 inorganic and organic arsenic compounds in marine biota by means of high-performance liquid chromatography/inductively coupled plasma mass spectrometry. *Rapid Commun. Mass Spectrom.* **2002**, *16* (10), 965–974.

(32) Sloth, J. J.; Julshamn, K. Survey of total and inorganic arsenic content in blue mussels (Mytilus edulis L.) from Norwegian fiords: Revelation of unusual high levels of inorganic arsenic. *J. Agric. Food Chem.* **2008**, *56*, 1269–1273.

(33) Ciardullo, S.; Aureli, F.; Raggi, A.; Cubadda, F. Arsenic speciation in freshwater fish: Focus on extraction and mass balance. *Talanta* **2010**, *81*, 213–221.

(34) Šlejkovec, Z.; Bajc, Z.; Doganoc, D. Z. Arsenic speciation patterns in freshwater fish. *Talanta* **2004**, *62*, 931–936.

(35) Miyashita, S.; Shimoya, M.; Kamidate, Y.; Kuroiwa, T.; Shikino, O.; Fujiwara, S.; Francesconi, K. A.; Kaise, T. Rapid determination of arsenic species in freshwater organisms from the arsenic-rich Hayakawa River in Japan using HPLC-ICP-MS. *Chemosphere* **2009**, *75* (8), 1065–1073.

(36) Soeroes, C.; Goessler, W.; Francesconi, K. A.; Kienzl, N.; Schaeffer, R.; Fodor, P.; Kuehnelt, D. Arsenic speciation in farmed Hungarian freshwater fish. *J. Agric. Food Chem.* **2005**, *53*, 9238–9243.

(37) Halder, D.; Biswas, A.; Šlejkovec, Z.; Chatterjee, D.; Nriagu, J.; Jacks, G.; Bhattacharya, P. Arsenic species in raw and cooked rice: Implications for human health in rural Bengal. *Sci. Total Environ.* **2014**, *497-498*, 200–208.

(38) Naito, S.; Matsumoto, E.; Shindoh, K.; Nishimura, T. Effects of polishing, cooking, and storing on total arsenic and arsenic species concentrations in rice cultivated in Japan. *Food Chem.* **2015**, *168*, 294–301.

(39) Raab, A.; Baskaran, C.; Feldmann, J.; Meharg, A. A. Cooking rice in a high water to rice ratio reduces inorganic arsenic content. *J. Environ. Monit.* **2009**, *11*, 41–44.

(40) Sengupta, M. K.; Hossain, M. A.; Mukherjee, A.; Ahamed, S.; Das, B.; Nayak, B.; Pal, A.; Chakraborti, D. Arsenic burden of cooked rice: Traditional and modern methods. *Food Chem. Toxicol.* **2006**, *44*, 1823–1829.

(41) Cubadda, F.; Raggi, A.; Zanasi, F.; Carcea, M. From durum wheat to pasta: effect of technological processing on the levels of arsenic, cadmium, lead and nickel-a pilot study. *Food Addit. Contam.* **2003**, *20* (May 2015), 353–360.

(42) She, L. K.; Kheng, L. C. Arsenic Contents in Some Malaysian Vegetables. *Pertanika* **1992**, *15* (2), 171–173.

(43) Devesa, V.; Macho, M. L.; Jalón, M.; Urieta, I.; Muñoz, O.; Súñer, M. a.; López, F.; Vélez, D.; Montoro, R. Arsenic in cooked seafood products: Study on the effect of cooking on total and inorganic arsenic contents. *J. Agric. Food Chem.* **2001**, *49*, 4132–4140.

(44) Laparra, J. M.; Vélez, D.; Montoro, R.; Barberá, R.; Farré, R. Bioaccessibility of inorganic arsenic species in raw and cooked Hizikia fusiforme seaweed. *Appl.*

549 *Organomet. Chem.* **2004**, *18*, 662–669.

550 (45) Devesa, V.; Súñer, M. a.; Algora, S.; Vélez, D.; Montoro, R.; Jalón, M.; Urieta, I.; Macho,
551 M. L. Organoarsenical species contents in cooked seafood. *J. Agric. Food Chem.* **2005**,
552 *53*, 8813–8819.

553 (46) Dahl, L.; Molin, M.; Amlund, H.; Meltzer, H. M.; Julshamn, K.; Alexander, J.; Sloth, J. J.
554 Stability of arsenic compounds in seafood samples during processing and storage by
555 freezing. *Food Chem.* **2010**, *123* (3), 720–727.

556 (47) Devesa, V.; Martínez, A.; Súñer, M. A.; Benito, V.; Vélez, D.; Montoro, R. Kinetic study
557 of transformations of arsenic species during heat treatment. *J. Agric. Food Chem.*
558 **2001**, *49* (V), 2267–2271.

559 (48) Baer, I.; Baxter, M.; Devesa, V.; V??lez, D.; Raber, G.; Rubio, R.; Llorente-Mirandes, T.;
560 Sloth, J. J.; Robouch, P.; de la Calle, B. Performance of laboratories in speciation
561 analysis in seafood - Case of methylmercury and inorganic arsenic. *Food Control* **2011**,
562 *22* (12), 1928–1934.

563 (49) Pétursdóttir, Á. H.; Gunnlaugsdóttir, H.; Krupp, E. M.; Feldmann, J. Inorganic arsenic in
564 seafood: does the extraction method matter? *Food Chem.* **2014**, *150*, 353–359.

565 (50) Moreira, C. M.; Duarte, F. A.; Lebherz, J.; Pozebon, D.; Flores, E. M. M.; Dressler, V. L.
566 Arsenic speciation in white wine by LC-ICP-MS. *Food Chem.* **2011**, *126* (3), 1406–1411.

567 (51) Raab, A.; Ferreira, K.; Meharg, A. A.; Feldmann, J. Can arsenic-phytochelatin complex
568 formation be used as an indicator for toxicity in Helianthus annuus? *J. Exp. Bot.* **2007**,
569 *58* (6), 1333–1338.

570 (52) Foster, S.; Maher, W.; Krikowa, F.; Apte, S. A microwave-assisted sequential extraction
571 of water and dilute acid soluble arsenic species from marine plant and animal tissues.
572 *Talanta* **2007**, *71* (2), 537–549.

573 (53) CEN. *EN 16802:2016 - Determination of inorganic arsenic in foodstuffs of marine and*
574 *plant origin by anion-exchange HPLC-ICP-MS*; 2016.

575 (54) Narukawa, T.; Inagaki, K.; Kuroiwa, T.; Chiba, K. The extraction and speciation of
576 arsenic in rice flour by HPLC-ICP-MS. *Talanta* **2008**, *77* (1), 427–432.

577 (55) Hajeb, P.; Sloth, J. J.; Shakibazadeh, S.; Mahyudin, N. A.; Afsah-Hejri, L. Toxic elements
578 in food: Occurrence, binding, and reduction approaches. *Compr. Rev. Food Sci. Food*
579 *Saf.* **2014**, *13* (4), 457–472.

580 (56) Gray, P. J.; Conklin, S. D.; Todorov, T. I.; Kasko, S. M. Cooking rice in excess water
581 reduces both arsenic and enriched vitamins in the cooked grain. *Food Addit. Contam.*
582 *Part A* **2015**, *0049* (February), 1–8.

583 (57) Raab, A.; Baskaran, C.; Feldmann, J.; Meharg, A. a. Cooking rice in a high water to rice
584 ratio reduces inorganic arsenic content. *J. Environ. Monit.* **2009**, *11*, 41–44.

585 (58) Wishart, D. S.; Tzur, D.; Knox, C.; Eisner, R.; Guo, A. C.; Young, N.; Cheng, D.; Jewell, K.;
586 Arndt, D.; Sawhney, S.; et al. HMDB: The human metabolome database. *Nucleic Acids*
587 *Res.* **2007**, *35* (SUPPL. 1), 521–526.

588 (59) European Food Safety Authority. Dietary exposure to inorganic arsenic in the
589 European population. *EFSA J.* **2014**, *12* (3), 3597.

590 (60) European Food Safety Authority. Scientific Opinion on Arsenic in Food. *EFSA J.* **2010**, *7*

(10), 1351.

(61) Gray, P. J.; Conklin, S. D.; Todorov, T. I.; Kasko, S. M. Cooking rice in excess water reduces both arsenic and enriched vitamins in the cooked grain. *Food Addit. Contam. Part A. Chem. Anal. Control. Expo. Risk Assess.* **2016**, *33* (1), 78–85.

(62) Lai, V. W. M.; Sun, Y.; Ting, E.; Cullen, W. R.; Reimer, K. J. Arsenic speciation in human urine: Are we all the same? *Toxicol. Appl. Pharmacol.* **2004**, *198*, 297–306.

(63) Visciano, P.; Perugini, M.; Manera, M.; Abete, M. C.; Tarasco, R.; Salese, C.; Amorena, M. Total arsenic in raw and boiled portions of Norway lobster (Nephrops norvegicus) from the Central Adriatic Sea. *J. Agric. Food Chem.* **2013**, *61*, 12445–12449.

List of Tables

Table 1 : Overview of the treatments of sampled foodstuffs

	Raw	Boiled (no excess water)	Boiled (excess water discarded)[a]	Boiled (soup)[a]	Steamed	Fried	Prepared in microwave	Soaked
White rice	x	x	x					
Brown rice	x	x	x					
Carrots	x		x		x			
Leeks	x		x		x			
Onion	x					x[b,c]		
Mushroom	x					x[b,c]		
Cod	x					x[b,c]	x	
Trout	x					x[b,c]	x	
Scampi	x					x[d]		
Mussels	x				x[3]			
Scallops	x					x[d]		
Nori	x			x				
Hijiki	x		x	x				x

[a] Water brought to boiling temperature before addition of the sample
[b] Vegetable butter added and melted before addition of the sample
[c] No water added
[d] No fat added

Table 2 : Limits of quantification of the methods used for determination of As_{tot} and As species.

	Weight (mg)	LOQ in solution As_{tot} ($\mu g\ L^{-1}$)	LOQ in matrix As_{tot} ($mg\ kg^{-1}$ WW)	LOQ in solution As species ($\mu g\ L^{-1}$)	LOQ in matrix As species ($mg\ kg^{-1}$ WW)
Rice	500	0.01	0.0020	0.10	0.0020
Vegetables, wine	1000	0.01	0.0010	0.10	0.0010
Seaweed	250	0.01	0.0040	0.10	0.0040
Fish, Crustacean, Molluscs	500	0.01	0.0020	0.50	0.010

Table 3 : Overview of HPLC properties per method. Rice and vegetables are extracted with diluted acid; wine, fish, molluscs and crustacean are extracted with ultra pure water. Arsenic speciation analysis in rice, vegetables and wine is performed using anion exchange chromatography. Arsenic speciation in fish, molluscs and crustacean is performed using ion-pair anion exchange chromatography.

	Method 1			**Method 2**			**Method 3**					
Matrices	Rice Vegetables Algae			Wine			Fish Molluscs Crustacean					
Extraction solution	0.63 M HNO_3 3% H_2O_2			ultra pure-H_2O			ultra pure-H_2O					
Column	PRP-X-100, Hamilton						IonPac AS7, Dionex					
Injection volume (µL)	60						60					
Mobile phase A	60 mM ammoniumcarbonate						0.05 mM benzenedisulfonic acid, 0.5 mM HNO_3, 0.5 % MeOH					
Mobile phase B	ultra pure-H_2O						0.05 mM benzenedisulfonic acid, 50 mM HNO_3, 0.5 % MeOH, (pH 1.8)					
Flow rate	1.0 mL min^{-1}						1.0 mL min^{-1}					
Elution gradient												
Time (min)	0	0.5	10	13	15	16	0	3	4	16	17	21
%A	20	20	100	100	20	20	100	100	30	30	100	100
%B	80	80	0	0	80	80	0	0	70	70	0	0

1 **Table 4 : Concentrations of total As and As species (mg kg^{-1} WW) and % DW in the raw matrices ('as sold'). The standard deviation of three replicates is indicated between brackets.**

MATRIX		Dry weight %	As	As_i	AB	DMA	MA
			mg kg^{-1} WW ('as sold')				
Rice							
White rice	Lot 1	89	0.169 (± 0.003)	0.145 (± 0.006)	-	0.048 (±0.005)	0.0027 (± 0.0003)
	Lot 2	89	0.169 (± 0.005)	0.131 (± 0.004)	-	0.061 (± 0.006)	0.0053 (± 0.0006)
	Lot 3	90	0.279 (± 0.003)	0.109 (± 0.005)	-	0.172 (± 0.020)	0.0065 (± 0.0004)
Brown rice	Lot 1	90	0.162 (±0.004)	0.141 (± 0.004)	-	0.038 (± 0.002)	0.0025 (± 0.0008)
	Lot 2	90	0.143 (± 0.003)	0.150 (± 0.002)	-	0.022 (± 0.001)	0.0020 (± 0.0004)
	Lot 3	90	0.352 (± 0.009)	0.109 (± 0.004)	-	0.223 (±0.012)	0.0086 (± 0.0003)
Vegetables							
Carrot	Lot 1	8.3	0.0037 (± 0.0004)	0.0030 (± 0.0001)	-	<0.0010	<0.0010
	Lot 2	8.5	0.0016 (± 0.0004)	0.0010 (± 0.0002)	-	<0.0010	<0.0010
	Lot 3	10.4	0.0176 (±0.0034)	0.0126 (± 0.0002)	-	<0.0010	<0.0010
Leek	Lot 1	7.2	0.0107 (± 0.0011)	0.0119 (± 0.0011)	-	-	-
	Lot 2	9.9	0.0070 (± 0.0013)	0.0054 (± 0.0002)	-	<0.0010	-
	Lot 3	9.3	0.0091 (± 0.0023)	0.0101 (± 0.0023)	-	-	-
Onion	Lot 1	10.4	0.0029 (± 0.0008)	0.0015 (± 0.0003)	-	<0.0010	<0.0010
	Lot 2	17.1	0.0099 (± 0.0029)	0.0088 (± 0.0015)	-	-	-
	Lot 3	13.0	0.0174 (± 0.0016)	0.0125 (± 0.0022)	-	-	-
Mushrooms							
Mushroom	Lot 1	6.7	0.0175 (± 0.0089)	0.0019 (± 0.0001)		0.0045 (± 0.0002)	-
	Lot 2	6.2	0.0345 (± 0.0055)	0.0024 (± 0.0002)		0.0067 (± 0.0008)	-
	Lot 3	6.3	0.0111 (± 0.0006)	0.0016 (± 0.0002)		0.0044 (± 0.0004)	-
Drinks							
Wine	Lot 1		0.0069 (± 0.0004)	0.0063 (± 0.0003)	-	<0.0010	-
	Lot 2		0.0046 (± 0.0002)	0.0041 (± 0.0003)	-	<0.0010	-
	Lot 3		0.0114 (± 0.0003)	0.0099 (± 0.0005)	-	<0.0010	-
Algae							
Nori	Lot 1	90	35.5 (± 1.5)	0.151 (± 0.007)	11.6 (± 1.3)[a]	0.173 (± 0.006)	0.121 (± 0.039)
	Lot 2	94	34.0 (± 1.0)	0.0703 (± 0.0101)	8.99 (± 0.95) [a]	0.0804 (± 0.0028)	0.127 (± 0.021)
	Lot 3	83	29.5 (± 0.3)	0.0506 (± 0.0174)	9.35 (± 1.76) [a]	0.0464 (± 0.0011)	0.107 (± 0.045)
Hijiki	Lot 1	85	102.9 (± 3.86)	77.5 (± 2.9)	5.10 (± 0.88) [a]	2.2 (± 0.1)	-
Fish and crustacean							
Cod	Lot 1	18	10.4 (± 2.7)	< 0.010	9.69 (± 1.98)	< 0.010	< 0.010
	Lot 2	19	3.40 (± 3.86)	< 0.010	3.01 (± 0.84)	< 0.010	< 0.010
	Lot 3	20	2.09 (± 0.06)	< 0.010	1.90 (± 0.11)	< 0.010	< 0.010
Trout	Lot 1	25	0.361 (± 0.041)	< 0.010	0.279 (± 0.037)	< 0.010	< 0.010
	Lot 2	23	0.422 (± 0.036)	< 0.010	0.413 (± 0.005)	< 0.010	< 0.010
	Lot 3	25	0.865 (± 0.088)	< 0.010	0.801 (± 0.244)	< 0.010	< 0.010

Scampi	Lot 1	20	0.337[§]	< 0.010	0.277 (± 0.011)	< 0.010	< 0.010
	Lot 2	20	0.333[§]	< 0.010	0.222 (± 0.030)	< 0.010	< 0.010
	Lot 3	17	5.14[§]	< 0.010	4.72 (± 0.74)	< 0.010	< 0.010
Molluscs							
Mussel	Lot 1	12	2.01 (± 0.11)	0.0128 (± 0.0006)	0.825 (± 0.012)	0.0129 (± 0.0012)	< 0.010
	Lot 2	8.5	1.53 (± 0.02)	0.0234 (± 0.0039)	0.298 (± 0.004)	0.0164 (± 0.0027)	< 0.010
	Lot 3	10	1.99 (± 0.04)	< 0.010	0.444 (± 0.007)	0.0198 (± 0.0031)	< 0.010
Scallop	Lot 1	22	1.24 (± 0.03)	< 0.010	0.905 (± 0.017)	< 0.010	< 0.010
	Lot 2	14	0.593 (± 0.035)	< 0.010	0.314 (± 0.001)	< 0.010	< 0.010
	Lot 3	14	0.577 (± 0.019)	< 0.010	0.346 (± 0.007)	< 0.010	< 0.010

[a] AB and/or other cationic and uncharged species

Table 5 : Total As and As species after application of the various food processing methods expressed in % of the concentration in the raw foodstuff (DW base). Standard deviation of three independent replicas is given in brackets. Significant effects are indicated with * in case an interaction was observed between the factor lot and treatment, and with $ if a main effect of treatment was seen.

MATRIX			Dry weight (%)	As	As_i	AB	DMA
				% of concentration in raw matrix			
				Rice			
White rice	Boiling with excess water discarded (5/32)	Lot 1	37	49 (±3)*	34 (±1)*		46 (±5)*
		Lot 2	37	60 (±4)*	47 (±1)*		65 (±7)*
		Lot 3	39	59 (±4)*	42 (±1)*		63 (±8)*
	Boiling until dryness (10/32)	Lot 1	37	106 (±10)	79 (±3)		108 (±13)
		Lot 2	37	111 (±6)	96 (±5)		97 (±12)
		Lot 3	39	101 (±9)	93 (±7)		65 (±10)*
Brown rice	Boiling with excess water discarded (5/32)	Lot 1	40	43 (±5)*	35 (±4)*		25 (±11)*
		Lot 2	35	53 (±2)*	40 (±3)*		54 (±3)*
		Lot 3	52	75 (±6)*	46 (±5)*		75 (±11)*
	Boiling until dryness (10/32)	Lot 1	38	89 (±25)	101 (±3)		94 (±5)
		Lot 2	42	115 (±19)	89 (±11)		86 (±11)
		Lot 3	40	116 (±20)	99 (±14)		97 (±14)
				Vegetables			
Carrot	Boiling with excess water discarded	Lot 1	6.6	79 (±10)	79 (±14)		
		Lot 2	8.3	88 (±21)	88 (±23)		
		Lot 3	8.6	69 (±7)*	69 (±7)*		
	Steamed	Lot 1	12	78 (±16)	84 (±15)		
		Lot 2	12	86 (±24)	99 (±23)		
		Lot 3	12	59 (±9)*	59 (±7)*		
Leeks	Boiling with excess water discarded	Lot 1	6.5	63 (±6)$	58 (±17)$		
		Lot 2	6.8	67 (±25)$	73 (±8)$		
		Lot 3	6.0	76 (±27)$	82 (±26)$		
	Steamed	Lot 1	8.1	66 (±16)$	57 (±10)$		
		Lot 2	8.8	62 (±21)$	75 (±21)$		
		Lot 3	9.8	82 (±28)$	75 (±26)$		
Onion	Fried	Lot 1	20	79 (±41)	73 (±37)		
		Lot 2	26	107 (±52)	85 (±37)		
		Lot 3	23	112 (±21)	102 (±18)		
Mushroom	Fried	Lot 1	11	101 (±8)	98 (±9)		91 (±10)
		Lot 2	19	49 (±8)*	56 (±5)*		51 (±7)*
		Lot 3	11	91 (±8)	83 (±12)		82 (±12)
				Drinks			
Wine	Heated	Lot 1	-	110 (±11)	101 (±7)		
		Lot 2	-	95 (±7)	98 (±9)		
		Lot 3	-	101 (±6)	103 (±7)		
				Algae			
Nori	Boiled (soup)	Lot 1	2.4	94 (±4)		95 (±15)[a]	
		Lot 2	2.8	76 (±22)		91 (±17)[a]	
		Lot 3	2.3	77 (±8)		81 (±16)[a]	
Hijiki	Soaked (excess water removed)	Lot 1	6.8	72 (±5)*	60 (±6)*	93 (±20)[a]	30 (±5)*
	Boiling with excess water discarded	Lot1	6.5	50 (±3)*	35 (±3)*	89 (±14)[a]	41 (±6)*
	Boiled (soup)	Lot1	3.2	97 (±6)	93 (±14)	108 (±22)[a]*	98 (±7)
				Fish and crustacean			
Cod	Fried	Lot 1	24	106 (±53)		107 (±25)	
		Lot 2	24	102 (±11)		82 (±11)	
		Lot 3	24	96 (±15)		86 (±4)	
	Prepared in Microwave	Lot 1	22	107 (±25)		110 (±23)	
		Lot 2	23	82 (±11)		87 (±25)	
		Lot 3	22	86 (±4)		88 (±6)	
Trout	Fried	Lot 1	26	82 (±16)		86 (±20)	

		Lot 2	24	80 (±8)		72 (±4)	
		Lot 3	25	118 (±)23		115 (±37)	
	Prepared in Microwave	Lot 1	28	79 (±15)		74 (±14)	
		Lot 2	24	71 (±21)		66 (±12)	
		Lot 3	25	117 (±42)		92 (±38)	
Scampi	Fried	Lot 1	21	110 (±7)		111 (±13)	
		Lot 2	27	81 (±2)		72 (±12)	
		Lot 3	22	78 (±4)		82 (±13)	
				Molluscs			
Mussel	Steamed	Lot 1	28	87 (±6) [$]	58 (±16)*	64 (±7)*	56 (±17)*
		Lot 2	29	88 (±9) [$]	88 (±9)*	68 (±12)*	
		Lot 3	31	76 (±6) [$]		76 (±9)*	
Scallop	Fried	Lot 1	27	94 (±4)		109 (±5)	
		Lot 2	17	92 (±2)		100 (±5)	
		Lot 3	17	98 (±5)*		115 (±6)*	

[a] AB and other cationic and uncharged species

Table 6 : Decrease (% - DW base) of arsenicals after different food processing methods: current findings compared with various literature sources (n.s.: not significant).

Food matrix	Food processing	element	% decrease	Reference
Rice	Washing	As_{tot}	n.s.	[37]
	"	As_i	n.s.	
	Boiling with discarding excess of water	As_{tot}	7.5-66%	
	"	As_i	0.3-74%	
Rice	Washing	As_{tot}	2-24 %	[38]
	"	As_i	1-29%	
	"	DMA	n.s.	
	Boiling till dryness	As_{tot}	n.s.	
	"	As_i	n.s.	
	"	DMA	n.s.	
Rice	Washing + boiling with discarding excess of water	As_{tot}	28-45%	[39]
	"	As_i	40-49%	
	"	Organic As	n.s.	
Rice	Washing + boiling with discarding excess of water	As_{tot}	54%	[40]
Rice	Washing	As_{tot}	0-12%	[56]
	"	As_i	0-19%	
	Boiling till dryness	As_{tot}	n.s.	
	"	As_i	n.s.	
	Boiling with discarding excess of water	As_{tot}	15-65%	
	"	As_i	25-70%	
White rice	Boiling with discarding excess of water	As_{tot}	40-51%	This study
	"	As_i	53-66%	
	"	DMA	35-54%	
	Boiling till dryness	As_{tot}	n.s.	
	"	As_i	n.s.	
	"	DMA	n.s.	
Brown rice	Boiling with discarding excess of water	As_{tot}	25-57%	
	"	As_i	54-65%	
	"	DMA	25-75%	
	Boiling till dryness	As_{tot}	n.s.	
	"	As_i	n.s.	
	"	DMA	n.s.	
Pasta	Boiling with discarding excess of water	As_{tot}	60%	[41]
Vegetables	Boiling with discarding excess of water	As_{tot}	17-60%	[42]
Carrots	Boiling with discarding excess of water	As_{tot}	12-32%	This study
	"	As_i	12-31%	
	Steaming with discarding excess of water	As_{tot}	14-41%	
	"	As_i	1-41%	
leeks	Boiling with discarding excess of water	As_{tot}	24-37%	This study
	"	As_i	18-42%	
	Steaming with discarding excess of water	As_{tot}	18-38%	
	"	As_i	25-43%	
Onions	Frying	As_{tot}	n.s.	This study
	"	As_i	n.s.	
Mushrooms	Frying	As_{tot}	0-51%	This study
	"	As_i	0-44%%	
	"	DMA	0-49%	
Mussels	Steaming with discarding excess of water	As_{tot}	43%	[62]
	"	AB	67%	
Mussels	Steaming with discarding excess of water	As_{tot}	12-24%	This study
	"	As_i	12-42%	
	"	AB	24-36%	
Scallop	Frying	As_{tot}	n.s.	This study
	"	AB	n.s.	
Lobster	Boiling with discarding excess of water	As_{tot}	17%	[63]
Cod	Friying	As_{tot}	n.s.	This study
	"	AB	n.s.	
Cod	Preparing in microwave	As_{tot}	n.s.	This study
	"	AB	n.s.	
Trout	Friying	As_{tot}	n.s.	This study

	“	AB	n.s.	
Trout	Preparing in microwave	As_{tot}	n.s.	This study
	“	AB	n.s.	
Scampi	Friyng	As_{tot}	n.s.	This study
	“	AB	n.s.	
Hijiki	Boiling with discarding excess of water	As_{tot}	30-43%	[44]
	“	As_i	46-50%	
Hijiki	Soaking with discarding excess of water	As_{tot}	36-47%	[23]
	“	As_i	38-62%	
	Boiling with discarding excess of water	As_{tot}	65-79%	
	“	As_i	80-95%	
Hijiki	Soaking with discarding excess of water	As_{tot}	28%	This study
	“	As_i	40%	
	“	AB	7%	
	“	DMA	70%	
	Boiling with discarding excess of water	As_{tot}	50%	This study
	“	As_i	65%	
	“	AB	11%	
	“	DMA	59%	
	Boiling (soup)	As_{tot}	n.s.	This study
		As_i	n.s.	
		AB	n.s.	
		DMA	n.s.	
Nori	Boiling (soup)	As_{tot}	n.s.	This study
		AB	n.s.	

List of Figures

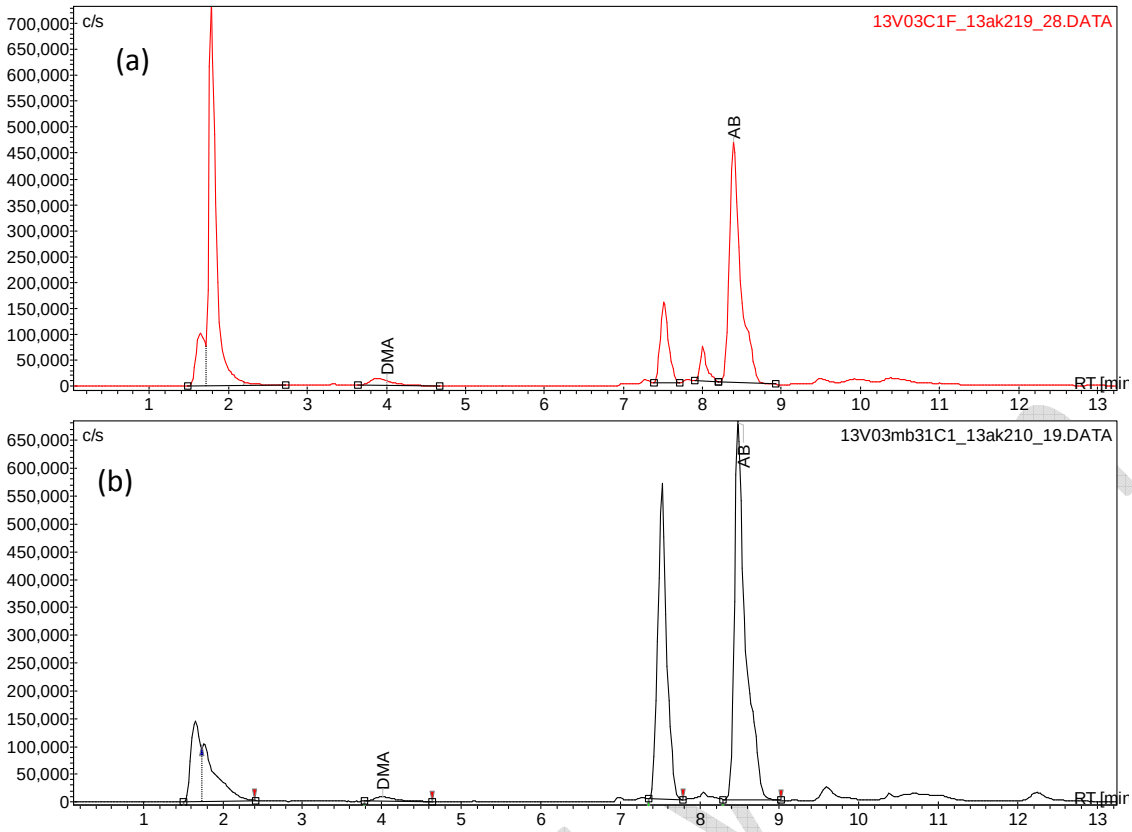

Figure 1 : Chromatograms of mussel extracts before (a) and after (b) steaming. Retention time (minutes) is given in the X-axis and the counts per second (c/s) are shown in the y-axis. The relative peak areas clearly differ before and after the steaming treatment.

TOC Graphic