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Advanced extraction methods for arsenic speciation in environmental samples

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Abstract

Total element concentrations of Li, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, As, Se, Rb, Sr, Mo, Cd, Ba, Ce, Gd, Hg, Tl, Pb, Bi and U have been determined in five different Wakame and four different Nori samples from five different manufacturers by inductively coupled plasma mass spectrometry (ICPMS), with a special focus on arsenic. No significant deviations in means of total metal/metalloid concentrations were observed when compared to published literature.

Total inorganic arsenic was determined in two different algae samples (Wakame and Nori), based on DIN EN 16802:2016-07. The results proved that the sum of arsenous acid and arsenic acid in those two algal species is less than 0.1 mg As/kg dry weight, thus human consumption is considered safe.

Further on, the extraction of arsenic from the brown macroalgae *Undaria pinnatifida* (commercially available as Wakame product) was studied and optimised. Low extraction yields (~5 %) in the case of conventional polar extracting solvents like water and methanol have been improved more than 10-fold utilizing aqueous Cholinium Lysinate as the extracting solvent. In contrast, yields with nonpolar solvents were improved only minimally by the addition of 1-ethyl-3-methylimidazolium acetate.

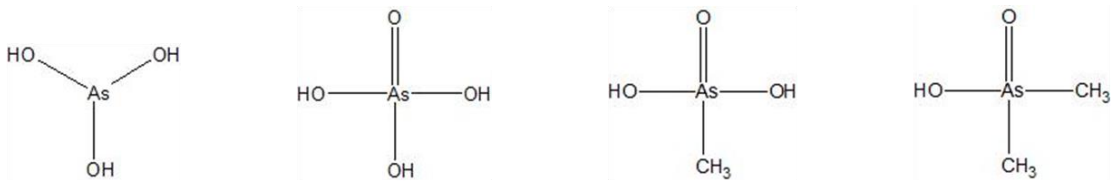
Arsenic speciation analysis of the extracts and conclusive spike experiments with arsenosugar standards proved that the major arsenic species found in Nori and Wakame is the phosphate arsenosugar (PO_4-Sugar). However, in the case of the latter it is assumed that the arsenosugar is an alkaline decomposition product of its arsenic containing phospholipid analogue, which is the major arsenical found in fresh *Undaria pinnatifida* algae. Stability tests of the Cholinium Lysinate extracts showed that the PO_4-Sugar hydrolyses during storage to form the glycerol sugar (OH-Sugar).

Lastly, spike recovery tests showed that Wakame adsorbs arsenate and partly also methylarsonic acid in the presence of Cholinium Lysinate, thus the combination proving itself to be an interesting candidate for water purification systems.

1 Introduction

Arsenic is a well-known and well-studied metalloid which can be found in nature in many forms and oxidation states (ranging from -3 through 0, +3, +5). Its abundance in earth's crust is only around 2 mg As/kg, but even so it is readily found in lots of minerals (especially sulphide ores), being present either as the pure element, as a sulphide compound, in ores with other metals or in its oxidised form (arsenite or arsenate salts) [1, 2].

Natural phenomena (such as erosion), as well as anthropogenic activities (industrial pollution in developing countries) lead to an increase of arsenic concentrations in the atmosphere and in all types of waters. This is indeed worrying since inhalation and/or contaminated water consumption represents a major risk for human health. It has been reported that inorganic arsenic is carcinogenic, causing skin cancer as well as other types of cancer [3]. The trivalent arsenite is more toxic than arsenate and the corresponding MA (methylarsonic acid) and DMA (dimethylarsinic acid) are less toxic [1]. Chemical structures of these trivalent and pentavalent arsenic species are shown in Figure 1.



Arsenous acid	Arsenic acid	Methylarsonic acid	Dimethylarsinic acid
As(III)	As(V)	MA	DMA

Figure 1: Chemical structures of arsenous acid [As(III)], arsenic acid [As(V)], methylarsonic acid (MA) and dimethylarsinic acid (DMA)

Nonetheless, toxicity is mainly related to oxidation state and molecular structure but is also dependent on the bioavailability of the arsenicals. Furthermore, the bioavailability may also vary from individual to individual not having the same capability to metabolize arsenic

species [4, 5]. Lastly, particle size and exposure time do also play an important role on the toxicity of different arsenicals [1, 6, 7].

The recommended limit of arsenic in drinking water is 10 µg/L (set by the WHO [8]), but it is often the case that concentrations exceed this threshold (Bangladesh, India, Mexico, some states in USA [8, 9]). This limit refers to the total arsenic concentration in drinking water, and since As in drinking water mainly occurs in the form of inorganic arsenic (iAs) species, in terms of risk assessment it is enough to establish limits based on the total arsenic concentration [1, 10].

However, not only water intake represents a risk in terms of arsenic uptake for humans, but also food consumption that contains high amounts of arsenic. Environmental samples accumulate and transform inorganic arsenic species into various other forms of arsenic species [11]. It is also possible that the soil/sediment already contains organic arsenicals (produced by microorganisms) which are absorbed by fauna. Thus, arsenic species can be found in all sorts of merchandise, like rice, dietary products, vegetables, fungi, and so on. Especially the later can have high hyperaccumulation rates up to 7000 mg As/kg dm (even edible mushrooms as in the case of *Laccaria laccata´*s caps which contained 143 ± 8 mg As/kg dm) [12, 13].

Similarly, marine, and freshwater organisms readily absorb the dissolved arsenic, creating a biological chain of arsenic transfer from plant to small herbivorous animals all the way to human consumers. Although one would expect a biomagnification of arsenic along the food chain, luckily that is not the case. Seaweeds usually contain more total arsenic than fish, shellfish, and shrimps [11, 14]. It should also be noted that arsenic compounds biochemically readily change in this food chains. The major components of seaweeds are arsenosugars in contrast to fish, which contain mostly arsenobetaine [15].

Since arsenic is called the “King of Poisons” it should set alarm bells ringing that the biota and especially commercial food contain such high amounts of this toxic element. However, for accurate risk assessment it is not enough to determine total arsenic levels in food samples, due to the difference in toxicity between arsenic species. For instance arsenosugars are considered to be non-toxic [4, 5, 7]. Therefore, it is of great interest to develop accurate and robust analytical methods for quantitative arsenic speciation in environmental samples and

adjust existing regulations or formulate new ones for the maximum allowed As species concentration in these food samples. Unfortunately, due to different matrix constitution and different species content, same extraction procedures will not yield same efficiencies. Furthermore, extraction parameters should be set properly to avoid altering the chemical composition of the analyte of interest (for instance oxidation of As(III) to As(V) due to harsh conditions) [16]. Also, the lack of appropriate CRMs and/or standards makes it difficult to check the trueness of the given analytical method. It is necessary to develop proper certified reference materials with qualitative and quantitative species information, as well as provide cautions regarding species stability during sample preparation, handling and storage [14, 15, 17].

It is important to define the analytical question properly in each case. What information, what analytes are to be extracted and analysed? Is it important to maximise the extraction efficiency of arsenic by using severe conditions and thus running into risk of partially destroying organic arsenicals? Or is it more of interest to use mild conditions for species preservation risking non-quantitative extraction? Whichever of the above it may be, it is significant to consider performing more than one extraction procedure to address such complex research questions the right way [16].

The upcoming chapters will mainly focus on commercially available macroalgae and the speciation of arsenic in those samples. The role of sampling and sample preparation for the accurate arsenic species determination will be discussed. Moreover, it should be pinpointed that sampling fresh algae from water sources and purchasing commercially available, dried, and processed algae from local stores can already result in different speciation consequently. Again, it must be underlined, that As speciation in environmental samples is always connected to the core analytical research question.

Special emphasis will be put on evaluating different extraction procedures for As species extraction from seaweed samples, especially less commonly applied extraction procedures like the use of ionic liquids and supercritical fluids will also be discussed.

1.1 The role of algae in our daily life

Algae are important nutrients in the eastern culture, exhibiting numerous health beneficial properties. In the rise of globalism, this food has become commercially available for consumers all around the world. Beside the usage of marine algae for direct consumption, there are branches that exploit their complex chemical composition, for instance extracting biologically active compounds or antioxidants, for its own products as in the case of pharmaceutic and cosmetic goods. Supercritical fluid extraction (with the usage of ethanol as a co-solvent) of the brown algae *Undaria pinnatifida* yields an oil extract containing fucoxanthin which functions as an anticancer carotenoid [18]. Another study extracted galactofucan sulphate from the same algae with diluted sulfuric acid. Galactofucan sulphate is believed to act as an antiviral compound [19]. In the case of cosmetic industry algal extracts are exploited as moisturizers, thickening agents, anti-aging lubes. Since the algae are natural and sustainable products, possibly producing less chemical waste while extraction in comparison with conventional synthetic methods in cosmetics, they have become a rather interesting opportunity also for larger industrial production [20].

1.1.1 Arsenic species in algae

In terms of speciation analysis algae appear to be bioaccumulators of arsenic resulting in high content of different species in all types of seaweeds. The most important and abundant class of arsenic species present in seaweeds are arsenosugars followed by other organic arsenicals such as aliphatic arsenolipids and arsenosugar phospholipids. Arsenous acid, dimethylarsinic acid and methylarsonic acid are present at low concentrations, whereas arsenic acid can be present also at higher concentrations as in the case of *Laminaria digitata* [21]*, Sargassum puliferum* [22] and Hijiki samples (up to 100 mg As(V)/kg dm) [23].

Nevertheless, many other forms of arsenosugars have also been identified and characterised. Therefore, a proper nomenclature needs to be established and there have been some attempts to abbreviate arsenosugars, because IUPAC-nomenclature is not sufficiently clear enough for communication. For instance the IUPAC name of SO_4-Sugar is (2S)-3-[5-deoxy-5-(dimethylarsinoyl)-β-D-ribofuranosyloxy]-2-hydroxypropyl hydrogen sulphate [24], which is too long and complicated for routine communication.

Generally speaking, arsenosugars consist of a 5-deoxy ribose moiety and different substituents at the C1 (side chain moiety) and C5 (arsenic containing group). The arsenic containing group can be a dimethylated arsinoyl or thioarsionyl substituent or it can be even trimethylated and positively charged. As for the C1 substituent, the most common are the glycerol, glycerol-phosphate ester, sulfonate and sulphate groups [25 – 27]. For a clearer overview, their structural formulas, as well as their abbreviations used thorough the master thesis are illustrated in Figure 2.

Other species, such as arsenocholine (AC), the tetramethylarsonium ion (TETRA) and arsenobetaine (AB) are not incorporated in algae, but especially the latter can be falsely detected, when sampling and sample pre-treatment was not carried out properly (removing sediments, bacteria and epiphytes coexisting with the algae) [1, 28].

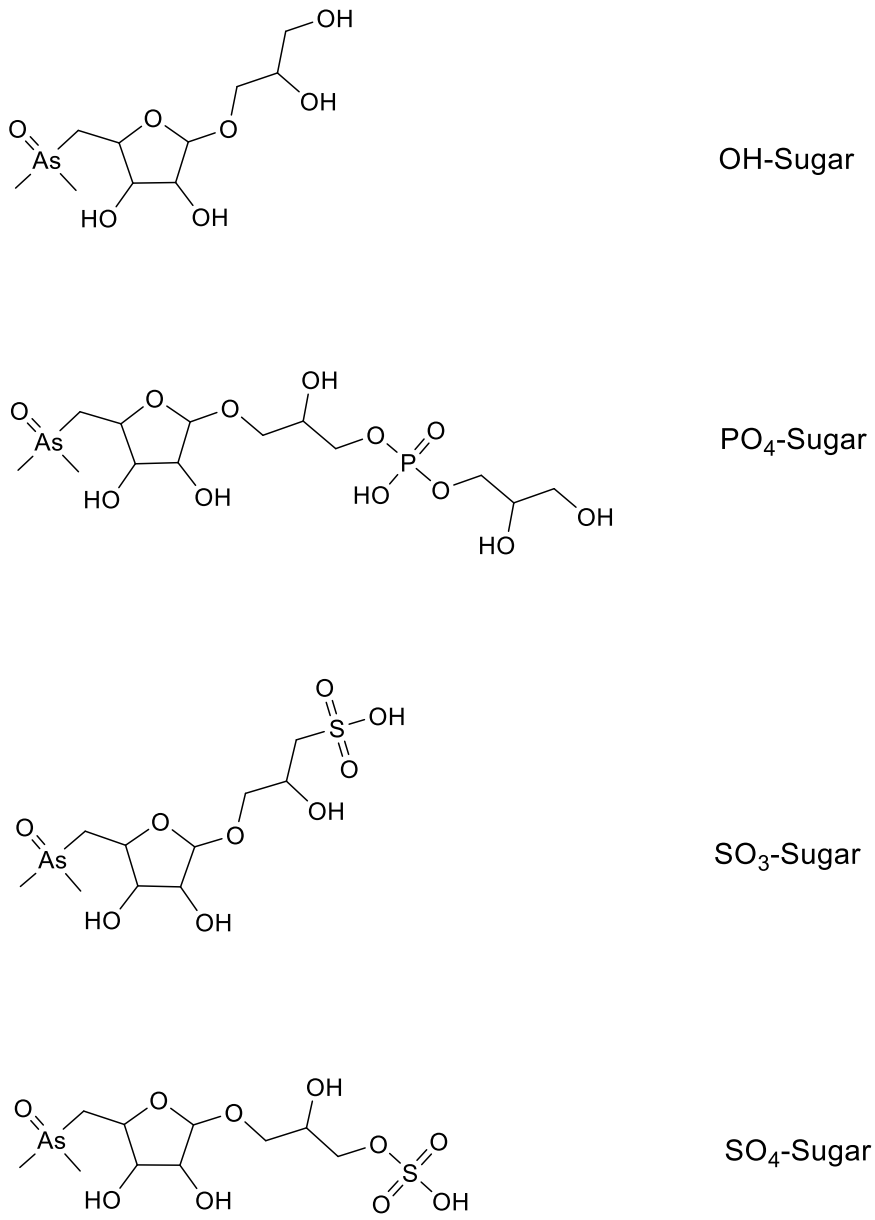


Figure 2: Chemical structures of dimethylarsinoylribosides commonly present in seaweed samples

But how is it possible that seaweeds contain such a vast variety of different species, although seawater contains mainly inorganic arsenic?

It is proposed that seaweeds cannot distinguish between PO_4^{3-} and AsO_4^{3-} (in seawater both anions mostly present as $H_2PO_4^-$/ HPO_4^{2-} and $H_2AsO_4^-$/$HAsO_4^{2-}$ anions). Seawater being rich on both anions makes this combination an excellent „brewing system" for arsenic species (especially arsenosugars and arsenosugar phospholipids). The biotransformation of arsenate to other species is yet to be fully understood. Nevertheless, it is proposed that seaweed transforms the absorbed inorganic As via enzymatically catalysed methylation to MA and

further to DMA, which are then further processed to yield high amounts of sugars and lipids [6, 29]. However different species of macroalgae have variable rates of bioaccumulation and biotransformation of arsenic, making each sample a unique case of study. Furthermore, uptake does not depend only on the type of algae (age, type and absorption capacity) and the arsenic content in seawater, but also on synergistic effects, such as symbiosis of algae with other lifeforms, sampling site and other factors ranging from salt and other elements concentrations, pH factor, temperature of water and so on [1, 10, 30, 31].

Comparing different types of algae, most commonly brown (*Phaeophyta*), red (*Rhodophyta*) and green (*Chlorophyta*) algae, it is evident that there are taxonomic differences in terms of maximal arsenic uptake and different species concentrations. This is the consequence of different macrostructures of the mentioned types of algae. As a rule of thumb brown algae yield the highest total arsenic concentrations followed by red algae and lastly green ones. *Phaeophyta* are also more variable and total arsenic concentration expand over a wider range (2 – 240 mg As/kg dm) than in the case of *Rhodophyta* and *Chlorophyta*. The same trend is observable for inorganic arsenic content in this three Phylums [6]. However many exceptions can be found, and even concentration levels of the same seaweed species bought from a different manufacturer may scatter drastically [30, 32].

Another interesting difference becomes obvious when comparing seawater and freshwater algae. For instance, Raab et. al investigated arsenic species in the water-soluble fraction of algal extracts, extracted at room temperature and being compared in an interlaboratory trial. The extraction efficiencies ranged from 3 – 96 %. Seawater Hijiki samples having the highest total arsenic concentration (up to 120 mg As/kg dm) nearly yielded a quantitative extraction, whereas fresh water black moss species with lower total arsenic concentration (up to 35 mg As/kg dm) only gave extraction efficiencies up to 5 % [33]. Although the extraction yield could be independent of the water type, and purely of taxonomic difference nature, it should be further investigated.

It is also baffling that arsenic in some samples is nearly non-extractable with water, water/methanol extractants, even at elevated temperatures. For instance, Almela et. al studied the extraction efficiency of As in edible seaweed samples. One of the samples (*Undaria pinnatifida*) showed poor extraction efficiencies in H_2O/MeOH mixtures both at

room temperature and even after cooking (boiling) the algae. Furthermore, the same brown algal species was purchased from another manufacturer and treated the same way as the previous one, yielding ten times higher extraction efficiencies of arsenic [34]. This controversy indicates that the arsenic extractability is already influenced by the different sampling site of the algae and/or is altered due to different pre-processing procedures of the different manufacturers, before the algae is distributed into shops for commercial purchase.

Both the black moss and Wakame samples are intriguing candidates for further investigations, especially to determine the cause of the low extraction yield, as well as the identification of the non-extractable arsenic species.

1.1.2 Stability of arsenosugars

To obtain reliable chromatographic results on arsenosugar concentrations in all kinds of algal extracts, one should consider the stability of these species. Although there is little research conducted in this area of speciation, arsenosugars are prompt to degradation only upon severe conditions (severe acidic conditions and high temperatures). Mild conditions, like moderate pH values and ambient temperatures assure species preservation during extraction [7].

The combination of low pH (gastric juice simulations) and high temperature leads to the inter-conversion of arsenosugars, PO_4-Sugar, SO_4-Sugar and SO_3-Sugar degrades to the OH-Sugar and further on the C5 group of the OH-Sugar is hydrolytically cleaved to yield the plain dimethylarsinoyl fructose as reported by Gamble et. al [35].

Storage stability tests have shown that the arsenosugars are generally stable regardless of storage conditions (dry or aqueous extract, temperature range from -18°C to 60°C). However, as matrix composition changes from sample to sample, to avoid the hydrolysis of PO_4-Sugar and SO_3-Sugar to the OH-Sugar Madsen et. al recommends the addition of minor portions of methanol to the extracts, which acts as an antimicrobial agent, thus preventing biological degradation of the analytes [36].

1.1.3 Stability of arsenolipids

Besides arsenosugars, arsenolipids make up the second largest fraction of arsenic species in many marine algal samples. Therefore, a link between the two must exist. However, there

are even less data on arsenolipid biosynthesis, its role in the specimens and it is not even clear if arsenophospholipids (AsPLs) are synthesized from arsenosugars or is it the other way around, that during the life cycle of the algae the AsPLs break down to arsenosugars [4, 37].

As for arsenolipid stability during extraction, Pétursdóttir et. al noticed that extracting fish samples first with water before hexane extraction, the extractability with hexane was drastically reduced. This suggests that nonpolar arsenolipids are prompt to hydrolysis with water [38]. But what about polar arsenolipids, as in the case of arsenic containing phospholipids? Raab et. al proved, that upon hydrolysing AsPLs (both under acidic and basic conditions), no arsenic containing fatty acids were formed. Moreover, the results showed that the fatty acid ester bonds of the AsPLs are cleaved off, living behind PO_4-Sugar and OH-Sugar, while the latter is thermodynamically more stable [39]. For a deeper understanding however more research is needed, especially on the biosynthetic connection between sugars and lipids. Furthermore, the lack of appropriate CRMs for species studies makes this task nearly impossible, since there is no way of proving whether species interconversion occurs due to different parameters used for extraction.

1.1.4 Overview: analytical process

The following subchapters discuss all necessary steps in the analytical process for arsenic speciation analysis in seaweed samples. These steps are thoroughly examined in several review articles, therefore they are discussed only briefly here. Special focus will be put on the two less commonly applied extraction methods used in the master thesis (ionic liquids and supercritical fluids as solvents of interest).

1.1.4.1 Sampling and sample processing

The first and the most important step in an analytical process is the sampling procedure. However, the current research is not dealing with fresh algal samples, but commercially available, dried, and pre-processed edible kelp, therefore sampling and fresh sample clean-up was not necessary. Nevertheless, already the proper sample handling of the living algal samples can have a huge impact on the accuracy of the arsenic species determination. For instance, separating the algae from other symbiotic living beings (epiphytes, microbes) [10].

By purchasing commercial products, these steps are unfortunately impossible to conduct and the only information about the sampling is provided by the manufacturer.

1.1.4.2 Sample pre-treatment

After the algae have been collected, a drying or freeze-drying process is necessary to prevent fouling of the algae, which might already change speciation. Thereafter the remaining processes are the same as for the dried commercial samples, namely grinding and sieving the material to a pre-defined corn size (homogenization). The size of the granulate is often omitted, but of great importance, since the surface area defines the ability of the solvents to interact with the material. The higher the surface area the better the wetting, however one should not overshoot it, since too small particles could induce sticking and/or clumping [1, 3].

1.1.4.3 Extraction

A highly delicate topic in arsenic speciation is the extraction procedure. While most algae treated with soft extractants yield high amounts of arsenic, even at mild conditions, there are many-many exemptions to this belief. Non-extractable fractions of arsenic are nowadays still “untouchable” since existing techniques just will not do it. If drastic conditions are employed (high temperatures and severe conditions), speciation certainly will change and that is counterproductive. NanoSIMS (secondary ion mass spectrometry) analysis of algae tissues may highlight where the arsenic species are located in the algae and this may explain their behaviour, however without actual species information [40]. Perhaps the future holds new approaches, which will allow the identification and characterisation of such complex matrix bound arsenic species. Whether they are protein/lipid/cellular bound or entrapped remains a mystery [8].

1.1.4.4 Separation and detection

Since the analytical methodologies for arsenic speciation in environmental samples have been well established and optimised, only a small playing field can be explored for its improvement (especially in the case of polar extracts).

Primary ion-exchange chromatography is used for the separation of polar arsenic species. Complementary both cation- and anion-exchange columns are used to comprise all possible analytes. Chromatographic column recoveries are important to calculate, since low recoveries indicate unretained analytes, which are stuck to the stationary phase. Regeneration of the column with methanol might elute the adsorbed species, thus the column can be used for a longer period. Other columns used for separation are scarcer, however still employable. For instance, reverse-phase ion pairing columns for the separation of polar arsenicals. Other reverse-phase columns are used for the separation of arsenolipids and other less polar arsenicals [15].

Next to HPLC systems, gas chromatography (GC) systems and rarely capillary zone electrophoresis (CZE) are also employed [10].

As for the detection systems used there are coupled and direct methods. Chromatographic separation coupled to element-selective detection system like ICPMS is the most widely spread method of choice. However, for the identification of new species, molecular spectroscopic methods are used. Atmospheric pressure ionization coupled to single quadrupole or triple quadrupole mass analysers (MS/MS) gives excellent qualitative information on species structures, complementary to the element selective detection system, which is excellent for quantification due to its wide dynamic range and low limits of detection achievable [7, 15]. In contrast, direct methods give instant information on arsenic and its neighbouring matrix, where previously described steps can be skipped, saving lot of time and costs [15]. However, these techniques give little to no molecular information and are only employable semi-quantitative. Nevertheless, they are great complementary methods for coupled techniques.

1.2 Ionic liquids

Ionic liquids (ILs) are usually described as green alternatives for volatile and hazardous organic solvents. They exhibit interesting properties such as negligible vapor pressure and high thermal stability. Interestingly, ILs are organic salts, but in liquid state, even at ambient temperature, so one may say they are “salty solvents”. This makes them attractive for various applications in most fields of chemistry and other disciplines such as pharmacy, metallurgy, and material science [41].

As for the treatment of biological material, ILs are used as dissolution agents for cellulose and generally in biomass applications (delignification) [42, 43]. However many applications have incorporated ILs as extractants to improve yields of one specific class of analytes, as in the case of lipid extraction from *Chlorella vulgaris* using 1-buthyl-3-methylimidazolium trifluoro sulfonate, which improved the fatty acid yield almost twofold in comparison with conventional methods [44]. Furthermore, upon extracting the lipid fraction, the residue may be further extracted with enzymes to yield sugars through hydrolysis [45].

As one can see the playing field is very wide and applying ILs in extraction procedures may even result in increased concentrations of arsenolipids and/or arsenosugars in the extracts.

1.3 Supercritical fluids

Another alternative for mild extractions is the so-called supercritical fluid extraction (SCF). The supercritical aggregate state can be described as a state between liquid and gaseous, since it has liquid-like form but also incorporates properties from the gas phase, for instance no phase boundary is present, therefore the surface tension is negligible. As the system reaches its critical point (critical temperature and pressure) it instantaneously changes from liquid to supercritical fluid. The transition from gas to supercritical fluid is rarer. This state of matter has a higher density than gases and is similarly compressible than gases but at the same time much less viscous than simple fluids. Furthermore, the polarity of the solvent is tuneable with the change in pressure, which is directly connected with change in density and permittivity. So, the SCFs act as solvents and have way higher diffusion coefficients than normal liquids, thus solvating and penetrating the matrix of the solid or liquid samples more easily [46].

For environmental samples this is very convenient, since high extraction efficiencies of active compound without physicochemical degradation are achievable. However, the solubility and solvent strength of SCFs are limited. Organometallic compounds are difficult to extract, but the addition of chelating agents or co-solvents may induce extractability [47]. Therefore, polar arsenic species should be extractable only by the use of co-solvents, surfactants or additives [48]. An interesting example is the SCF extraction of arsenic from the CRM DORM-1 with methanol as co-solvent (25 % v/v). The maximum achieved yield was 65 ± 5 %, which is far from being quantitative. Moreover, it may be the case that methanol alone could extract the same amount, but that was not checked [49]. For nonpolar arsenicals no data are available in terms of extractability with SCF. The only reliable anchor point is to look at solubility diagrams of fatty acids and acid esters in SCFs. These show that solubility increases with increasing pressure, however this is not true for all fatty acids, therefore one should be cautious [50].

2 Materials and methods

2.1 Instrumentation

- Microwave digestion system, MLS Ultraclave IV®, EMLS Mikrowellen-Laborsysteme GmbH, Leutkirch im Allgäu, Germany
- ICPMS, Agilent 7700x, Agilent Technologies, Waldbronn, Germany
- ICPMS, Agilent 7900, Agilent Technologies, Waldbronn, Germany
- HPLC 1260, Agilent Technologies, Waldbronn, Germany
- Ultra-centrifugal mill, ZM 200, Retsch GmbH, Haan, Germany
- Analytical balance, Denver Instrument SI-234, Göttingen, Germany
- Ultrasonic bath, Transsonic T 700/H, Elma Schmidbauer GmbH, Singen, Germany
- Centrifuge, Rotina 420R, Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany
- pH-meter, Thermo ORION 5 STAR, Fisher Scientific GmbH, Vienna, Austria
- duoPUR sub-boiling system, EMLS Mikrowellen-Laborsysteme GmbH, Leutkirch im Allgäu, Germany
- PRP-X100 anion-exchange chromatographic column, 5-µm particles, 150 x 4.6 mm, stainless steel, Dr. Maisch HPLC GmbH, Ammerbuch-Entringen, Germany (serial number: 2041934803)
- Zorbax 300-SCX, cation-exchange chromatographic column, 5-µm particles, 150 x 4.6 mm, Agilent Technologies, Waldbronn, Germany
- Tubes, polypropylene, Cellstar®, 15 mL, Greiner Bio-One International GmbH, Kremsmünster, Austria
- Tubes, polypropylene, Cellstar® 50 mL, Greiner Bio-One International GmbH, Kremsmünster, Austria
- HPLC polypropylene microvials, 0.7 ml, Bruckner Analysentechnik, Linz, Austria

- HPLC snap rings, natural rubber red-orange, 11 mm, Lab Logistics Group GmbH, Meckenheim, Germany
- Syringe Filters, 25 mm, Nylon66 (polyamide), pore size: 0.22 µm, BGB®, Rheinfelden, Germany
- Pipette, Acura 825 autoclavable 10 – 100 µL Socorex, Ecublens, Switzerland
- Pipette, Acura 825 autoclavable 100 – 1000 µL Socorex, Ecublens, Switzerland
- Pipette, Acura 825 autoclavable 0.5 – 5 mL Socorex, Ecublens, Switzerland
- Aluminium heating block, self-made (85 x 76 x 51 mm, 16 holes: 40 mm deep and 11 mm in diameter)
- Shaking water bath, THERMOLAB® 1083, Gesellschaft für Labortechnik mbH, Burgwedel, Germany
- IKA® RTC basic, IKA®-Werke GmbH & Co. KG, Staufen, Germany
- IKA® ETS-D5, IKA®-Werke GmbH & Co. KG, Staufen, Germany
- Magnetic follower, 7 x 2 mm, Fisherbrand®, United Kingdom
- SCF extractor, produced by University of Maribor, Slovenia
- ISCO syringe pump, model 260D, Lincoln, Nebraska
- Parafilm® ´M´, Sigma-Aldrich, Vienna, Austria

2.2 Used chemicals and certified reference materials

- Ultrapure water, Milli-Q® for trace element analysis, 18.2 MΩ cm, Merck KGaA, Darmstadt, Germany
- Methanol, MeOH, 99.8 % HiPerSolv CHROMANORM®, VWR International S.A.S., Fontenay-sous-Bois, France
- Propan-2-ol, for HPLC, ≥99.9 %, CHROMASOLV™, Honeywell Austria GmbH, Riedel-de Haën™, Graz, Austria

- Trichloromethane, $CHCl_3$, ROTISOLV®, Carl Roth GmbH + Co. KG, Karlsruhe, Germany
- Pyridine, for HPLC, ≥99.9 %, Sigma-Aldrich, Vienna, Austria
- Nitric acid, HNO_3, ≥ 65 % p.a., ROTIPURAN®, Carl Roth GmbH + Co. KG, Karlsruhe, Germany; further subboiled by a duoPUR sub-boiling system
- Hydrochloric acid, HCl, 37 % p.a., Carl Roth GmbH + Co. KG, Karlsruhe, Germany
- Formic acid, HCOOH, ~ 98 %, Sigma-Aldrich, Vienna, Austria
- Hydrogen peroxide, H_2O_2, 30 %, Carl Roth GmbH + Co. KG, Karlsruhe, Germany
- Ammonium dihydrogen phosphate, $NH_4H_2PO_4$, Suprapur, Merck KgaA, Darmstadt, Germany
- Ammonia solution 25 %, NH_3, Suprapur, Merck KgaA, Darmstadt, Germany
- Argon, Ar, 5.0, Messer Austria GmbH, Gumpoldskirchen, Austria
- Carbon dioxide, CO_2, 5.0, Messer Austria GmbH, Gumpoldskirchen, Austria
- Helium, He, 6.0, Messer Austria GmbH, Gumpoldskirchen, Austria
- Hydrogen, H_2, 6.0, Messer Austria GmbH, Gumpoldskirchen, Austria
- Sodium hydroxide, NaOH, pro analysi, Merck KgaA, Darmstadt, Germany
- 1-ethyl-3-methylimidazolium acetate, [EMIm][OAc], purum, proionic GmbH, Graz, Austria
- 1-ethyl-3-methylimidazolium diethyl phosphate, [EMIm][DEP], purum, proionic GmbH, Graz, Austria
- Cholinium L-Lysinate, [Ch][Lys], technical grade, proionic GmbH, Graz, Austria
- Standard Reference Material (SRM) 1640a, Trace Elements in Natural Water, NIST, Gaithersburg, USA
- Reference Material ALVA-PA14/1, Compound Feed Oregano, Round Robin, ALVA, Linz, Austria

- Reference material IAEA-140/TM, Trace Elements in Seaweed Fucus Spiralis, Analytical Quality Control Services, Agency's Laboratories, Seibersdorf International Atomic Energy Agency, Vienna, Austria
- Standard Reference Material® 1568b Rice Flour, NIST, Gaithersburg, USA
- Metals and trace elements for water analysis, No M148, Environment Agency Austria, BOKU/IFA-Tulln, Austria
- Metals and trace elements for water analysis, No M149, Environment Agency Austria, BOKU/IFA-Tulln, Austria
- Acetic acid ≥99%, Sigma-Aldrich, Germany, Steinheim
- Carbon dioxide, CO_2, 2.5, Messer Slovenia, Ruše, Slovenia
- Ethanol, EtOH, pro analysi, Merck KgaA, Darmstadt, Germany

2.3 Standard solutions of elements

All single element standards were purchased from Carl Roth Gmbh & Co. KG, Karlsruhe, Germany. Matrix of each solution: 2 % HNO_3 (v/v).

Concentrations of Ag, Al, As, B, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Fe, Gd, Hg, In, Li, Lu, Mg, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sn, Sr, Te, Ti, Tl, U, V, Zn were 1000 mg/L.

In contrast to the above element standards, Ca, K, Mg, Na, P and S standards had 10 times higher concentrations.

2.4 Standard solutions of arsenic species

Arsenic species standards were prepared inhouse at the University of Graz, Institute of Chemistry [51]. All standards consisted of 1000 mg As L^{-1}, except for the arsenosugar standard, which concentration was quantified during the work.

- Arsenic acid, As(V): prepared by dissolution of $Na_2HAsO_4 \cdot 7H_2O$, Merck KGaA, Darmstadt, Germany.

- Methylarsonic acid, MA: prepared by the reaction of sodium dimethylarsinate and methyl iodide (adapted from [52]).
- Dimethylarsinic acid, DMA: prepared by the dissolution of sodium dimethylarsinate trihydrate, Fluka, Buchs, Switzerland
- Trimethylarsine oxide, TMAO: prepared by the reaction of trimethylarsine with hydrogen peroxide under argon [51].
- Tetramethylarsonium ion, TETRA: prepared from $(CH_3)_3As$ and CH_3I by mixing both reagents under Ar and mild conditions to yield white tetramethylarsonium iodide crystals [51].
- Arsenocholine, AC: synthesized from trimethylarsine and bromoethanol under inert conditions and to yield white crystals of arsenocholine bromide [51].
- Arsenobetaine, AB: synthesized from trimethylarsine and bromoacetic acid under inert conditions and recrystallised to yield white crystals of arsenobetaine bromide [51].
- Arsenosugar standard consisted of OH-Sugar, PO_4-Sugar, SO_4-Sugar and SO_3-Sugar.

Since the sugar standards are not commercially available, all above mentioned arsenosugars standards were isolated in-house from natural sources – kidneys of giant clams [53] and different seaweeds, like in the case of brown kelp *Ecklonia radiata*[54, 55]

The four isolated arsenosugars were characterised by ^1H NMR spectroscopy[53, 54] and in a different research also by Fast Atom Bombardment Tandem Mass Spectrometry [56].

Additionally, the OH-Sugar was separately synthesized and characterised [57].

2.5 Sample collection and preparation

Algal samples were obtained from local Asian stores in Austria, Hungary, and the UK. Three samples were purchased from the same producer (three times Wakame and Yakinori), but not in the same country. Additionally, 2 Wakame and 1 Yakinori product were bought from a different distributer.

List of manufacturers with the country of origin and the corresponding sample name and instructions found on the packaging labels:

- WELPAC, Fueru Wakame, Dried seaweed, farmed in the Republic of Korea – Wakame 1 - 3 (soak in cold water for 4 - 6 min, drain well, then add to salad) – purchased from local stores in Vienna and Budapest
- Herman Kuijper BV, Dried cut Wakame, farmed in China, – Wakame 4 (50 g in 2 L hot water for one day, pour, clean with fresh water, drain well, cook for 20 min, again clean and drain) – purchased from a local store in Graz
- Chungjungwon Namhaeansan Clean Seaweed, N Deasang Co., Ltd., farmed in the Republic of Korea – Wakame 5 (soak seaweed in cold water then drain and wash it) – purchased from a local store in Graz
- Heuschen&Schrouff OFT B.V, YAKI SUSHI NORI, farmed in Japan – Nori 1 – 3 (suitable for direct consumption) – purchased from local stores in Vienna and Budapest
- Clearspring Ltd. London, SUSHI NORI, Dried Sea Vegetable (*Porphyra tenera)*, farmed in Japan – Nori 4 (suitable for direct consumption) – purchased from local store in London

The products were sold as dried seaweed food either shredded to pieces (Wakame) or pressed into sheets (Yakinori). The dried products were further homogenized utilizing the Retsch Ultra Centrifugal Mill ZM200 with ultra-fine-grinding sieves and a 12 tooth Ti push-fit rotor. The resulting powdered material was then stored in polypropylene containers (Figure 3).

Figure 3: Commercially available ground algae samples: Wakame (left) and Yakinori (right)

2.6 Multielement determination in two commercially available algae samples: Wakame and Yakinori

To obtain total element concentrations in the given samples, a microwave assisted digestion procedure was applied prior to mass spectrometric analysis. Three separate digestions were carried out using different certified reference materials for quality control. An overview of the digestions is shown in Figure 4.

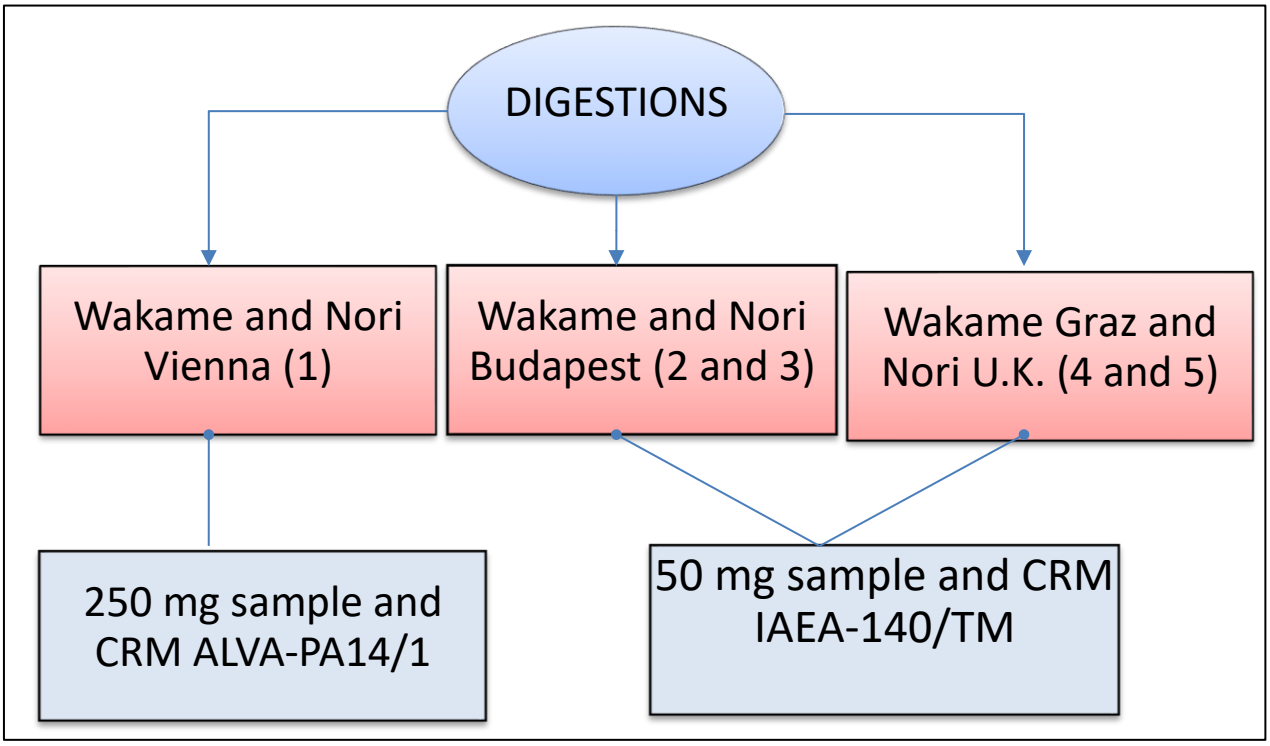


Figure 4: Diagram of digested samples and used CRMs

2.6.1 First digestion procedure – samples from Vienna

Approximately 250 mg (weighed to 0.1 mg) of the algae samples as well as the reference material ALVA-PA14/1 (feeding additive Oregano) was weighed directly into quartz tube digestion vessels and filled with 5 mL of HNO_3. Additionally, blank samples consisting only of concentrated HNO_3 were added and each sample was prepared in triplicates (n = 3). The tubes were then placed into the sample rack and inserted into the Ultraclave IV® microwave digestion system. After closing the vessel, a loading pressure of argon (40 bar) was applied. This was followed by starting the following temperature programme: 0-15 min, to 80°C; 15-30 min, to 150°C; 30-50 min, to 250°C; and finally held at 250°C for additional 30 min. The

absorbing solution for the microwave generated heat consisted of 1 % H_2SO_4 (w/w) in ultrapure water.

After cooling down to room temperature, the digests were transferred into 50 mL polypropylene tubes and diluted to an end volume of 50 mL with ultrapure water.

Total element concentrations of 27 elements (Li, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, As, Se, Rb, Sr, Mo, Cd, Ba, Ce, Gd, Hg, Tl, Pb, Bi, U) were determined utilizing the Agilent 7900 ICPMS. These were calculated based on an external calibration for each measured element. Spectrum mode options were chosen as follows:

- 1 point per isotope
- 5 replicates
- 50 sweeps per replicate
- Varying integration times for different masses (see appendix)

Prior analysis the instrument's performance in the used three tune modes (no gas mode, hydrogen reaction mode and helium collision mode) was checked by measuring a tuning solution consisting of 1.0 µg L^{-1} Li, Fe, Co, As, Se, Y, Ce, Tl in 2 % HNO_3 (v/v). These elements cover most of the mass range of the periodic table. Similar mass-to-charge ratios »behave similarly" (space charge effect), thus checking the performance for the low-mass, mid-mass and high-mass elements will guarantee that the sensitivity of the isotopes of interest will be similarly good [58]. Li @ m/z 7, Y @ m/z 89 and Tl @ m/z 205 response were measured in no gas mode, whereas Co @ m/z 59, Y @ m/z 89 and Tl@ m/z 205 performance was checked in helium collision mode and hydrogen reaction mode. Performance of ^{78}Se is frequently checked in H_2-mode as well. The integration times while tuning were 0.1 s for each mass.

Two more parameters are readily checked during instrument tuning, namely the oxide ratio ($^{156}CeO^+/^{140}Ce^+$) and the doubly charged ratio ($^{140}Ce^{2+}/^{140}Ce^+$). When those ratios are < 2 %, the plasma settings are optimal, generating low percentage of interferences.

Table 1 gives a rough estimation on the ICPMS tune parameters and the corresponding instrument performance. Calibration levels for each element are summarised in Table 2 and Table 3.

The trueness of the external calibration was checked by measuring SRM NIST 1640a (1+9, v/v), with a matrix adjustment to 10 % HNO_3 (v/v)) and in one case by measuring a reference water M149B (without matrix adjustment). Furthermore, a drift solution was prepared to examine the signal stability of the ICPMS during the measurement for each element. The element concentrations in the drift solution were set to match the level 3 points of the external calibration. The frequency of the drifts was set to be approximately $\sqrt{n}$, where *n* is the total number of samples measured. These drifts were distributed evenly through the whole set of the acquisition. It is important to pinpoint that the drift solutions matrix composition is different from the external calibration, hence slight deviations are possible between external calibration and drift solution for specific element sensitivities.

During the measurement, an internal standard solution was pumped and combined with the sample stream via a t-type connector and simultaneously dispersed into the spray chamber. 0.2 mg/L Be, Ge, In and Lu (prepared in 10 % HNO_3 (v/v)) were used to monitor and correct possible interferences due to the different matrix composition of the calibration solutions and all other samples.

Table 1: Typical instrument performance of ICPMS in three tune modes (integ. time = 0.1 s)

Parameter	**No gas mode**	**Reaction mode**	**Collision mode**
Flow rate	/	**H_2 (3.5 mL/min)**	**He (4.0 mL/min)**
^{7}Li	6.50*10^3 counts	/	/
^{59}Co	/	1.90*10^3 counts	4.35*10^3 counts
^{78}Se	/	200 counts	/
^{89}Y	22.1*10^3 counts	17.6*10^3 counts	4.75*10^3 counts
^{205}Tl	10.0*10^3 counts	18.1*10^3 counts	9.70*10^3 counts
Mean RSD	2.40 %	2.60 %	2.50 %
Oxide ratio $^{156}CeO^+/^{140}Ce^+$	1.20 %	/	/
Doubly charged ratio $^{140}Ce^{2+}/^{140}Ce^+$	1.60 %	/	/

Table 2: Calibration levels (µg/L) for elements prepared in 10 % HNO_3 (v/v)

Elements	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
Bi, Gd, Mo, Tl, U	0.001	0.005	0.01	0.05	0.1	0.5	1
B, Ba, Cd, Ce, Co, Li, Ni, Pb, Se, Sr, V	0.01	0.05	0.1	0.5	1	5	10
As, Cr, Mn	0.1	0.5	1	5	10	50	100
Al, Cu, Fe, Rb	1	5	10	50	100	500	1000

Table 3: Calibration levels for Hg (µg/L) and Ca, Mg, Na and K (mg/L) prepared in 10 % HNO_3 (v/v) and 1 % HCl (v/v)

Elements	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
Hg	0.01	0.05	0.1	0.5	1	5	10	/
Ca, Mg, Na	0.01	0.05	0.1	0.5	1	5	10	/
K	0.1	0.5	1	5	10	50	100	200

2.6.2 Second digestion procedure – samples from Vienna and Budapest

Similarly, as in the case of 2.6.1, algae samples were digested in nitric acid and diluted to obtain a final matrix composition of 10 % HNO_3 (v/v). This time however 50 mg of samples, as well as the certified reference material IAEA-140/TM (*Fucus Spiralis*) was used instead. Additionally, the humidity of the CRM was determined by weighing 1 g (with a precision of 0.1 mg) of the seaweed into a crystallizing dish and drying it for 24 h at 105°C. Thus, the trueness of the analytical method was calculated based on the dry weight of the CRM.

2.7 Arsenic species extraction from algae samples

For the extraction of arsenic species many different extraction procedures and extracting solvents or solvent mixtures were used. Figure 5 summarises the palette of extraction solvents used. Individual procedures are described in following subsections.

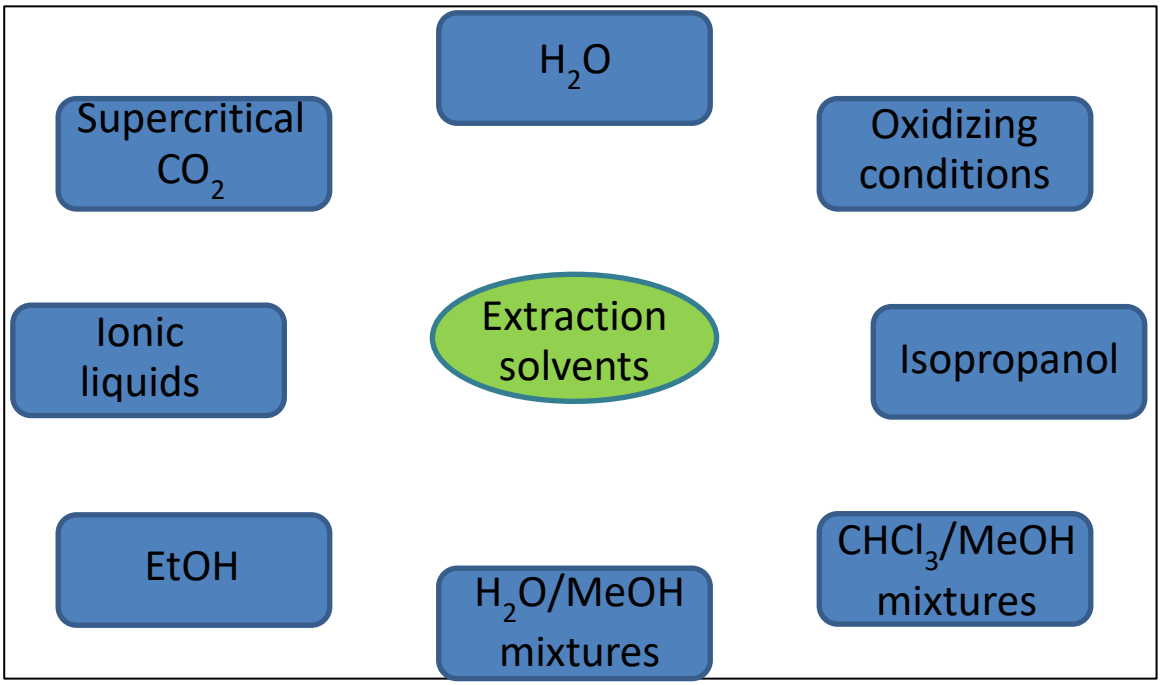


Figure 5: Solvents used for the extraction of arsenic species from seaweed samples

For a clearer overview, Table 4 summarises all procedures with the listed solvents and conditions that have been tested in the present thesis.

Table 4: Extraction experiments, used solvents and conditions

Experiment ID	Solvent	Conditions
MGy_002	H_2O and H_2O/MeOH 1+1 (v/v)	rt* and 60°C, ultrasound assisted extraction, 30 min
MGy_003	0.1 M HNO_3 and 3 % H_2O_2(v/v)	90°C, shaking water bath, 60 min
MGy_006	pure ionic liquids and 50 wt% aqueous solutions	75°C and 90°C, shaking water bath and beaker, different times
MGy_010	10 wt% [Ch][Lys]	60°C, shaking water bath, 2 h
MGy_010_ISO	Isopropanol	60°C, shaking water bath, 2 h
MGy_010_SON	H_2O and $NH_4H_2PO_4$ buffer at pH = 6.0	65°C, ultrasound assisted extraction, 2 h
MGy_011	pure [EMIm][OAc]	40°C, 5 min ultrasound and 2 h heating block
MGy_012	10 wt% [Ch][Lys]	40°C and 80°C, shaking water bath, different times and solution volumes
MGy_015	$CHCl_3$/MeOH 2+1 (v/v)	rt, rotary cross, 3 h
MGy_016	0.01 M NaOH	80°C, shaking water bath, 2 h
MGy_021	$scCO_2$ and EtOH	50°C, supercritical fluid extractor, different times and pressures
MGy_021_re-extract	$CHCl_3$/MeOH 1:1 (v/v)	rt, ultrasound assisted extraction, 1 h
MGy_022	[EMIm][OAc]: DCM/MeOH 1+1 (v/v)	rt, rotary cross, 20 h

*room temperature (rt) was kept at 23°C with the air conditioning system

2.7.1 Extraction of polar arsenicals

Approximately 200 mg of the given samples from Vienna ("Wakame-Tong green algae" and "Roasted Seaweed") were weighed into 15 mL polypropylene tubes (precision = 0.1 mg). 10 mL of an extraction solvent (either ultrapure water or methanol and ultrapure water mixture in a volumetric ratio of 1+1) was added and then subjected to an ultrasound-assisted extraction procedure for 30 min at room temperature. The samples were shaken before and after sonication. The samples, as well as the blanks were prepared in triplicates.

Following the extraction step the samples were centrifuged at 3260 g for 10 min at room temperature. All the samples were then filtered through an 0.22 µm polyamide filter.

Additionally, an ultrasound assisted extraction procedure was carried out on the same samples but deferring conditions:

- Ultrapure water, 65°C, 2 h, one Wakame sample and one blank
- $NH_4H_2PO_4$ buffer solution (pH = 6.0), 65°C, 2 h, one Wakame sample and one blank

Supplementary, the extraction of *Undaria pinnatifida and Porphyra umbilicalis* algae with ultrapure water, was conducted also in a shaking water bath for the following samples and at the below listed conditions:

- 40°C, 2 h, Wakame samples from Budapest (each prepared once) and one blank sample
- Ultrapure water, 60°C, 30 min, Wakame and Yakinori (Vienna) in triplicates, blank samples in triplicates
- 80°C, 2 h, Wakame samples from Vienna in triplicates, blank samples in triplicates

The extracts were then refrigerated and stored until analysis.

2.7.2 Acidic extraction under oxidizing conditions

200 mg of the Wakame and Yakinori samples from Vienna were diluted in 10 mL of the following extraction solution: 0.1 M HNO_3 and 3 % H_2O_2. (v/v). The mixtures were heated for 60 min in a shaking water bath at 90°C. Afterwards the samples were centrifuged (10 min, rt, 3260 g) and filtered through an 0.22 µm polyamide filter.

This extraction procedure was conducted based on DIN EN 16802:2016-07, for the determination of inorganic arsenic content in marine samples. The samples, as well as the blanks were prepared in triplicates. Extracts were stored at 4°C in a refrigerator.

For quality control the CRM 1568b Rice flour was also extracted at same conditions.

The next sections are focusing on the extraction of arsenic species mostly only out of Wakame samples.

2.7.3 Nonpolar extractions

The following solvents and solvent mixtures were used for the extraction of nonpolar arsenic species:

- Isopropanol
- $CHCl_3$/MeOH 2+1 (v/v)

For the extraction with isopropanol, approximately 200 mg of Wakame samples were diluted with 10 mL of solvent and extracted at 60°C for 2 h in a shaking water bath. The extracts were then centrifuged (10 min, rt, 3260 g) without dilution, filtered through an 0.22-µm polyamide filter and analysed on the same day for total arsenic concentration, to avoid solvent evaporation and thus biasing the trueness of the measurement.

In contrast, the extraction with chloroform/methanol mixture was carried out on a rotatory cross for 3 hours at room temperature. 300 mg of algae samples were diluted with 5 mL of the extracting agent and centrifuged (10 min, rt, 3260 g) upon extraction and subsequently filtered through an 0.22-µm polyamide filter. Again, the extracts were not diluted and stored, but analysed on the same day for their total arsenic content.

All samples, as well as the blanks were prepared in triplicates.

2.7.4 Extraction under basic conditions using aqueous sodium hydroxide

A 0.01 M solution of NaOH (pH = 12.0) was prepared to investigate if the poor arsenic extraction yield out of Wakame samples is caused by strong binding interactions between the arsenic species and the algae matrix. In contrast to ionic liquids, which possess

depolymerization properties [45], NaOH acts only as a hydrolytic agent, thus hydrolysing binding sites of arsenicals to the complex chemical composition of the algae.

200 mg of seaweed samples were diluted with 10 mL of the basic solution and subjected to an elevated temperature extraction procedure. Shaken for 2 h at 80°C, the extracts were centrifuged (10 min, rt, 3260 g) and filtered through an 0.22-µm polyamide filter. The extracts were stored at 4°C till analysis on their total arsenic content and arsenic speciation analysis.

All samples, as well as the blanks were prepared in triplicates.

2.8 Advanced extraction methods

The purpose of exploring new approaches for arsenic species extraction was to achieve:

- Quantitative extraction or at least increase the extraction yield to reasonable percentage
- Species preservation during extraction by using mild process conditions

2.8.1 Ionic liquids as extractants

The first approach was to use ionic liquids (ILs) as alternative solvents for toxic organic ones and thus develop a "green" alternative for arsenic speciation analysis in environmental samples. The following ionic liquids were used:

- 1-ethyl-3-methylimidazolium acetate, [EMIm][OAc]
- 1-ethyl-3-methylimidazolium diethyl phosphate, [EMIm][DEP]
- Cholinium L-Lysinate, [Ch][Lys]

These ILs were synthesized by proionic GmbH via the patented CBILS®-Synthesis route (Carbonate Based Ionic Liquid Synthesis) [41, 59]. Figure 6 shows their chemical structures. As one can see solely from structural point of view, these organic salts contain both positive and negative charges, as well as a carbon backbone, thus being miscible both with polar and nonpolar solvents such as chloroform. Although this is true for these three cases, the miscibility/immiscibility of ionic liquids with various solvents is not straightforward and thus cannot be generalised [60].

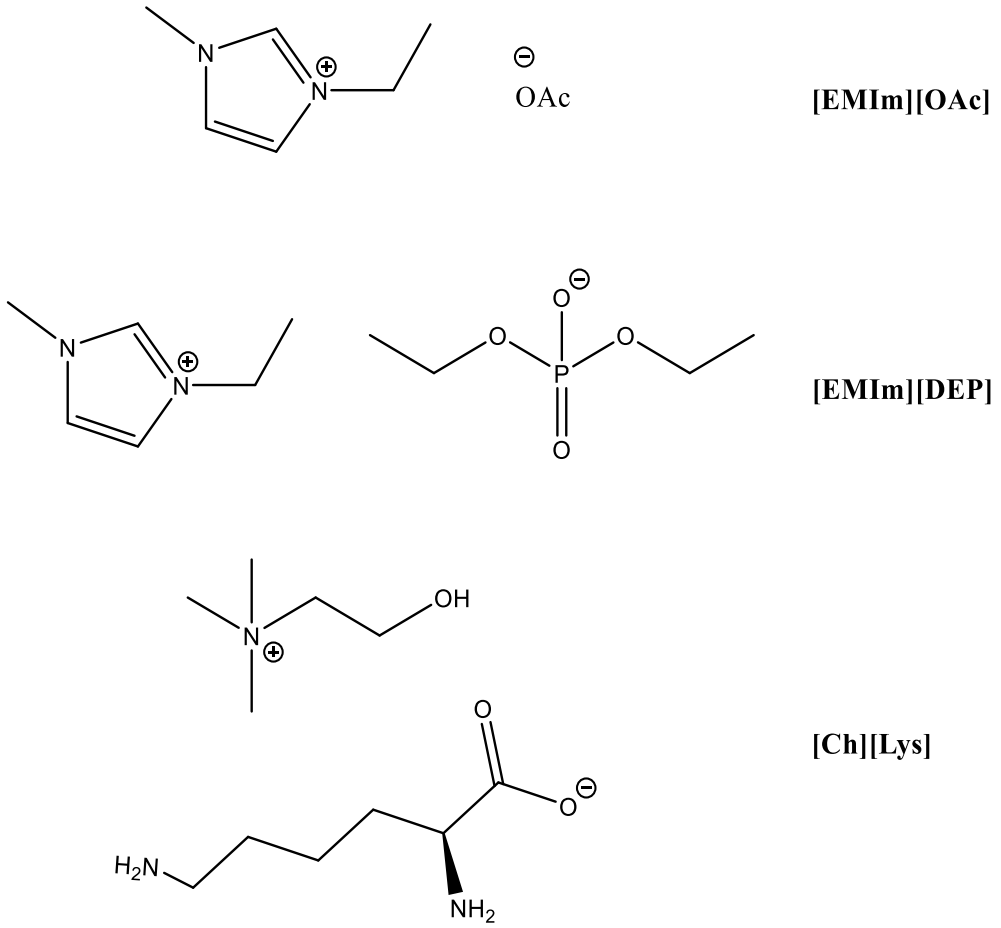


Figure 6: Chemical structures of ILs used for extraction

2.8.1.1 Preliminary tests

First experiments were performed with pure ionic liquids as solvents. The ratio of IL and biomass was chosen to be approximately 30+1 (w/w), so that the weight percent of the algae ranged from 2.5 – 3.5 wt%. Due to the high viscosity of the ILs, which prevented sufficient mixing and surface wetting of the algae, also 50 wt% mixtures of ILs and ultrapure water were prepared and used at the same dilution as the pure ILs.

For instance, 300 mg of seaweed was weighed into a 50 mL polypropylene tube and 9.7 g IL was added. The mixture was then placed into a shaking water bath and the biomass was extracted for 4 h at 90°C.

Afterwards the samples were diluted to a final volume of 50 mL, shaken and centrifuged (10 min, rt, 3260 g). After filtration (0.22-µm polyamide filter) the extracts were stored at 4°C till analysis.

The following extraction were carried out:

- Pure [Ch][Lys], 90°C, 4 h, shaking water bath, 1 Wakame, 1 Yakinori and 1 blank sample
- Pure [EMIm][OAc], 75°C, 1 h, beaker and magnetic stirrer, 1 wakame sample and 1 blank sample
- 50 wt% [Ch][Lys] in ultrapure water, 90°C, 4 h, shaking water bath, 1 Wakame and 1 blank sample
- 50 wt% [EMIm][DEP] in ultrapure water, 90°C, 4 h, shaking water bath, 1 Wakame and 1 blank sample
- 50 wt% [EMIm][OAc] in ultrapure water, 90°C, 4 h, shaking water bath, 1 Wakame and 1 blank sample

Additionally, one sample of Wakame was diluted only in [EMIm][OAc] and stirred and heated on a magnetic stirrer at approx. 80°C. The algae completely dissolved in the IL. Even after dilution with ultrapure water no precipitate was observable.

2.8.1.2 Aqueous solutions of [Ch][Lys] as extractant

In the preliminary investigations [Ch][Lys] proved to be the most promising IL in terms of ability to extract arsenicals out of *Undaria pinnatifida* samples. Since many individual extractions were planned and the amount of [Ch][Lys] was limited, approximately 4 x 250 mL of aqueous [Ch][Lys] solution with a concentration of 10 wt% was prepared. This stock solutions were then used for the treatment of the different Wakame samples. The following parameters of extraction were investigated:

- Extraction temperature (40°C, 60°C, 80°C)
- Time of extraction (2 h, 24 h)
- Ratio extractant versus biomass (50 and 125)
- Effect of wetting the algae in the extractant for a defined amount of time prior extraction (no wetting versus 2 h wetting)

The stepwise extraction was as follows: approximately 200 mg (weighed to 0.1 mg) of the seaweed samples were diluted in the desired volume of 10 % [Ch][Lys] and subjected to extraction under elevated temperature in a shaking water bath. After this process, the mixtures were cooled to room temperature and centrifuged (diluted or undiluted) at 3260 g for 10 minutes, followed by filtration of the supernatants through an 0.22-µm polyamide filter. Extracts were stored at 4°C until analysis.

Additionally, the extraction procedure at 80°C for Wakame samples from Vienna was repeated three times, and for the Wakame samples from Hungary two times each (all three batches were from the same manufacturer: WELPAC). This was conducted to check the robustness of the extraction procedure and whether there are significant deviations comparing arsenic species concentrations between two different batches.

In all experiments blank samples were prepared to evaluate any possible contamination and thus for blank subtraction. Furthermore, all extractions were carried out in triplicates.

2.8.1.1 Experiments with [EMIm][OAc]

Since pure [EMIm][OAc] did perform similarly well as 10 wt% [Ch][Lys] in terms of arsenic extractability at elevated temperature, mild conditions were investigated. It was used as a pure extractant and together with nonpolar solvents to allow leaching of arsenicals without hydrolysis or degradation reactions.

For this purpose, a self-made aluminium heating block was dimensioned and 40 mm holes with 11 mm diameters were drilled. The 15 mL polypropylene tubes fitted smoothly.

The setup of the extraction was as follows: 100 mg of Wakame samples were diluted in 2 mL of [EMIm][OAc], sonicated for 5 minutes and afterwards placed into the heating block. This assembly was then placed onto a magnetic stirrer with hot plate. The temperature was set to 40°C. After 2 hours the extracts were diluted with ultrapure water, rigorously shaken and centrifuged (10 min, rt, 3260 g). The supernatants were filtered through an 0.22-µm polyamide filter and stored at 4°C till analysis.

As a complementary method, extraction at room temperature was conducted utilizing a rotatory cross. Pure [EMIm][OAc] and a mixture of [EMIm][OAc] and DCM/MeOH (2+1 v/v)

were used as extractants. Samples and blanks were evenly fixed on the rotatory cross with elastic straps. The samples were rotated for 20 h at room temperature. Pure [EMIm][OAc] samples were diluted with DCM/MeOH (2+1 v/v) prior centrifugation. All the samples were then centrifuged at 3260 g for 10 minutes and filtered through an 0.22-µm polyamide filter. The obtained extracts were analysed on the same day to avoid solvent evaporation while being stored.

In all experiments blank samples were prepared to evaluate any possible contamination and thus for blank subtraction. Furthermore, all extractions were carried out in triplicates.

2.8.2 Supercritical fluid extraction with carbon dioxide

First, two separate supercritical fluid extraction (SFE) procedures were carried out to estimate the rough extraction efficiency of arsenic in pure CO_2.

The first experiment was carried out by weighing 0.9 g of Wakame algae with a precision of 0.1 mg into a HPLC column tube (4 mm x 250 mm). The autoclave was then fixed into the SFE setup shown in figures from 7 to 9 (semi continuous extractor). CO_2 was pressurised to 20 MPa by a high-pressure syringe pump and the apparatus was filled with the fluid. The system was heated to 50°C and upon reaching the desired pressure equilibrium, the first expansion valve was slightly opened (circled in Figure 7). The CO_2 flow rate was regulated with a second, micrometric precision valve. The extract was collected in 30 min interval, completing 4 cycles of refilling the syringe pump with carbon dioxide. The expansion valves were flushed with ethanol to obtain the remaining extract in the tubing.

A blank sample was also collected by flushing the extraction apparatus with ethanol prior extraction.

For the second experiment the same sample was pressurised with 39 MPa CO_2 and left-over night at 50°C. In an attempt to damage the cellular structure of the algae, the pressure was rapidly released. The remaining steps were the same as in the case of the first experiment, but in this case 12 cycles of refilling and extraction were carried out. The obtained extracts were then stored at -18°C until analysis.

The residual algae in the autoclave were also collected and one portion (50 mg) of it was digested in a quartz tube digestion vessel filled with 5 mL of HNO_3. Additionally, a blank sample consisting only of the concentrated HNO_3 was added. The digestion procedure was the same as described in 2.6.1. This procedure was necessary to confirm or neglect any loss of arsenic during the SFE (i.e., adsorption on the 316L stainless steel type tubes used as the connectors between valves and autoclave).

After cooling down to room temperature, the digests were transferred into 50 mL polypropylene tubes and diluted to a final volume of 50 mL with ultrapure water.

One more portion of the remaining algae (200 mg) was re-extracted using chloroform/MeOH mixture in a ratio of 1+1 (v/v). The sample/solvent mixture was placed into an ultrasonic bath and sonicated for 1 h. In this step blank samples were also prepared to estimate the arsenic background in the solvents used. For comparison, the same amount of "unprocessed" Wakame algae was also extracted using the same solvent and extraction procedure. This rough estimation will give information on whether the pressurizing step was successful and arsenic species release is now more feasible in the case of pressurised algae.

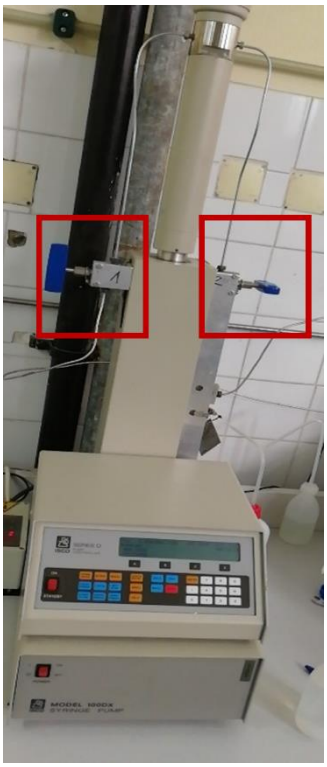


Figure 7: CO_2 inlet (left)/outlet (right) valve and the syringe pump

Figure 8: Temperature controlled extraction column and barometer

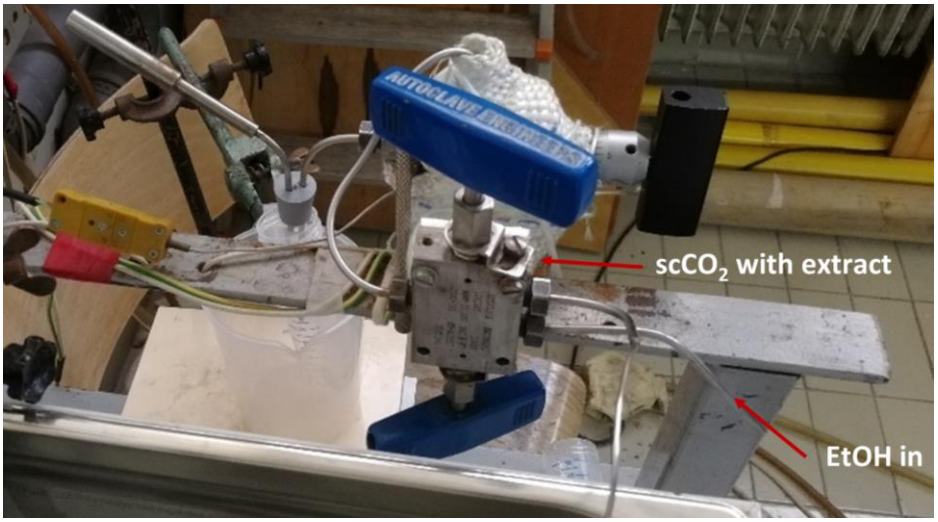


Figure 9: Expansion valves, heat mantle and sample collector

2.8.3 Supercritical fluid extraction using co-solvents

Alternatively, EtOH as a polar solvent was used to promote extraction of arsenicals. 900 mg of *Undaria pinnatifida* sample was weighed into a polypropylene vial and filled with 5 mL of EtOH and preliminary subjected to an ultrasound assisted extraction at 30°C for 30 min. Afterwards the mixture was transferred to the autoclave and the algae was subjected to a SFE procedure, following the same steps as in the case of 2.8.2, second experiment. The extract as well as the blank sample was collected into a polypropylene vial and kept at -18°C. The residual algal sample was again used for digestion to estimate the loss of arsenic in the SFE apparatus.

2.9 Spike experiments

To investigate the integrity of different arsenic species during the extraction procedures, spike experiments were carried out. A focus was put on the case of As(V) which could adsorb onto algal matrix via biosorption even at ambient conditions as kinetically investigated by Hansen et. al in the case of dry *Lessonia nigrescens* samples [61].

2.9.1 Spiking samples with arsenic acid

A 10 mg/L spiking solution of As(V) was prepared by diluting 1 g/L arsenate stock solution in ultrapure water. Prior extraction small portions of the spiking solution were added to the prepared extraction mixtures (solvent + algae). Samples without spikes were prepared as well. For background subtraction, blank samples were prepared consisting only of the extracting solvents. These were then subjected to an extraction procedure under elevated temperature (80°C) in a shaking water bath for 2 h. The solvent/biomass ratio was kept constant, that is 50 (10 mL + 200 mg). After extraction, the extracts were diluted with ultrapure water to reach a final dilution factor of 2500. These mixtures were then centrifuged (10 min at 3260 g) and filtered through an 0.22-µm polyamide filter. Extracts were kept at 4°C till analysis.

Table 5 summarises all used extracting solvents and the amount of arsenate added.

Table 5: Arsenic acid spike experiments

Solvent	Spike amount of As [µg]
10 wt% [Ch][Lys]	5
Ultrapure water	0.5
0.01 M NaOH solution	2

For the purpose to check, whether the arsenate did indeed adsorb during the extraction and did not get lost while filtration of the extract or sample preparation for ICPMS, the [Ch][Lys] sample mixtures were further processed. Samples with residue (3 blank samples, 3 samples without spikes and the 3 spiked samples) were carefully decanted (end volume of 20 mL without loss of residue) to lower the total volume of the solvent to be evaporated. These

tubes were then put into a rotational vacuum concentrator and the solvent was evaporated under vacuum conditions (10 mbar, 80°C, 24 h).

2.9.2 Spiking samples with an equimolar mixture of DMA, MA and As(V)

To investigate whether [Ch][Lys] does affect also other arsenic species during sample treatment, a 30 mg/L mixture of DMA, MA and As(V) in ultrapure water was prepared as a spiking solution.

Moreover, three different extracting solvents were prepared as follows:

- 10 wt% [Ch][Lys] in ultrapure water with a pH of 12.0
- 10 wt% [Ch][Lys] in ultrapure water, pH = 7.0, adjusted with dropwise addition of acetic acid
- 10 wt% [Ch][Lys] in ultrapure water, pH = 4.0, adjusted with dropwise addition of acetic acid

For each extracting solution 3 blank samples (extractant only), 3 Wakame samples, 3 spiked blanks and 3 spiked Wakame samples were prepared by addition of 9.8 mL of extractant, 200 mg of dried seaweed and 250 µL of spiking solution. These extracts were then placed into a shaking water bath, preheated to 80°C and were extracted for 2 h. After extraction, the extracts were diluted with ultrapure water to 50 mL end volume, shaken rigorously and lastly centrifuged at 3260 g for 10 minutes. The supernatants were then filtered of (0.22-µm polyamide filter) into smaller polypropylene containers. Extracts were kept refrigerated until analysis.

2.10 Total element determination in aqueous algal extracts

Aqueous algae extracts extracted with ultrapure water, ultrapure water/methanol mixture (1+1 v/v) at room temperature and 60°C (refer to 2.7.1) were analysed for their total element concentrations to estimate and compare extraction yields for different elements. Hence total element concentrations of Li, Na, Mg, K, Ca, V, Mn, Fe, Co, Ni, Cu, As, Se, Rb, Sr, Mo, Cd, Ba, Ce, Pb, U were determined on the Agilent 7700x ICPMS.

Methodological details on the monitored isotopes, their respective limit of detection, tune modes and internal standards used can be found in the Appendix.

Typical instrument settings and tuning parameters are listed in Table 6.

Table 6: Typical instrument performance of 7700x ICPMS in three tune modes (integration time = 0.1 s)

Parameter	**No gas mode**	**H_2 mode**	**He mode**
Flow rate	/	**H_2 (3.5 mL/min)**	**He (4.0 mL/min)**
^{7}Li	$3.40*10^3$ counts	/	/
^{59}Co	/	$1.30*10^3$ counts	$1.90*10^3$ counts
^{78}Se	/	100 counts*	/
^{89}Y	$7.60*10^3$ counts	$7.50*10^3$ counts	$2.10*10^3$ counts
^{205}Tl	$4.60*10^3$ counts	$4.90*10^3$ counts	$4.10*10^3$ counts
Mean RSD	2.80 %	3.50 %	3.20 %
Oxide ratio $^{156}CeO^+/^{140}Ce^+$	1.60 %	/	/
Doubly charged ratio $^{140}Ce^{2+}/^{140}Ce^+$	1.40 %	/	/

*Without the use of CO_2 as optional gas

For quantification, an external calibration method was used. Elements of interest were diluted in ultrapure water and matrix adjusted to 10 % HNO_3 (v/v). Concentration levels were the same as in the case of total element determination in digested algae samples (section

2.6.1). The trueness of the external calibration was checked by measuring SRM NIST 1640a (1+9, with a matrix adjustment to 10 % HNO_3 (v/v) respectively).

2.11 Total arsenic measurement in various algal extracts

For all other extracts only the arsenic concentration was calculated after measurement with ICPMS (either Agilent 7700x or Agilent 7900). This was done to determine the extraction efficiency of arsenic out of seaweed samples depending on different process parameters used. The instrument´s performance was monitored and optimised in the same manner as described in 2.10, except that H_2 reaction mode was not included.

Prior to the measurements the extracts were further diluted by adjusting the matrix to 10 % HNO_3 (v/v). For quantification, an external calibration was prepared in the same manner as the samples were diluted. The trueness of the calibration was checked by the measurement of SRM NIST 1640a (1+9, with a matrix adjustment to 10 % HNO_3 (v/v) respectively). Additionally, a calibration point from the midrange of the calibration curve was chosen as a drift solution to monitor any possible changes in signal intensities during each run. The frequency of the drifts was distributed evenly through the whole acquisition. Lastly, 0.2 mg/L ^{74}Ge and ^{115}In (prepared in 10 % (v/v) HNO_3) were used to monitor and correct possible non-spectral interferences due to the different matrix composition of the calibration solutions and all other samples.

The arsenic signal was monitored as ^{75}As in helium collision mode to eliminate the $^{40}Ar^{35}Cl^+$ polyatomic interference on the mass-to-charge ratio 75.

In these measurements the so-called carbon enhancement effect was also utilized by mixing a flow of 0.20 L/min CO_2 (1 % (v/v) in Ar) with the sample aerosol stream carried by the nebulizer gas (Ar) just before it reaches the torch. The nebulizer gas flowrate was decreased to maintain a constant flow of sample aerosol into the plasma. This guarantees constant positioning of the normal analytical zone in the plasma, which results in minimal changes in sensitivity.

The signal enhancement of arsenic is a consequence of the higher rate of ionization of ^{75}As in presence of carbon atoms [62]. This influence however is only valid for elements exhibiting a high first ionization potential (as example: As – 9.81 eV, Se – 9.75 eV, Te – 9.01 eV), but are

still lower than for carbon – 11.26 eV [63]. The similarity in energy levels results in charge transfer mechanisms, where the plasma saturated with C^+ ions catch electrons from the above mentioned atomized elements, resulting in formation of carbon atoms and ions of interest, thus improving the sensitivity manifold is discussed as one possible mechanism [64]. This however should then apply also for other elements, but in contrast to As and Se, a manifold increase in sensitivity for instance in the case of Zn – 9.39 eV is not observable [65]. Thus, the mechanism of the carbon enhancement effect is much more complex and is dependant also on the mean free path of the analyte in the plasma, as well as its collision cross section and the its number of possible ionization states [66].

However, CO_2 was not only used to achieve higher sensitivity for arsenic [67], but also to compensate for the different matrix composition of the extracts, calibration solutions and internal standard solution. The latter two consisted only of 10 % aqueous HNO_3 solution (v/v), whereas the matrix composition of the extracts were complex mixtures, containing a significant amount (up to 1% v/v) of organic molecules (ionic liquids or other organic solvents like isopropanol for instance). This means that the matrix already contributes to the charge exchange reactions in the plasma, resulting in a higher ionization rate of As in the case of extracts than in the case of calibration solution. To overcome this issue, the sample stream is saturated with carbon dioxide to give equal rates of ionization.

The second issue that arises with different matrices for samples and the calibration standards is the impedance change in the plasma during sample introduction (disruption of the secondary magnetic field generated by the plasma), thus desynchronising the RF generator and plasma frequencies (“mismatched state”). The extent of this is directly correlated to the sample composition. As an example, if the sample matrix changes from 10 % HNO_3 (v/v) to 10 % HNO_3 (v/v) + 1 % total organic content (v/v), the latter will have a different impact on the plasma, thus the RF generator will adjust its power supply to the RF coil (“matched state”) to a different extent comparing both cases. This ultimately results in slight deviations of the generated energies of the plasma [68, 69]. This issue is bypassed by saturating the sample aerosol stream with CO_2, resulting in the equivalent carbon content in all measured samples.

2.12 Quantification of inorganic arsenic via HPLC-ICPMS

Yakinori and Wakame extracts from 2.7.2 were diluted with ultrapure water (1+2) and transferred into HPLC vials. For quality control the CRM 1568b Rice flour extract was diluted in the same manner. An external calibration of 4 arsenic species (AB, DMA, MA and As(V)) was prepared in ultrapure water and also transferred into HPLC polypropylene vials (concentration range: 0.1 – 50 µg/L). The trueness of the calibration was checked by the measurement of SRM NIST 1640a (1+9). All samples were measured via HPLC coupled to ICPMS using the following system parameters summarised in Table 7:

Table 7: HPLC and ICPMS system parameters during acquisition

Column	PRP-X100 (150 x 4.6 mm; 5 µm, stainless steel)
Flow rate [mL/min]	1.00
Column compartment T [°C]	40
Draw speed [µL/min]	100
Injection volume [µL]	10
Mobile phase	20 mM $NH_4H_2PO_4$ buffer at pH = 6.0
Back pressure [bar]	approximately 80
Peristaltic pump speed [rpm]	0.5
Nebulizer	MicroMist
Optional gas	0.20 L/min CO_2 (1 % (v/v) in Ar)
Collision gas	He
Monitored isotopes	$^{40}Ar^{12}C^+$, $^{75}As^+$, $^{40}Ar^{37}Cl^+$

Similarly, as in the case of total arsenic measurement, the nebulizer gas flowrate was reduced to 0.90 L/min and a 0.20 L/min CO_2 stream (1 % (v/v) in Ar) was mixed with the sample stream prior reaching the torch, so that the gas stream had a constant flow of 1.10 L/min to the plasma. Arsenic was measured in helium collision mode to minimize the polyatomic interference of $^{40}Ar^{35}Cl^+$ on ^{75}As. The extent of chlorine interference removal was monitored by measuring the mass-to-charge ratio 77 ($^{40}Ar^{37}Cl^+$). Finally, to check if the carbon background stays constant during acquisition, the mass-to-charge ratio 53 was also monitored ($^{40}Ar^{13}C^+$). To warrant signal stability during measurement, a calibration point from the midrange of the calibration curve was chosen as a drift solution to monitor any

possible changes in signal intensities. The frequency of the drifts was distributed evenly through the whole acquisition.

2.13 Speciation of polar arsenicals in Wakame and Yakinori extracts

Various Wakame extracts were analysed on their arsenic species content on a HPLC system coupled to an element selective detector (ICPMS). Both anion- and cation-exchange chromatography was conducted, however the latter only to exclude possible cationic species that are not found in algae.

2.13.1 Cation-exchange chromatography

Starting with cation exchange chromatography, aqueous Wakame extracts were diluted in ultrapure water and transferred into HPLC polypropylene vials. A method adapted from Braeuer et. al [70] was used to separate AB, TMAO, AC, TETRA. The system parameters are listed in the following Table 8:

Table 8: HPLC and ICPMS system parameters during acquisition

Column	Zorbax 300-SCX (150 x 4.6 mm; 5 µm, stainless steel)
Flow rate [mL/min]	1.50
Column compartment T [°C]	30
Draw speed [µL/min]	100
Injection volume [µL]	10
Mobile phase	10 mM pyridine buffer at pH = 2.3
Nebulizer	MicroMist
Monitored isotopes	$^{40}Ar^{12}C^+$, $^{75}As^+$, $^{40}Ar^{37}Cl^+$

To increase the sensitivity for arsenic, the nebulizer gas flowrate was reduced to 0.90 L/min and a 0.20 L/min CO_2 stream (1 vol% in Ar) was mixed with the sample stream prior reaching the torch, so that the gas stream had a constant flow of 1.10 L/min to the plasma. Arsenic was measured in helium collision mode to minimize the polyatomic interference of $^{40}Ar^{35}Cl^+$ on ^{75}As. The extent of chlorine interference removal was monitored by measuring the mass-to-charge ratio 77 ($^{40}Ar^{37}Cl^+$). Finally, to check if the carbon background stays constant during acquisition, the mass-to-charge ratio 52 was also monitored ($^{40}Ar^{12}C^+$).

2.13.2 Anion-exchange chromatography

The main scope of the master thesis focussed on anionic arsenic species, and in the case of OH-Sugar the neutral molecule. Algae extracts from most experiments were further diluted in ultrapure water and transferred into HPLC vials. The method used is described in 2.12 in Table 8. The optimum pH was adopted from Raber et. al [57], therefore pH = 5.6 and pH = 6.0 were used for buffer preparation. An external calibration of four arsenic species (AB, DMA, MA and As(V)) was prepared in ultrapure water and also transferred into HPLC plastic vials (concentration range: 0.1 – 50 µg/L – depending on the measured samples the range was adjusted). The blank sample consisted of ultrapure water only. The trueness of the calibration was checked by measuring the SRM NIST 1640a diluted in ultrapure water (1+9). A drift solution was also measured (mid-range calibration point) to monitor the stability of the measurements. The drifts were evenly distributed through the whole acquisition.

For the identification of the unknown arsenosugar peaks, a frozen PO_4-Sugar standard was melted and diluted in ultrapure water (1+499). Similarly, the OH-Sugar standard was melted and diluted in ultrapure water (1+9). Since the shelf life of such standard is unknown, the arsenosugar concentrations were also unknown, however for qualitative analysis and sample spiking they were ideal. Additionally, the concentration of the OH-Sugar, as well as the PO_4-Sugar was determined via compound independent calibration (CIC) [71]. Also, the concentrations of DMA, MA and As(V) were determined, and the species were identified by spiking experiments with As(V), MA and DMA standards by using the injector program of the HPLC system.

3 Results and discussion

3.1 Results of the digestion procedures

Total element concentrations of the measured digested samples are shown in Table 9 (Wakame samples) and Table 10 (Nori samples). Different concentration ranges are shaded with different colours. For instance, the concentration range of mg/kg dry mass is shaded with a green colour. It is important to pinpoint that all results are based on the dry mass of the seaweed samples. Therefore, results in mg/kg dry mass are calculated as follows:

$$c\left[\frac{mg}{kg}\right] = \gamma\left[\frac{mg}{L}\right] \times df \text{ [L/kg]} \quad 1$$

Where *df* is the combined dilution factor of a particular digest and *γ* is the mass concentration of the diluted digest calculated via external calibration directly in the MassHunter software. Typical dilution factors of samples were approximately 1000.

3.1.1 Trueness for SRM NIST 1640a and M149B

The trueness of the external calibration, checked by comparing the measured values versus the certified values for Li, B, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, As, Se, Rb, Sr, Mo, Cd, Ba, Tl, Pb, U of a diluted NIST 1640a sample, varied from 82 – 99 %, except for Al where it was below 80 % for the first digestion procedure.

Similarly, the trueness of the external calibration for the second and third digestion procedure varied from 88 – 114 % and 81 – 119 % (same SRM). However, for the third digestion experiment there was a carry-over effect observed, which occurred from previous samples measured. Consequently, the trueness of Li and K was over 120 %, but since the algae samples consisted of much higher levels of Li and K than the drift solution contained, and the carryover was only observable in the SRM NIST 1640a sample, but not in the digestion blanks, Li and K concentration were also reported.

Additionally, the trueness of the external calibration in the second set of acquisition was also checked by the measurement of a reference water M149B. The accuracy for Al, Cr, Mn, Fe, Co, Ni, Cu, As, Se, Cd, Pb and U varied from 86 – 115 %, except for mercury where it was below 40 %. Although the result for Hg is extremely low, it can be interpreted as a loss of the

element through evaporation due to the long storage of the reference water and since the external calibration solutions, as well as the digests were prepared fresh, mercury concentrations are reported.

3.1.2 *Trueness of CRM ALVA-PA14/1 and CRM IAEA-140/TM*

Furthermore, results of the two digested reference materials, which were measured to assess the integrity of the digestion procedures, ranged from 51 – 153 % for CRM ALVA-PA14/1 (Oregano) and 56 - 137 % for CRM IAEA-140/TM Seaweed (*Fucus spiralis*). However, most elements were in the range of 80 – 120 %.

To show some exceptions two elements are picked out. The first one is Hg, which had a trueness over 300 % in the case of *Fucus spiralis*. The only possible explanation of this behaviour is a contamination of the CRM, since no interference was detectable on the ^{201}Hg isotope. Another unusual result was the low percentage of Ba (again in the case of *Fucus spiralis)* that was only 55.8 %. But since, the trueness of Ba was over 90 % for the Oregano CRM in the first digestion experiment and the results for Ba of the remeasured Wakame Vienna sample was 32.9 ± 0.7 mg/kg dm, which is comparable to the barium concentration obtained in the first digestion experiment (33.6 ± 0.3 mg/kg dm), all results for this element were reported. To elucidate this unusual results anomaly further experiments would be necessary to either prove or exclude possible analyte loss during sample storage or sample preparation of the certified reference material *Fucus spiralis*.

For clarity a visual example, the results set for different analytes on the trueness of the second digestion procedure and the ICPMS method is displayed in Figure 10.

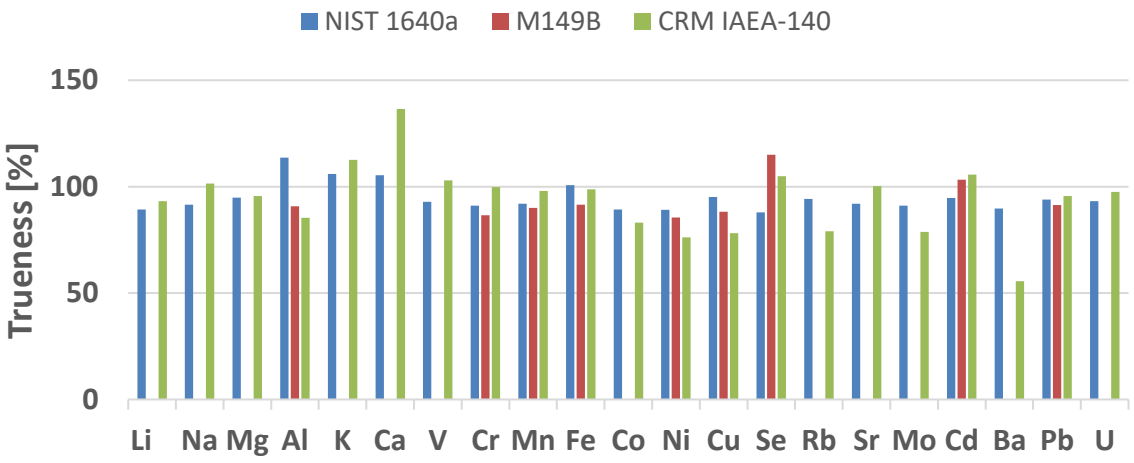


Figure 10: Trueness of the second digestion experiment for different elements determined from 3 different CRMs

Results on the trueness of all three experiments for arsenic are separately presented in Figure 11.

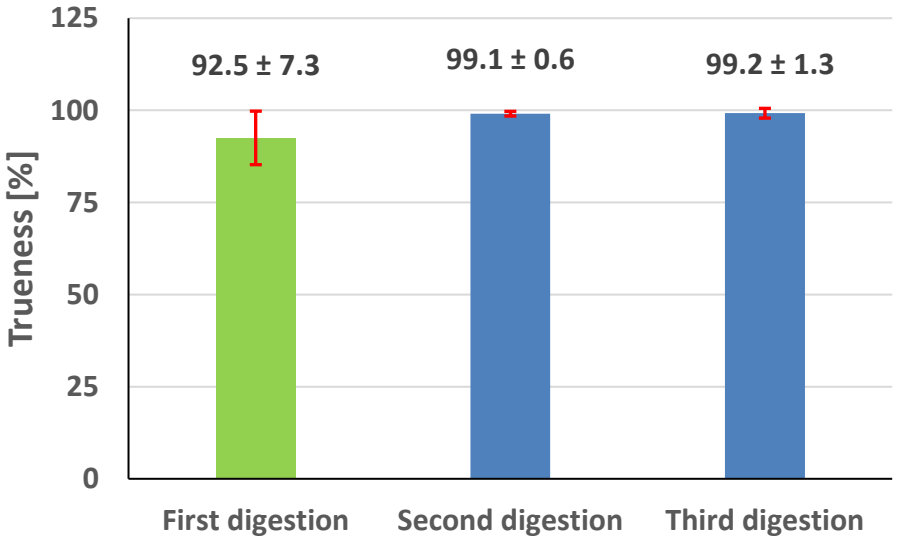


Figure 11: Trueness of digestion experiments for arsenic: Oregano (first digestion - green) and *Fucus spiralis* (second and third digestion - blue)

3.1.3 *Stability of the measured drift solutions*

The stability of all three sets of acquisitions was sufficient for all elements. The concentration of the measured drift solutions stayed stable during the whole measurement. The only exception that occurred was in the case of boron, where a clear carryover effect was observable in all three digestion experiments. The carryover was the consequence of the

poor washout of the autosampler system after measuring the Wakame and Yakinori samples. Possibilities to overcome this issue are to either set the concentration of B in the drift solution at least one order of magnitude higher or adjust the time of the rinse programme or maybe even include an extra rinse step (rinse solution containing small amounts of hydrofluoric acid of sodium fluoride [72]). As an additional option for reducing the boron carry-over flushing the spray chamber with NH_3(g) was discussed. Additionally, an increase of the sensitivity for boron was observed [73]. This is achieved by converting the H_3BO_3 (remaining on the surface of the spray chamber) to its ammonia salt that is then sluiced down into the waste container. Although the boron washout is enhanced, the authors neglect the fact that boron might be sticking also in the tubing of the sample introduction system. Therefore, the research group of Analytical Chemistry for Health and Environment (ACHE) suggests measuring boron in alkaline environment (both external calibration and samples).

As a visual example, the scattering of drift concentrations of selected elements in the case of the second experiment is depicted in Figure 12.

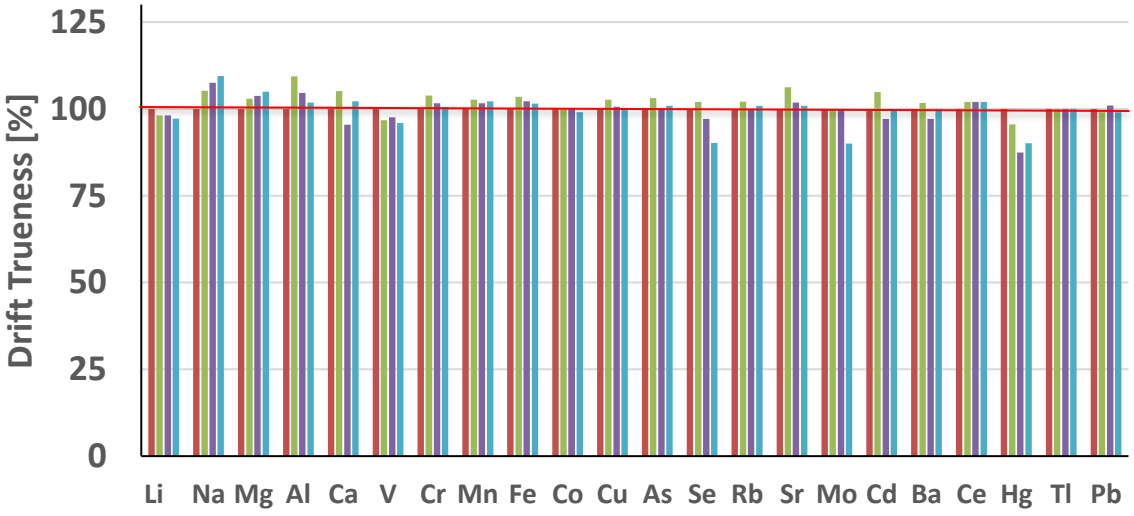


Figure 12: Stability of the drift solution for selected elements in the case of the second digestion experiment

3.1.4 *Stability of the internal standard*

The recovery diagram of internal standard solutions can be found in the appendix. The recoveries were in the range of 80 – 120 % throughout the whole measurements. The highest

deviation occurred in the case of the M149B reference water, that has a slightly less acidic matrix composition than the calibration blank.

3.1.5 *Comments on the result set of Wakame and Nori digests*

As one can see, alkaline metals and earth alkaline metals have the highest abundance in both algal samples. This is in conjunction with the fact, that minerals commonly occur in high concentrations in sea water [74], from which these types of algae are harvested.

For a clearer visualisation, normalised concentrations of Li, Na, Mg, Al, K, Ca, Mn, Fe, Co, Cu, As, Se, Rb, Sr for different Wakame and different Nori samples are plotted in Figure 13 and Figure 14 as bar graphs to compare differences or similarities between different distributers based on total element concentrations.

Starting with Wakame samples and comparing the results between different manufacturers one can see, that same elements are within the same order of magnitude. Smaller differences are seen in the case of manganese and lead, where the Deasang manufacturer´s seaweed (Wakame Graz 2) contains up to five times less Mn and Pb than Wakame samples from the WELPAC manufacturer, although both originate from the Republic of Korea. In contrast, comparing the Wakame Vienna sample with the two samples from Hungary, produced by the same manufacturer (WELPAC), it is evident that the latter two contain lower concentrations of Al, Mn, Fe, Cu, Sr, Cd, Pb and U. This can be explained by the fact that although the producer is the same, the algae were sampled in a different year (3 years gap), where the environment of algae growth could also change. However, due to the lack of information on sampling site and sample handling it is difficult to explain these differences. One may attribute those fluctuations to geographical origin [75].

Similar conclusions can be drawn in the case of Nori samples. The Nori product from U.K. however shows somewhat lower concentrations for most elements, especially in the case of Cd (5 times less than the other samples). The only exception is uranium concentration, which deviates to a large extent, because its concentration is 20 times higher than in the initial Yakinori sample from Vienna.

Nevertheless, the order of magnitude of the determined elements is comparable and in good correlation with data from the literature. Slight deviations however occur since sampling

sites and manufacturers are differing. For instance, Fe, Ni and Pb concentrations are in some cases up to 10 times higher. Although also the seasonal variation can influence the total element concentrations found in brown algal species, its span is much narrower than found in the current research (for instance in the case *Sargassum kjellmanianum*, the Fe concentration decreases only by 1.2 when harvesting fully grown algae in comparison with young fresh algae analysed [76]), thus the variations might be the consequence of different sampling sites of the manufacturers.

As an in-depth study evaluation Miedico et al investigated 21 trace elements in 54 dried Nori and 22 dried Wakame, commercially available products, where most of the elements were 2 – 6 times more abundant than in the case of this master thesis (except for As and for Pb). Quality control was assured by measuring the NIST SRM 1570a Spinach Leaves for total element concentration and by recovery experiments for elements with no certified value. However, each sample was prepared only in duplicates. Furthermore, ammonia reaction gas was employed to minimise polyatomic interferences. While this works good for $^{75}As^{+}$-> $^{91}As(NH_2)^{+}$ eliminating the $^{40}Ar^{35}Cl^{+}$ and not producing new interferences (assuming there is little zirconium in the seaweeds), for other elements it might create new interferences, since a single quadrupole instrument was employed. Even more, mercury is measured at mass-to-charge ratio 202 which is prompt to interference by $^{186}W^{16}O^{+}$ (material often used for grinding) and another example vanadium is measured at mass-to-charge ratio 51 which is inappropriate, since the matrix most certainly contains a lot of chlorine (seawater samples), thus $^{35}Cl^{16}O^{+}$ interfering with vanadium signal. Therefore, one should be cautious when looking for information on total element content in seaweeds, often the concentrations may be overestimated, even though the accuracy of the method was verified by CRMs [75].

Lastly, total arsenic concentrations are summarised in Figure 15. It is evident that in the case of the same manufacturer the arsenic content is constant. Furthermore, comparing different manufacturers one can see that the total arsenic content is of the same order of magnitude and only slight deviations are present. Comparing results with the literature shows a similar picture, while total arsenic concentrations range from 40 – 52 mg As/kg dm in the case of *Undaria pinnatifida* and 26 – 42 mg As/kg dm in the case of *Porphyra tenera* samples [34, 75, 77].

Table 9: Concentrations (means ± SD) in 5 different Wakame samples

Element	Unit	Wakame Vienna	Wakame Budapest 1	Wakame Budapest 2	Wakame Graz 1	Wakame Graz 2
Li	[mg/kg]	0.85 ± 0.01	0.88 ± 0.01	0.86 ± 0.02	0.83 ± 0.02	0.68 ± 0.01
B	[mg/kg]	27.2 ± 0.4	26.4 ± 0.5	25.9 ± 0.5	27.0 ± 0.2	24.3 ± 0.3
Na	[g/kg]	66 ± 1	69 ± 1	67 ± 2	74 ± 2	72 ± 1
Mg	[g/kg]	9.90 ± 0.03	11.1 ± 0.3	10.83 ± 0.03	8.80 ± 0.09	7.26 ± 0.13
Al	[mg/kg]	102 ± 2	40 ± 10	33.1 ± 0.2	87 ± 3	90 ± 9
K	[g/kg]	3.10 ± 0.03	3.23 ± 0.01	3.19 ± 0.05	3.31 ± 0.11	3.38 ± 0.07
Ca	[g/kg]	14.9 ± 0.1	14.5 ± 0.1	14.4 ± 0.2	16.3 ± 0.1	14.5 ± 0.2
V	[mg/kg]	0.29 ± 0.01	0.153 ± 0.002	0.148 ± 0.001	0.28 ± 0.06	0.35 ± 0.04
Cr	[mg/kg]	< 2	0.11 ± 0.01	0.12 ± 0.02	0.82 ± 0.19	0.24 ± 0.01
Mn	[mg/kg]	14.7 ± 0.2	8.4 ± 0.1	8.5 ± 0.3	6.1 ± 0.1	3.9 ± 0.1
Fe	[mg/kg]	155 ± 6	89 ± 3	87 ± 2	130 ± 1	115 ± 11
Co	[mg/kg]	0.101 ± 0.001	0.112 ± 0.001	0.110 ± 0.002	0.151 ± 0.001	0.086 ± 0.004
Ni	[mg/kg]	< 2	0.53 ± 0.01	0.55 ± 0.03	1.73 ± 0.10	0.57 ± 0.1
Cu	[mg/kg]	1.40 ± 0.04	1.07 ± 0.02	1.12 ± 0.03	1.86 ± 0.06	1.00 ± 0.02
As	[mg/kg]	39.5 ± 0.3	38.2 ± 0.6	39.1 ± 0.2	35.7 ± 0.7	40.1 ± 0.1
Se	[µg/kg]	85 ± 5	103 ± 28	90 ± 5	87 ± 5	70 ± 2
Rb	[mg/kg]	0.99 ± 0.01	0.89 ± 0.09	0.87 ± 0.05	0.92 ± 0.04	0.96 ± 0.03
Sr	[mg/kg]	875 ± 12	692 ± 3	691 ± 10	925 ± 10	829 ± 14

Element	Unit	Wakame Vienna	Wakame Budapest 1	Wakame Budapest 2	Wakame Graz 1	Wakame Graz 2
Mo	[mg/kg]	0.081 ± 0.008	0.094 ± 0.009	0.091 ± 0.009	0.120 ± 0.004	0.145 ± 0.017
Cd	[mg/kg]	2.82 ± 0.02	1.71 ± 0.01	1.74 ± 0.01	1.98 ± 0.03	1.28 ± 0.02
Ba	[mg/kg]	33.6 ± 0.3	36.3 ± 0.6	36.5 ± 0.5	48.1 ± 0.4	17.1 ± 0.2
Ce	[mg/kg]	0.170 ± 0.030	0.070 ± 0.001	0.064 ± 0.005	0.151 (*n* = 2)	0.226 (*n* = 2)
Gd	[µg/kg]	n.d.*	12.2 ± 0.6	11.7 (*n* = 2)	21.2 (*n* = 2)	19.5 ± 4.2
Hg	[µg/kg]	17.6 ± 0.8	< 20	< 20	< 85	< 85
Tl	[µg/kg]	1.00 ± 0.01	< 2	< 2	< 1	< 1
Pb	[mg/kg]	0.91 ± 0.01	0.50 ± 0.01	0.49 ± 0.01	0.57 ± 0.01	0.18 ± 0.01
Bi	[µg/kg]	6.9 ± 0.4	< 5	6.6 ± 1.2	8.8 ± 0.4	14.4 ± 0.4
U	[µg/kg]	419 ± 7	321 ± 6	324 ± 6	347 ± 5	412 ± 7

*not determined

Table 10:Concentrations (means ± SD) in 4 different Yakinori samples

Element	Unit	Nori Vienna	Nori Budapest 1	Nori Budapest 2	Nori U.K.
Li	[mg/kg]	0.25 ± 0.01	0.34 ± 0.01	0.36 ± 0.01	0.19 ± 0.01
B	[mg/kg]	8.3 ± 0.1	8.7 ± 0.2	9.4 ± 0.2	< 1.3
Na	[g/kg]	5.0 ± 0.1	5.11 ± 0.03	5.73 ± 0.07	1.70 ± 0.03
Mg	[g/kg]	3.3 ± 0.03	2.57 ± 0.01	2.63 ± 0.03	2.69 ± 0.05
Al	[mg/kg]	30 ± 2	50 ± 3	40 ± 2	9 ± 1
K	[g/kg]	20.0 ± 0.1	23.5 ± 0.3	27.8 ± 0.4	24.7 ± 0.5
Ca	[g/kg]	3.00 ± 0.02	4.53 ± 0.05	4.71 ± 0.05	3.88 ± 0.15
V	[mg/kg]	0.80 ± 0.01	1.00 ± 0.02	0.95 ± 0.02	15.4 ± 0.3
Cr	[mg/kg]	< 2	0.15 ± 0.03	0.19 ± 0.06	0.60 ± 0.41
Mn	[mg/kg]	28.9 ± 0.5	30.9 ± 0.6	33.2 ± 0.5	23.3 ± 0.5
Fe	[mg/kg]	123 ± 3	142 ± 3	145 ± 3	88 ± 3
Co	[mg/kg]	0.227 ± 0.001	0.166 ± 0.002	0.163 ± 0.003	0.085 ± 0.007
Ni	[mg/kg]	< 2	0.67 ± 0.02	0.68 (*n* = 2)	0.474 ± 0.262
Cu	[mg/kg]	15.0 ± 0.1	19.3 ± 0.3	19.0 ± 0.3	7.7 ± 0.1
As	[mg/kg]	23.0 ± 0.2	23.2 ± 0.5	23.2 ± 0.5	23.9 ± 0.6
Se	[µg/kg]	42 ± 1	45 ± 3	48 ± 3	55 ± 3
Rb	[mg/kg]	3.80 ± 0.01	4.49 ± 0.06	4.67 ± 0.06	4.90 ± 0.11
Sr	[mg/kg]	36 ± 1	23.4 ± 0.1	24.4 ± 0.2	27.1 ± 0.3

Element	Unit	Nori Vienna	Nori Budapest 1	Nori Budapest 2	Nori U.K.
Mo	[mg/kg]	0.62 ± 0.01	0.71 ± 0.01	0.77 ± 0.01	0.55 ± 0.02
Cd	[mg/kg]	1.70 ± 0.01	1.86 ± 0.04	2.02 ± 0.05	0.29 ± 0.01
Ba	[mg/kg]	24.5 ± 0.2	6.0 ± 0.6	6.1 ± 0.1	5.6 ± 0.1
Ce	[mg/kg]	0.27 ± 0.01	0.37 ± 0.02	0.36 ± 0.01	0.072 ± 0.001
Gd	[µg/kg]	n.d.*	73 ± 1	76 ± 1	150 ± 3
Hg	[µg/kg]	8.8 ± 0.2	< 20	< 20	< 85
Tl	[µg/kg]	0.60 ± 0.01	< 2	< 2	< 1
Pb	[mg/kg]	0.090 ± 0.001	0.105 ± 0.003	0.111 ± 0.004	0.222 ± 0.007
Bi	[µg/kg]	2.5 ± 0.9	< 5	< 5	< 5
U	[µg/kg]	6.20 ± 0.01	38.1 ± 0.3	21.71 ± 0.2	118 ± 2

*not determined

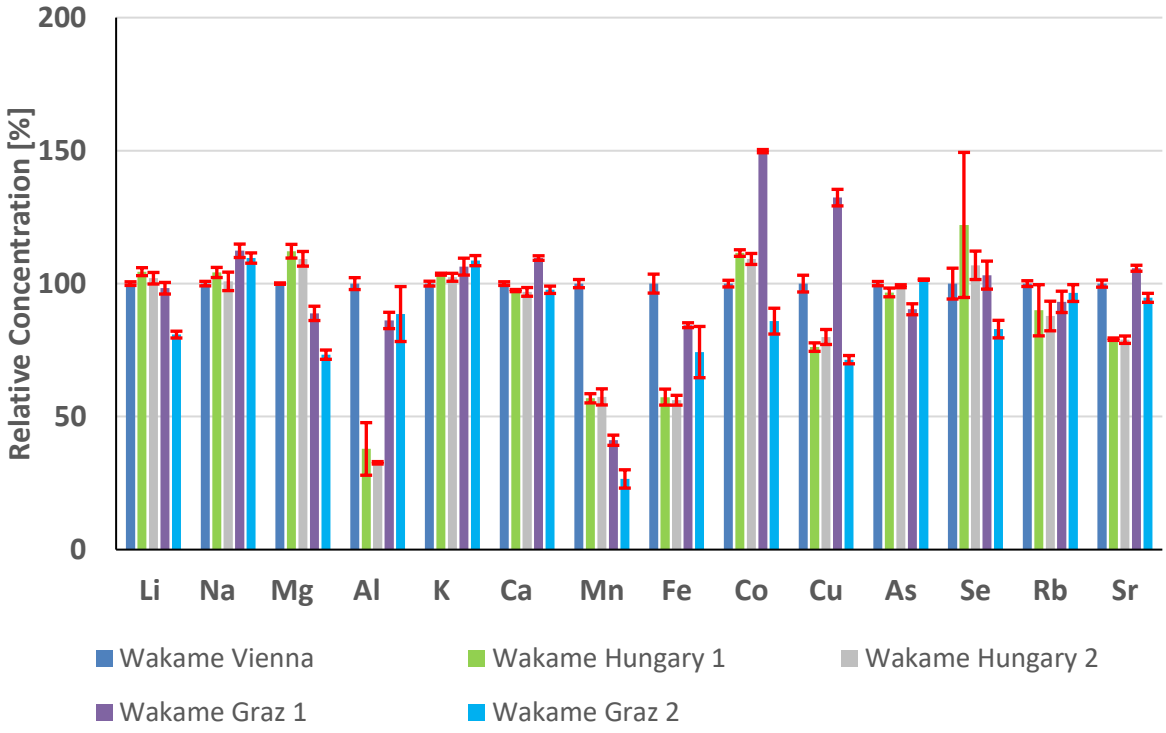


Figure 13: Normalised element concentrations of digested Wakame samples bought from three different distributers at three different local Asian stores

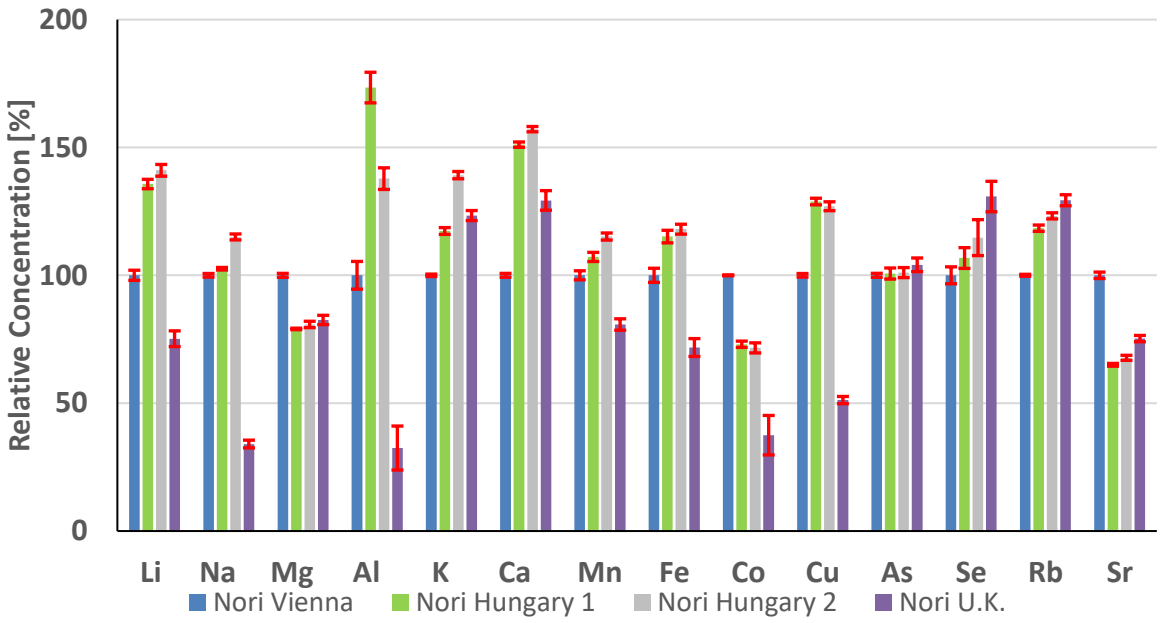


Figure 14: Normalised element concentrations of digested Nori samples bought from two different distributers at three different local Asian stores

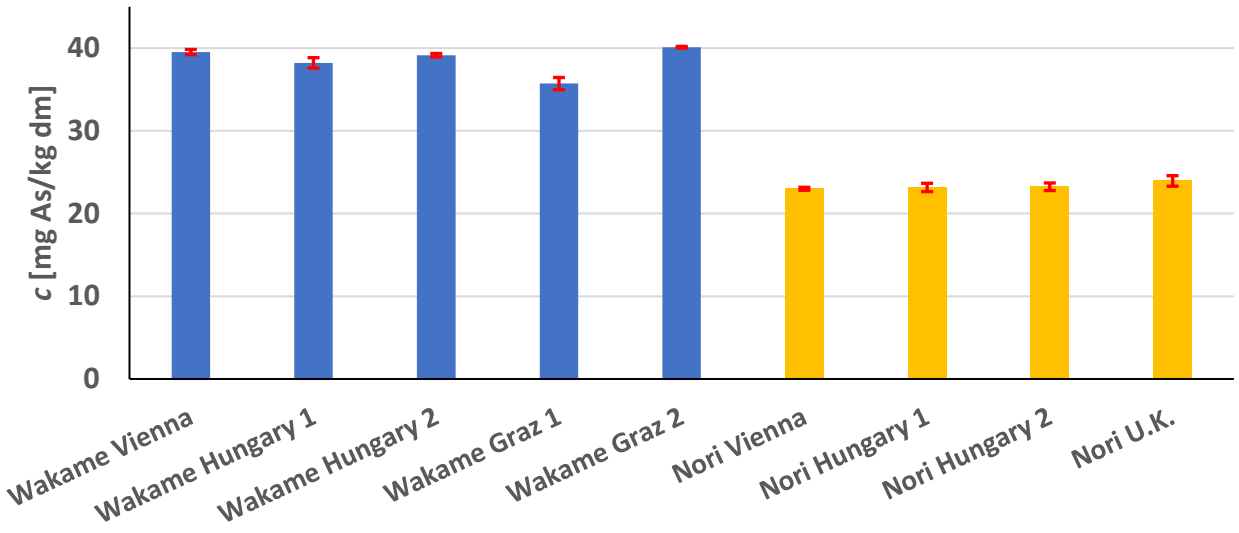


Figure 15: Results for total arsenic concentrations in 9 digested seaweed samples

3.2 Extractability of various elements from Wakame and Yakinori samples under mild conditions

Extraction efficiencies of various elements from Wakame and Yakinori samples are plotted in Figure 16, Figure 17 and Figure 18 based on the solvent and conditions used for extraction. Elements such as Ni, Se, Pb and U are not shown since these elements were not extractable. The extraction efficiency is calculated as follows:

$$ex\ [\%] = \frac{c_{extract}}{c_{digest}} \times 100 \qquad 2$$

Where ex [%] is the extraction efficiency in percent for a selected element and the concentrations of the extract and digest are expressed in mg/kg dm.

Note: the standard deviation of the extraction efficiency is the combined standard deviation of the standard deviations of extract and digest concentrations. It is calculated as follows:

$$s_{ex}\ [\%] = \frac{c_{extract}}{c_{digest}} \times \left(\frac{s_{digest}}{c_{digest}} + \frac{s_{extract}}{c_{extract}}\right) \times 100 \qquad 3$$

For example: the total arsenic concentration in Wakame seaweed is 39.5 ± 0.2 mg/kg dm and the total arsenic concentration in a aqueous extract of the same sample (60°C, 2 h, shaking water bath) is 1.37 ± 0.27 mg/kg dm. Thus, the standard deviation of the extraction efficiency is:

$$s_{ex}\ [\%] = \frac{1.37}{39.5} \times \left(\frac{0.2}{39.5} + \frac{0.27}{1.37}\right) \times 100 = 0.7\ \% \qquad 4$$

In the case of potassium for the same sample and conditions however, we obtain much larger error bars, due to the fact that the extracts concentrations scatter to a larger extent: 1.1 ± 0.3 g/kg dm in comparison to the digested: 3.1 ± 0.3 g/kg dm (see Figure 17).

The trueness of the external calibration was between 93 – 110 % for all elements (V, Mn, Fe, Co, Ni, Zn, As, Se, Sr, Mo, Ba, Pb and U). The internal standard recovery diagram showed no significant deviations for the aqueous extracts. Same goes for the stability of the drift solution, which was higher in the case of Ni (up to 176 %), which is explainable by the fact that the concentration of the drift solution (0.10 ng/mL) was below the detection limit (0.12 ng/mL). This however proved to be no issue because all extracts yielded less than 0.12 ng/mL Ni.

Results show that the ultrasound assisted extraction procedure yields better results than the extraction in a shaking water bath, even if the latter was conducted at elevated temperature. Only exception is in the case of cobalt, where the extraction at 60°C yielded almost two times more Co in the extract. This could be explained by the fact that Co(II) compounds are more soluble in water at elevated temperatures.

Comparing elements extracted with ultrapure water and methanol/ultrapure water mixture, one can see that for most elements the extraction efficiencies are of the same order of magnitude, however always slightly less in the case of methanol. Especially Ca, V, Mn, and Fe are poorly extractable in methanol/water. Furthermore, comparing Wakame extracts with Yakinori extracts, one can see that for the same elements the extraction efficiency for the red algal species is always higher. This is especially true for V, Fe, Co, Cu, As and Sr. For example, the extraction efficiency of As is 4.3 % ± 0.9 % in the case of *Undaria pinnatifida* and 60 % ± 12 % in the case of *Porphyra umbilicalis*, both extracted at room temperature with ultrapure water. In the case of Wakame this is rather concerning since most of the arsenic remains in the algae after extraction (soaking) with water. And preparing Wakame this way for consumption is very common in the eastern culture (for instance soaking the algae in water and after draining adding it to salads). This issue is not that simple since the risk is species dependent and for an appropriate risk assessment arsenic speciation analysis of the extracts is necessary. Furthermore, testing human urine after seaweed consumption would also be interesting, however this was beyond the scope of this master thesis.

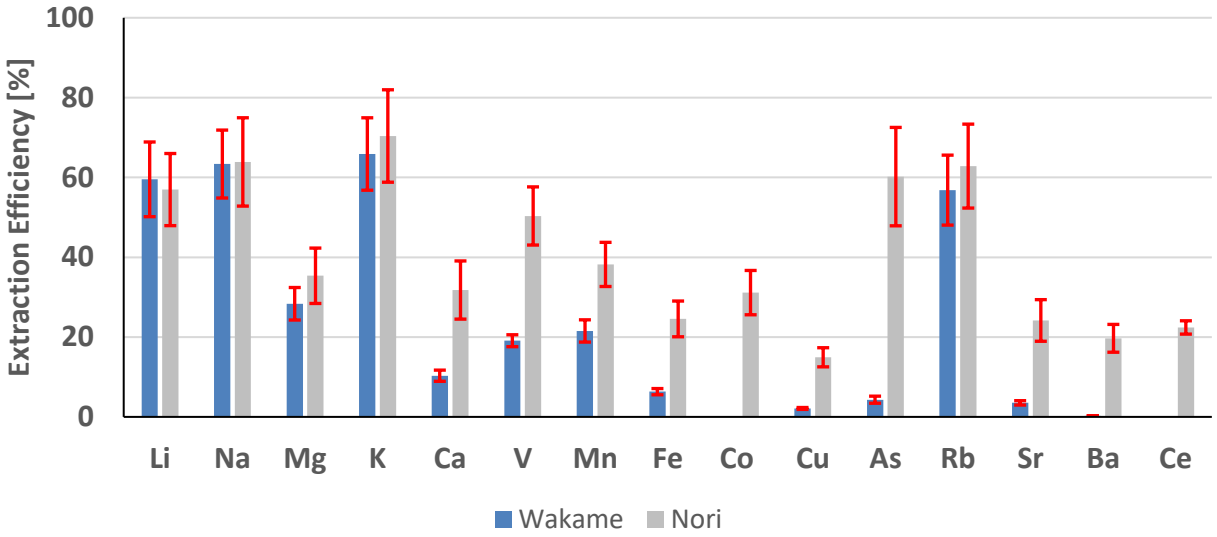


Figure 16: Extraction efficiencies for various elements extracted with ultrapure water at room temperature (Note: error bars are in percent and show the combined standard deviation of both the digested samples and extracted samples)

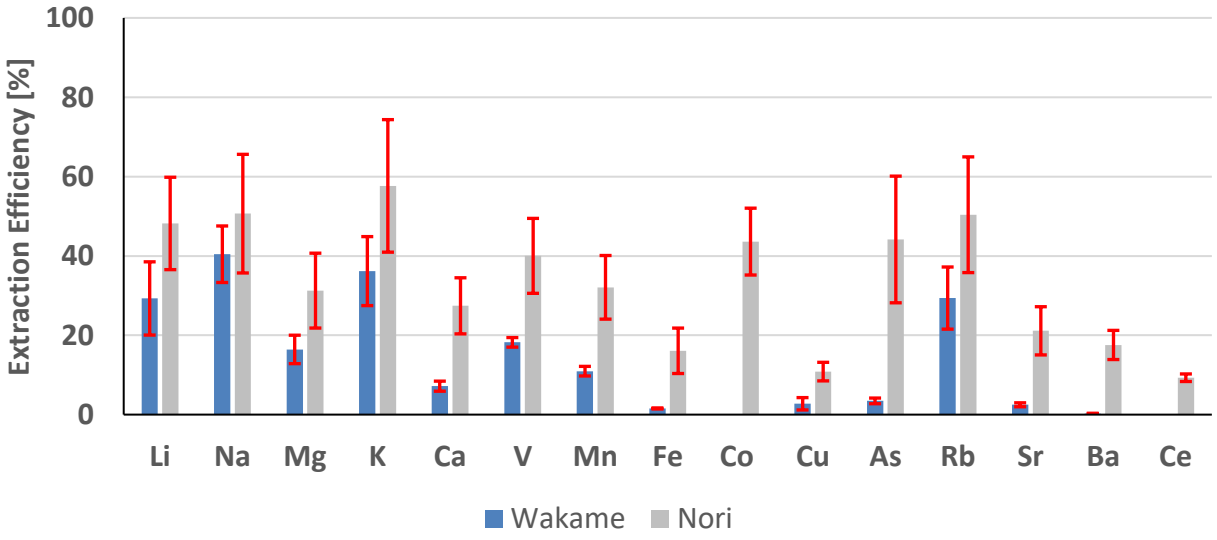


Figure 17: Extraction efficiencies for various elements extracted with ultrapure water at 60°C (Note: error bars are in percent and show the combined standard deviation of both the digested samples and extracted samples)

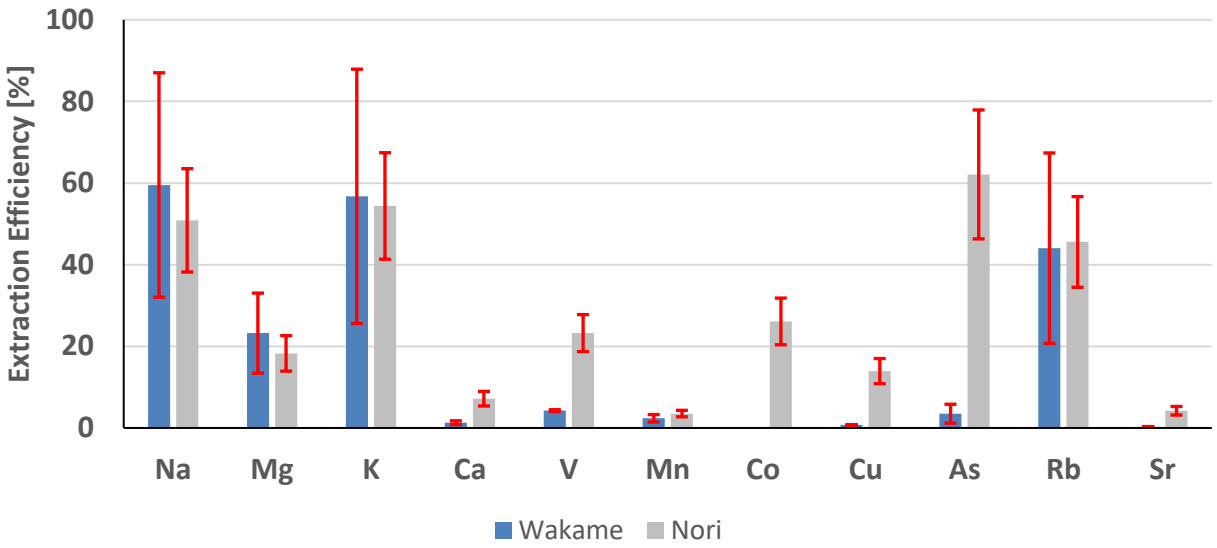


Figure 18: Extraction efficiencies for various elements extracted with (1+1 v/v) methanol/ultrapure water mixture at room temperature (Note: error bars are in percent and show the combined standard deviation of both the digested samples and extracted samples)

3.3 Inorganic arsenic content in two algae samples (Wakame and Nori Vienna)

Total arsenic concentrations of the extracts together with the extraction efficiencies were as follows:

- 27.1 ± 0.9 mg/kg for Wakame that corresponds to an ex [%] of 68.6 ± 3.0 %
- 18.0 ± 0.6 mg/kg for Yakinori that corresponds to an ex [%] of 78.4 ± 3.2 %

Results on the total arsenic concentration in the extracts were used to calculate column recoveries (cr) of the HPLC-ICPMS analysis for seaweed extracts. The relationship is described in the following equation:

$$cr\ [\%] = \frac{c_{As\ species}}{c_{extract}} \times 100 \qquad 5$$

The numerator is the sum of all arsenic species that are retained on the chromatographic column during acquisition (each peak integrated independently). Unknown peaks on the mass-to-charge ratio 75 were quantified via compound independent calibration [71].

Column recoveries were decent: 96 ± 13 % for Wakame and 98 ± 13 % for Yakinori.

The trueness of the external calibration both for total arsenic determination and arsenic speciation was above 93 %.

The trueness for the SRM 1568b (Rice flour) however was only 76 % in the case of total arsenic determination (total element concentration in the extract measured with ICPMS).

For the certified species concentrations (measured with ICPMS coupled to HPLC), the trueness for the arsenate peak was only 72 %. Furthermore, the trueness of DMA was 83 % and MA 91 %. The behaviour of the drift was stable throughout the whole set of acquisition.

The concentration of inorganic arsenic species is portrayed in Figure 19 (note the concentration unit which is in µg/kg dm).

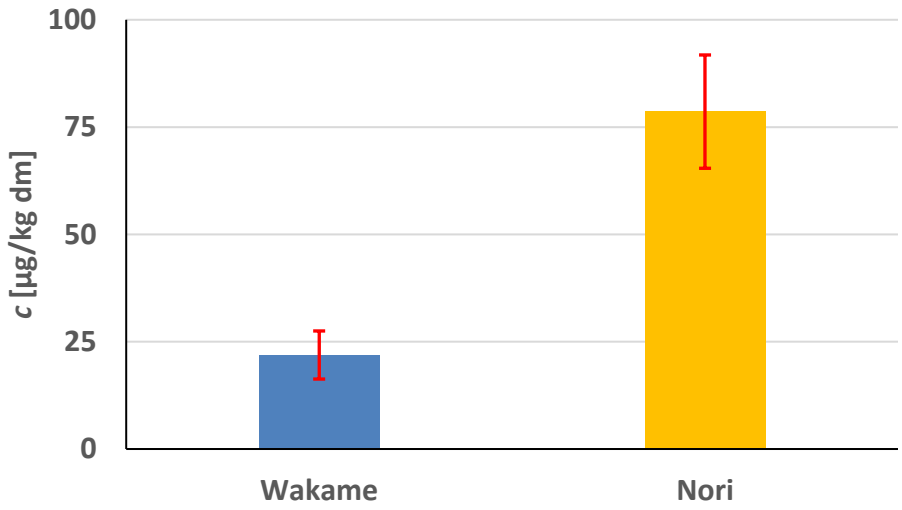


Figure 19: Inorganic arsenic content in two seaweed samples; Conditions: 0.1 M HNO_3 and 3 % H_2O_2(v/v), 90°C, shaking water bath, 60 min

These results show that the inorganic arsenic fraction in both algae is scarce. Its percentage in Wakame is only 0.05 % and somewhat higher 0.34 % in Yakinori. Furthermore, these results are below the established regulatory status in France and Europe, which set the inorganic arsenic limit to 3 mg/kg dm [78], thus the consumption of these seaweed is safe.

For a clearer overview of the arsenic speciation analysis of the oxidized Nori extracts, Figure 20 highlights its anion-exchange chromatogram overlayed with the chromatogram of an aqueous Nori extract, both measured at mass-to-charge ratio 75.

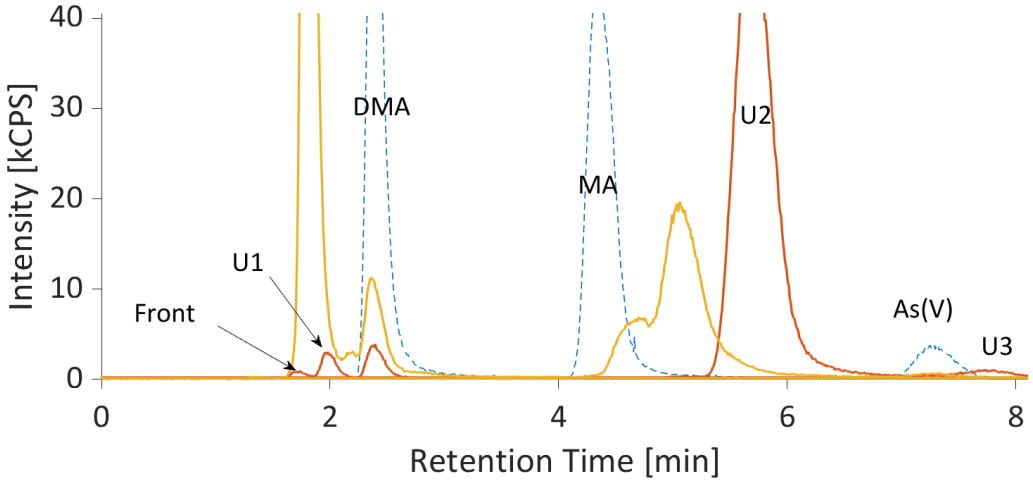


Figure 20: Anion-exchange chromatograms of Yakinori extracts extracted once with ultrapure water at 60°C (red) and once upon oxidizing conditions (yellow). The blue dotted line is a 50 µg/L standard solution of DMA and MA and 5 µg/L As(V); Conditions: PRP-X100 column (150 x 4.6 mm; 5 µm, stainless steel) at 30 °C, 20 mM $NH_4H_2PO_4$ buffer at pH = 6.0, flow rate = 1.0 mL/min and 10 µL injection volume

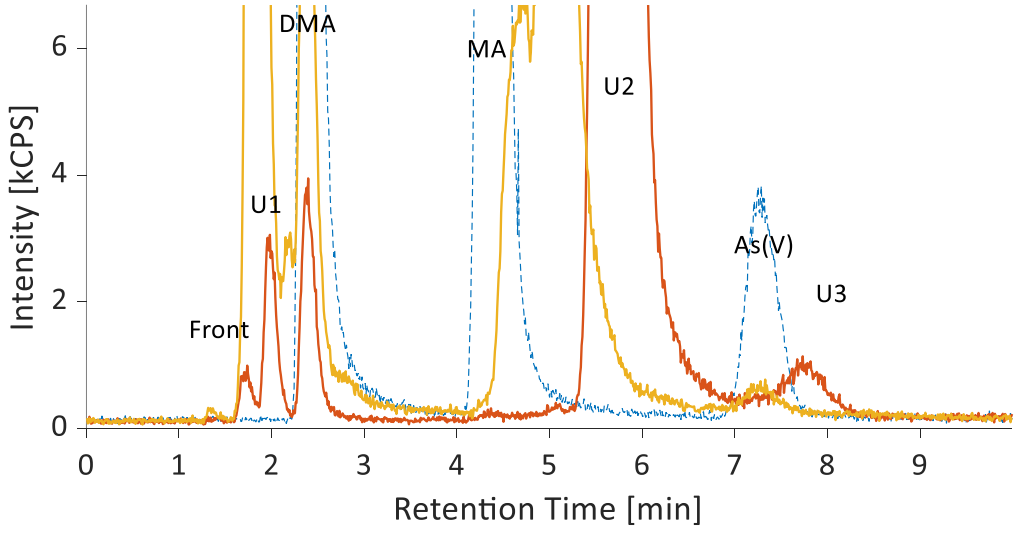


Figure 21: Closeup of figure 20 – yellow arsenate peak is the total inorganic arsenic found in Yakinori

As one can see for the aqueous extract, the major arsenic peak is an unknown substance eluting at 5.7 minutes. This peak does not occur in the oxidized extract. However, it is debatable that the double peak at 5.1 min is a degradation product of U2 maybe coeluting

with MA. Whether it really is a degradation product, or the retention time is shifted because of the acidified matrix composition of the extract needs to be checked by spiking experiments.

Nevertheless, what can be clearly stated is the fact that the major degradation product/products of the arsenic species found in Yakinori elute in or close to the solvent front. To clearly identify the unknown peaks and to confirm the arsenic species, spike experiments with standard solutions need to be conducted. Whether this unknown species are arsenosugars will be discussed in the following chapters.

3.4 Results on total arsenic and arsenic species concentrations in Wakame extracts

Figures presented in the following subsections summarise the extraction efficiencies of arsenic based on various extraction experiments conducted. The results are logically grouped based on the different parameters used (solvent, temperature, time, sample extraction method, repeatability, and so forth). Not all experiments are shown, nevertheless these illustrations give a good estimate on the arsenic extractability from the brown algal species *Undaria pinnatifida* and highlight similarities/deviations between different purchased batches. Detailed results on the total arsenic concentration of the extracts as well as the concentrations of arsenic species found in the extracts can be found in the appendix.

3.4.1 Wakame from Vienna

3.4.1.1 Polar solvents

Starting off with the initial material (Wakame Vienna) on which most of the research was conducted it is obvious that water and water/methanol mixtures do poorly release the arsenic into the tested solvents (Figure 22). The majority of arsenic remains bound to the algae in one way or another.

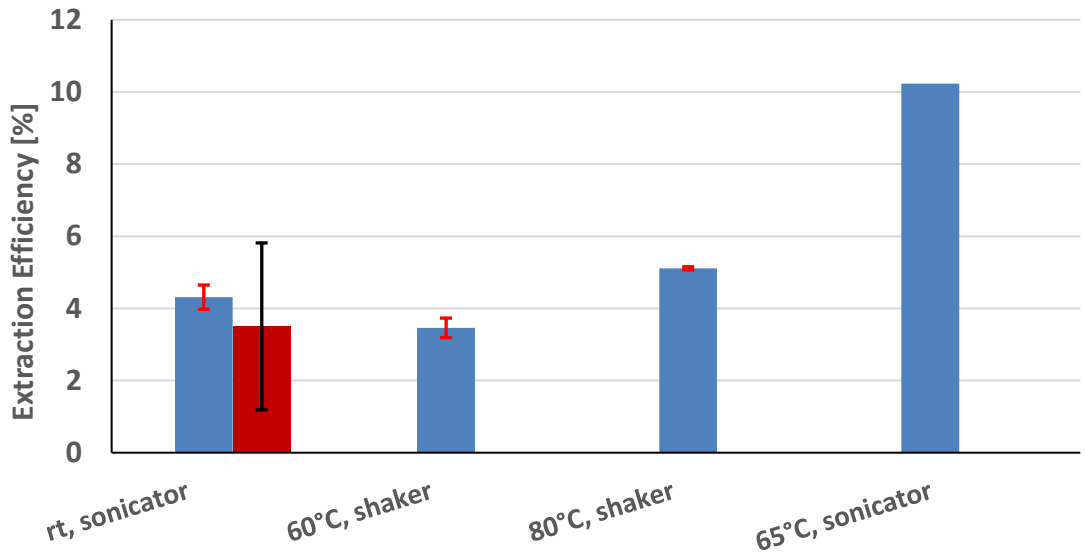


Figure 22: Arsenic extractability by polar solvents: water (blue bars), water/methanol (red bar) from Wakame samples in different extractors at different temperatures

By increasing temperature of the sonicator the extraction efficiency of arsenic magnifies to a larger extent than in the case of the shaking water bath. Furthermore, the sonicator appears to be the better option for extraction of polar arsenicals, probably because the ultrasound itself transfers energy to the samples, enhancing matrix breakdown and as a consequence solvent penetration [79]. The yield however is still poor and further experiments were performed mostly utilizing the shaking water bath due to its larger capacity (especially for the 50 mL polypropylene tubes), better temperature control and improved mixing (contact of algal surface area with the extractant).

Whether the non-soluble fraction is lipophilic or entrapped in the cellular structure of the algae is not clear yet. The results however are of great concern, since the traditional ways of cooking and preparing dishes from Wakame (miso soup, seaweed salads) do not get rid of the arsenic.

Gastric juice simulations on arsenic species release and their possible transformations as well as speciation analysis of urine samples from individuals on a “Wakame diet” could estimate the risk of such dietary exposure. This was however beyond the scope of the master thesis research.

Nevertheless, an interesting example from literature on this topic should give more clarity. Garcia Sartal et.al investigated different seaweed samples on their bioavailability by an *in vitro* digestion experiment. Results on Wakame samples showed that the raw as well as the cooked algae is poorly dialyzable (only up to 15 %), meaning that most arsenic residue is still bound in the algae and afterwards excreted. Furthermore cooking does not have any effect on arsenic's bioavailability [80]. Another study found a similar degree of As bioavailability (18 ± 1 %) [77].

3.4.1.2 Less polar and nonpolar extractions

Continuing with less polar and nonpolar solvents at mild conditions (rt and 40°C) one can see in Figure 23 that conventional organic solvents (isopropanol, dichloromethane, and methanol) extract arsenic very poorly. Extraction is enhanced by the addition of an ionic liquid, which tends to extract nonpolar arsenicals better than the polar ones. However, the extraction yield is not satisfying. Even a higher extraction yield (6 ± 1 %) is achieved by using organic solvent and ionic liquid mixtures as extractant. But still, even the latter is far from quantitative and is only a fourth of the total arsenolipid content extracted from fresh Wakame seaweed by Morita et. al [81].

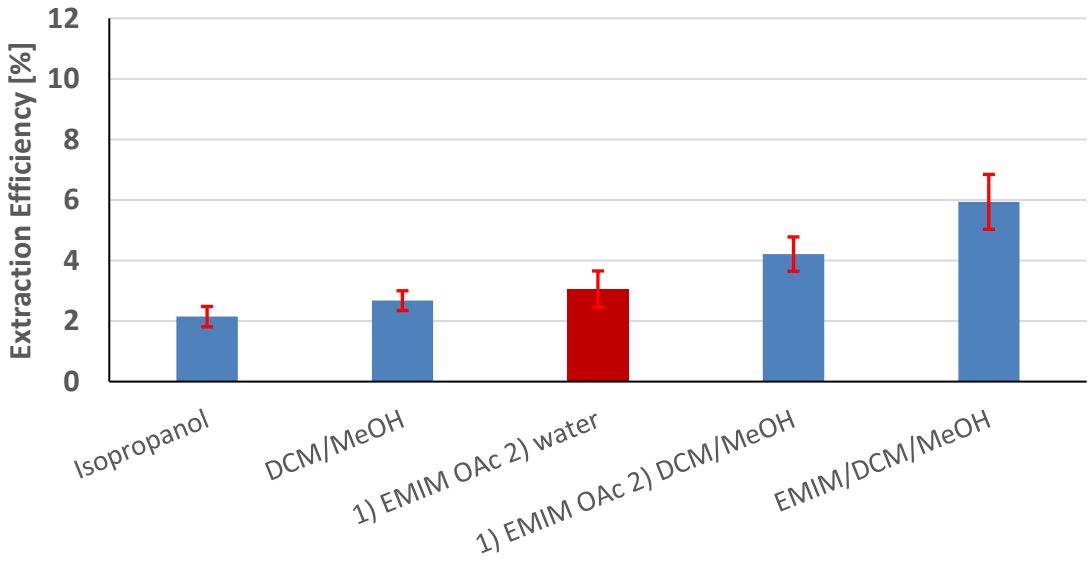


Figure 23: Arsenic extractability by nonpolar solvents and [EMIm][OAc] from Wakame samples performed at rt (blue bars) and 40°C (red bar)

Further experiments could focus on the investigation of organic solvent/IL mixtures for the extraction of lipophilic arsenicals from seaweed samples under mild conditions to prevent species degradation during the extraction procedure. [Ch][Lys] dissolved in different solvents or the mixture of two solvents like n-octanol, n-hexane, chloroform, dichloromethane, methanol could prove to be very promising.

3.4.1.3 Supercritical CO_2 with and without co-solvents

Another approach to extract nonpolar arsenic species without altering the speciation was the use of an SFE method. Results showed that the arsenic concentration was below 0.2 mg/kg dm in the case of using pure supercritical CO_2 as an extractant. This indicates that the metalloid species are poorly extractable with CO_2 under these conditions (400 bar, 50°C). Furthermore, the digested residue confirmed the hypothesis that the arsenic did remain in the algae and was not lost during extraction (either deposited on the SCF autoclave tubing or valves). Additionally, the re-extracted residue did not yield any improvement in terms of arsenic extractability since the chloroform/MeOH secondary extract consisted only of 4.1 % of the total digested arsenic.

Surprisingly not even the use of EtOH as a co-solvent proved to improve arsenic release into the alcoholic phase, the extract consisted only of 4.2 % of the total digested arsenic.

Therefore, the SCF extraction method has failed to improve extraction yield of As, but nevertheless it is a promising field of flow chemistry with a broad range of expansion possibilities. However due to the complexity of such setup and the long extraction times (up to one day) no further research has been conducted. Nonetheless, there is always room for improvement and next steps in this scenario would consist of increasing the pressure in the autoclave (up to 1000 bar), consequently increasing density that should also increase solubility [82]. Another approach would be to change the gas used for the SCF extraction (for instance propane) or try out other non-toxic co-solvents during extraction, since leakage in the setup could cause serious hazard for human health.

3.4.1.4 Ionic liquids

Continuing with harsher conditions, increasing temperature and exposure time might partially destroy not only the matrix of the algae but also the analyte, thus changing the

speciation. Nevertheless, preliminary tests showed that extracting arsenic with ionic liquids at higher temperatures gives excellent yields. For instance, using pure [EMIm][OAc] and partially dissolving the algae in it at approximately 80°C yielded an extraction efficiency of 62 %. Extracting with 50 wt% IL in ultrapure water (pH = 8.7) at the same temperature yielded only 15 %. However, using 50 wt% [EMIm][DEP] increased the yield to 36 % and even more using [Ch][Lys] increased it to 81 %. Since [EMIm][DEP] contains a large background of arsenic (4.5 mg As/kg pure IL – identified as As(V)) and using pure [EMIm][OAc] would not be economic, as well as the viscosity of the IL would aggravate effective mixing, the matter of choice falls on [Ch][Lys].

Additionally, two anion-exchange and one cation-exchange chromatogram are presented in Figure 24, Figure 25 and Figure 26. As one can see on Figure 24 DMA has been identified in the [EMIm][OAc] extract of *Undaria pinnatifida*. Additionally, arsenate was identified in the [Ch][Lys] extract (Figure 25) and furthermore 2 more unknown compounds (retention times at: 1.99 min and 4.64 min) were identified in the same extract (Figure 25).

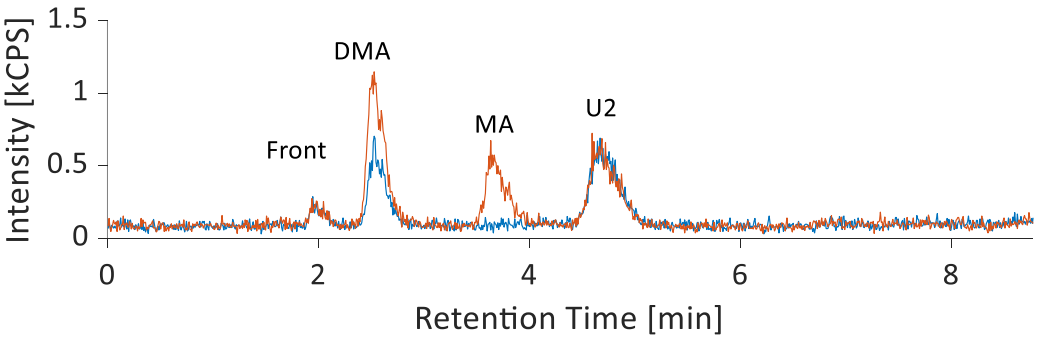


Figure 24: Anion-exchange chromatogram of pure [EMIm][OAc] Wakame extract extracted at 80°C for 30 min (blue) and the same [EMIm][OAc] Wakame extract spiked (brown) with 5 µg/L DMA and MA standard solution; Conditions: PRP-X100 column (150 x 4.6 mm; 5 µm, stainless steel) at 30 °C, 20 mM $NH_4H_2PO_4$ buffer at pH = 6.0, flow rate = 1.0 mL/min and 10 µL injection volume

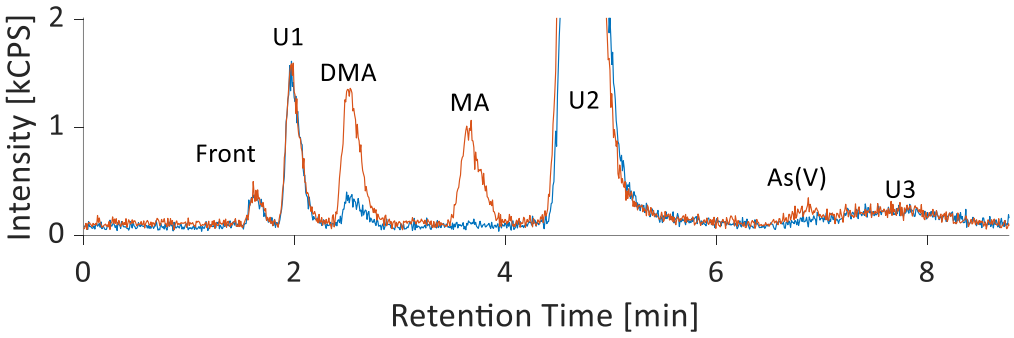


Figure 25: Anion exchange chromatogram of aqueous 50 wt% [Ch][Lys] Wakame extract extracted at 90°C for 1 h (blue) and the same [Ch][Lys] Wakame extract spiked (brown) with 5 µg/L DMA and MA and 0.5 µg/L As(V) standard solution; Conditions: PRP-X100 column (150 x 4.6 mm; 5 µm, stainless steel) at 30 °C, 20 mM $NH_4H_2PO_4$ buffer at pH = 6.0, flow rate = 1.0 mL/min and 10 µL injection volume

Moreover, the cation-exchange chromatogram (Figure 26) shows that none of the standards used is present in the Wakame extracts. Furthermore, there are two unidentified arsenic species present in both [Ch][Lys] extract and ultrapure water extract. Comparing the chromatograms of both extracts one can observe that the second unknown compound experiences a retention time shift (t_R =2.2 min for the aqueous extract to 2.0 min for the [Ch][Lys] extract - Figure 27). This could be the consequence of the coelution of the Cholinium cation (m/z = 52) and U2 (m/z = 75) – competing for the cation-exchange groups, meaning that the U2 is pushed from the exchange-sites sooner than in the case of the aqueous extract (Figure 27) – note the two brown peaks of m/z = 52 at 1.0 min and 2.0 min, which do not appear in the case of the aqueous extract (green chromatogram of m/z = 52). This behaviour indicates that cation-exchange chromatography is not suitable for arsenosugar speciation of ionic liquid extracts.

Further research focused only on the anion-exchange chromatography of IL extracts, since in this case carbon is not interfering due to elution in the front.

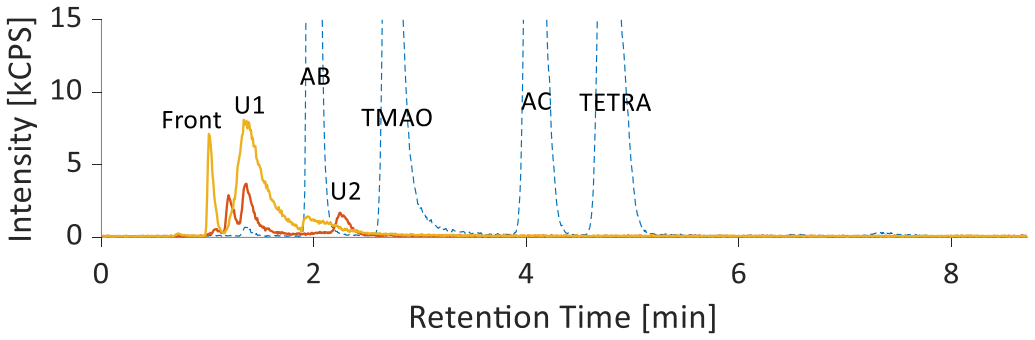


Figure 26: Cation exchange chromatogram of a 50 µg/L standard solution of AB, TMAO, AC and TETRA, as well as aqueous Wakame extract (red) extracted at 60 °C for 2 h and [Ch][Lys] Wakame extract extracted at 90°C for 1 h (yellow); Conditions: Zorbax 300-SCX column (150 x 4.6 mm; 5 µm, stainless steel) at 30 °C, 10 mM pyridinium buffer at pH = 2.3, flow rate = 1.5 mL/min a 10 µL injection volume

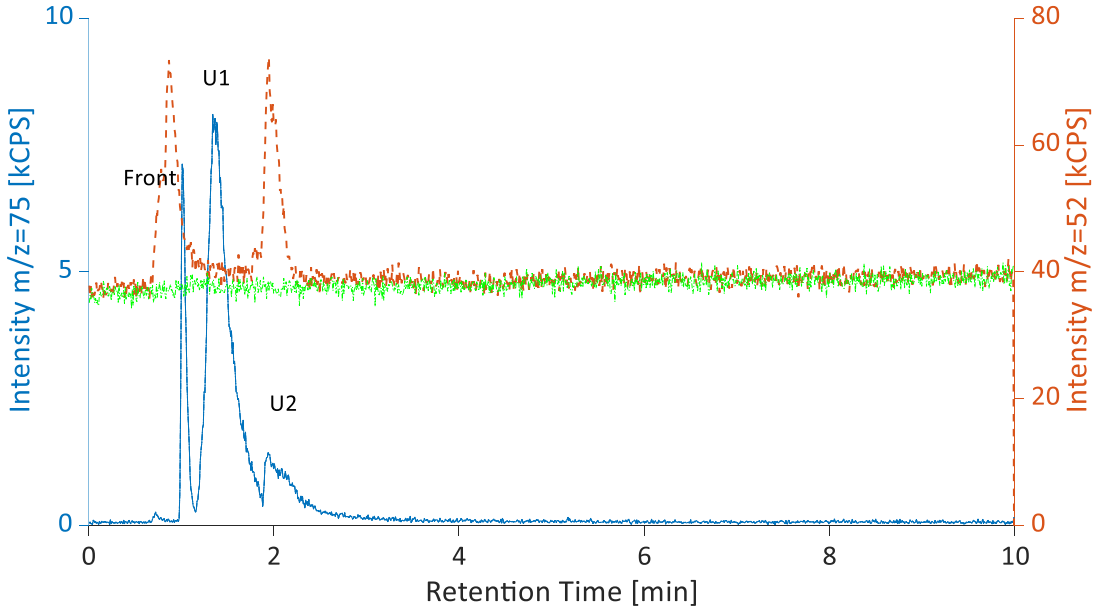


Figure 27: Cation exchange chromatogram of [Ch][Lys] Wakame extract extracted at 90°C for 1 h measured at mass-to-charge ratio 75 (blue) and 52 (brown); (Note: the green chromatogram is a direct comparison of the m/z = 52 background of an aqueous Wakame extract extracted at 60°C for 2 h); Conditions: Zorbax 300-SCX column (150 x 4.6 mm; 5 µm, stainless steel) at 30 °C, 10 mM pyridinium buffer at pH = 2.3, flow rate = 1.5 mL/min a 10 µL injection volume

3.4.1.5 Cholinium Lysinate as extractant

The following experiments were carried out with 10 wt% [Ch][Lys] with a pH of 12.1 ± 0.1 (*n* = 3). As a control group 0.01 M NaOH solution was prepared as the extracting solvent to check whether the high extraction yields are only pH dependent.

Results show that although NaOH increases the extraction yield 3 times in comparison to ultrapure water, it is still significantly lower than obtained for [Ch][Lys]. However, adjusting the pH of 10 wt% [Ch][Lys] with acetic acid to pH 7.0 and 4.0 did drastically decrease the extraction yield of arsenic. This indicates that the extraction is indeed pH dependent but depends also on the anions and cations present in the solution (Figure 1Figure 28).

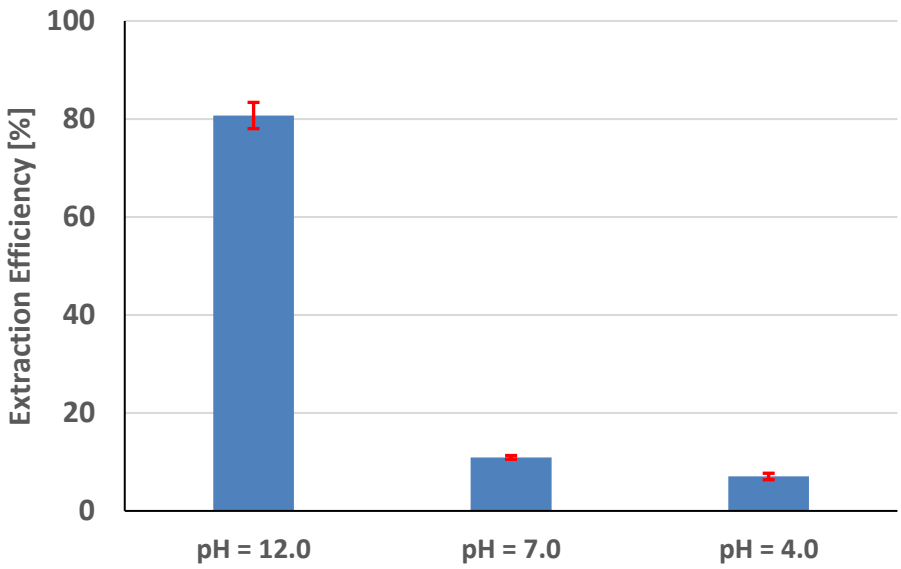


Figure 28: Extraction efficiency of arsenic from Wakame samples extracted with aqueous 10 wt% [Ch][Lys] with different pH of the solutions (adjusted with acetic acid); Note: all extractions were carried out in a shaking water bath at 80°C for 2 h

The following Figure 29 shows the impact of temperature on the extraction efficiency. It is clear that with increasing temperature the likelihood of arsenic species release into the solution increases.

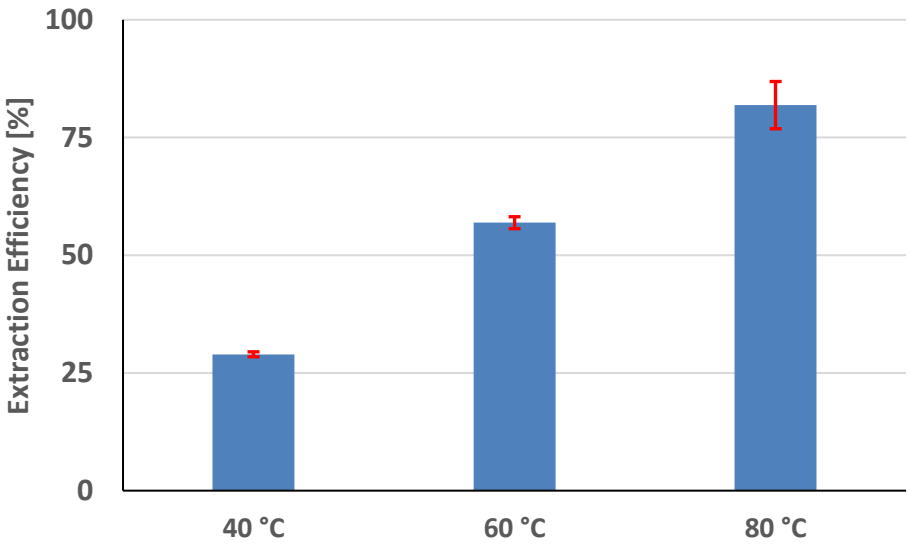


Figure 29: Temperature dependency of the As extraction yield from *Undaria pinnatifida* extracted with aqueous 10 wt% [Ch][Lys] for 2 h in a shaking water bath at different temperatures

Comparing results on pre-wetting the algae in 10 wt% [Ch][Lys] for 1 hour it is evident that the extraction happens already at room temperature without stirring the system, since the nonwetted algae yielded an extraction efficiency of arsenic of only 23 % at 40°C in comparison with the soaked algae that yielded an extraction efficiency of 29 ± 1 % at the same temperature. Furthermore, increasing the solvent-to-biomass ratio it is baffling that the extraction efficiency decreases by almost 9 % (Figure 30).

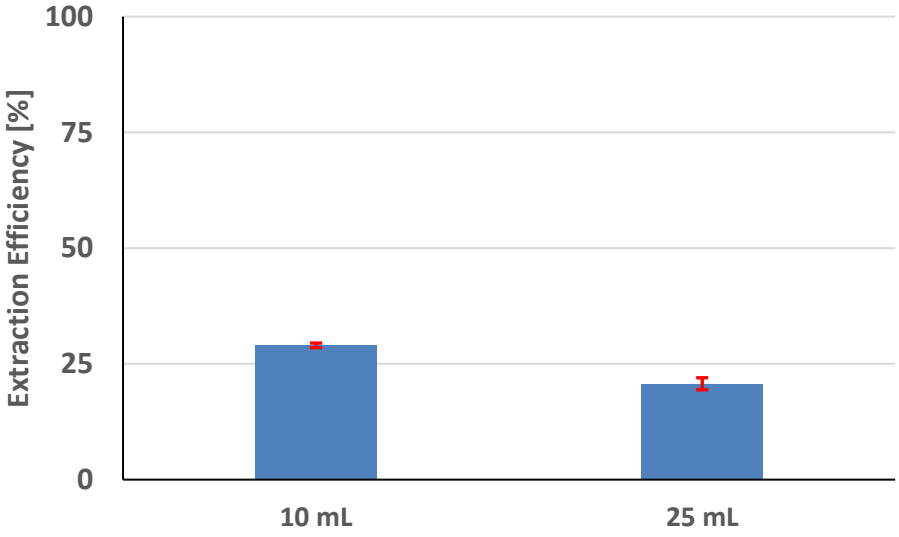


Figure 30: Effect of increased solvent-to-biomass ratio on As extraction yield from Wakame extracted with 10 wt% [Ch][Lys] in a shaking water bath extracted at 40°C for 2 h

What is the most interesting outcome of the research is the fact that using mild conditions (40°C) and elongate the time of extraction (from 2 h to 24 h) tremendously increases the extraction yield of arsenic from Wakame seaweed (Figure 31). That is a good anchor point for further research since the extractions could be performed maybe even at room temperature and possibly also with less [Ch][Lys] (1 wt% in ultrapure water or even less concentrated), thus giving an economic variant on the harsh conditions used.

Lastly, comparing the different batches of the same extraction procedure (at 80°C) one can see that the experiments are repeatable with little to no deviations visible (Figure 32). Thus, the method of choice for the extraction of arsenic from "difficult samples", which yield little arsenic using ultrapure water or other conventional solvents, would be the use of aqueous [Ch][Lys] solution. If it really is applicable in the case of other living organisms, such as fungi or crustaceans is yet to be investigated.

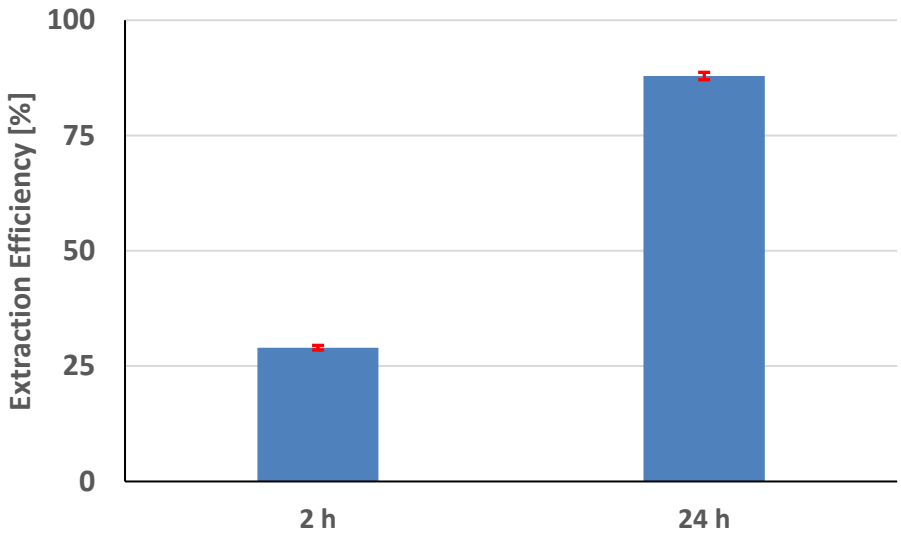


Figure 31: Effect of increased extraction time on As extraction yield from *Undaria pinnatifida* extracted with aqueous 10 wt% [Ch][Lys] in a shaking water bath at 40°C

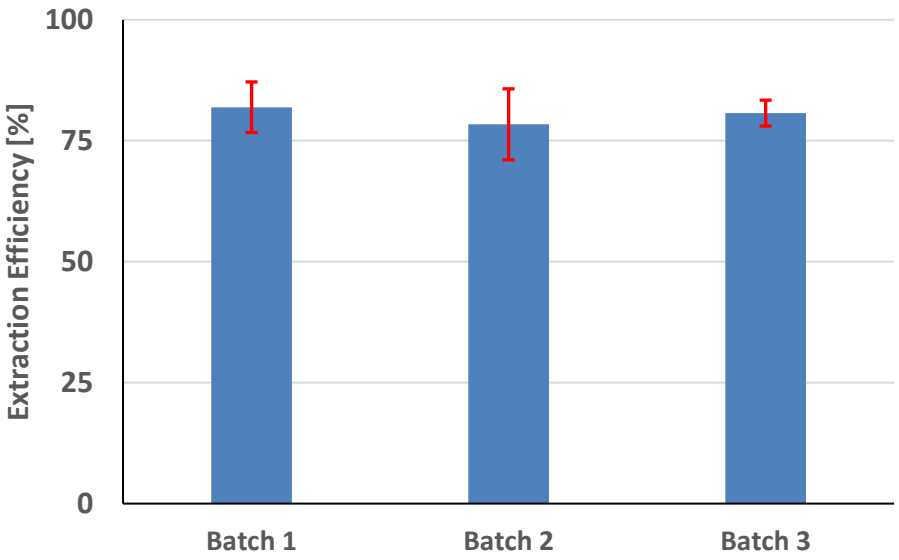


Figure 32: Comparison of the same extraction procedure (shaking water bath, 2 h and 80°C) carried out on three different batches of the same Wakame samples from the same manufacturer (WELPAC)

3.4.2 Comparison of three different Wakame samples

Results on Wakame samples bought in Budapest showed that they do yield similar extraction efficiencies for arsenic compared to the Wakame samples.

The extraction with pure [EMIm][OAc] at 40°C is summarised in Figure 33. As one can see the two batches from Hungary give the same extraction yields, however slightly less than the Wakame from Vienna. Similarly, extraction with ultrapure water results in a 5 % extraction yield of arsenic for both samples from Budapest. This is similar for the results of Wakame Vienna described in section 3.4.1.1 (5.1 ± 0.2 %).

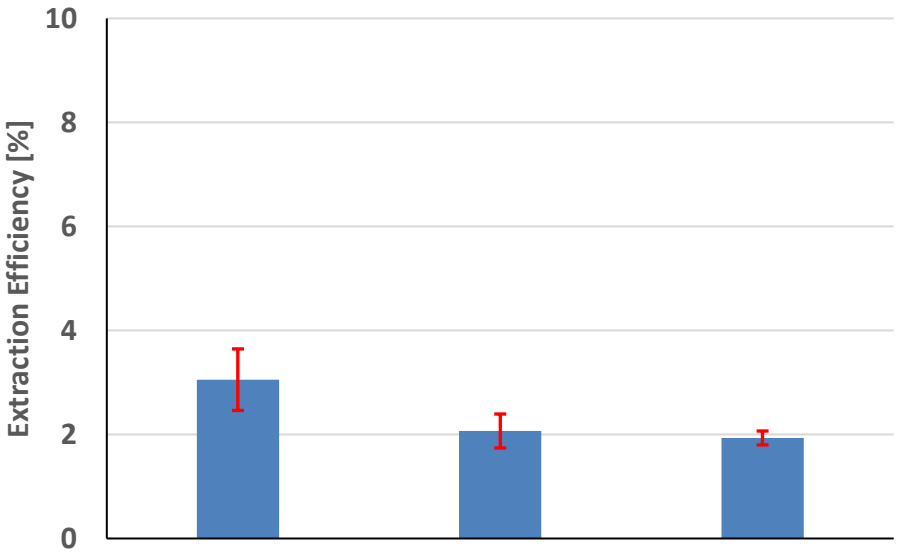


Figure 33: Comparison of three different batches of Wakame algae bought from the same manufacturer; Conditions: pure [EMIm][OAc], 40°C, 2 h, heating block

Finally results with 10 wt% [Ch][Lys] are summarised in Figure 34. Again, the tendency of increased extraction yield with higher temperatures is true for all three algae samples. Also, Wakame Vienna gives somewhat higher extraction yields at all three temperatures than the two samples from Budapest.

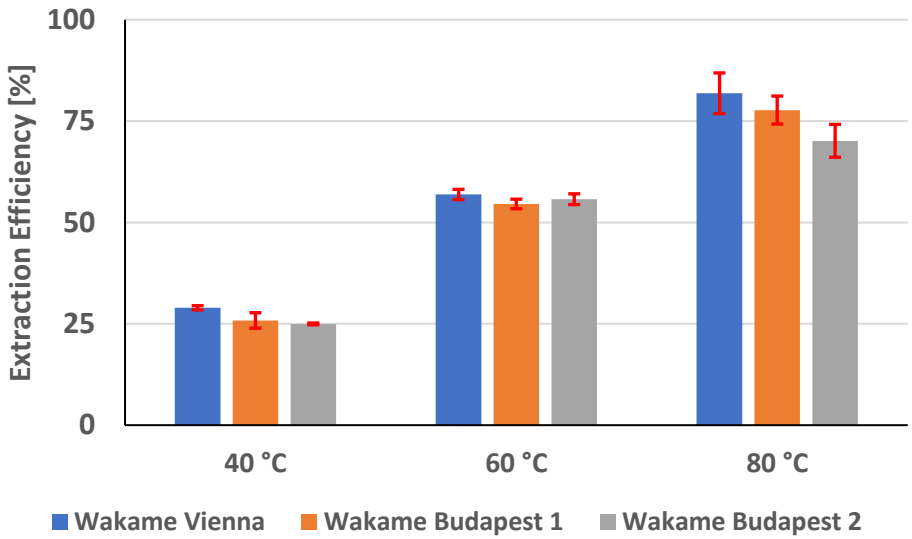


Figure 34: Comparison of three different batches of Wakame algae bought from the same manufacturer (WELPAC); Conditions: 10 wt% [Ch][Lys], 2 h, shaking water bath, different temperatures)

3.4.3 Anion-exchange chromatograms of various extracts, confirmation, and quantification of arsenosugars

As one can see from Figure 35 and the closeup on Figure 36, as well as on Figure 37 all previously unidentified peaks have been proved for their identity by spiking experiments. The OH-Sugar eluting before DMA, PO_4-Sugar eluting after MA and lastly the SO_3-Sugar coeluting with As(V). The latter is however difficult to quantify and even qualify due to its low abundance. Furthermore, a retention time shift to lower retention times occurs due to the presence of [Ch][Lys] in the matrix. (Detailed view on these chromatograms is found in the appendix).

To achieve baseline separation, one should change the HPLC parameters, for instance reduce the pH of the phosphate buffer used, this would however compromise the elution of OH-Sugar and DMA, which could coelute. Therefore, an optimization of the existing method with respect to the pH of the mobile phase is desired. Another alternative would be to apply a pH-gradient elution at a constant flowrate. For instance starting with pH = 6.0 until t = 4.5 min would baseline separate OH-Sugar, DMA, MA and PO_4-Sugar, and after 4.5 min the pH would drop to 5.0, achieving a baseline separation of As(V) and SO_3-Sugar [57].

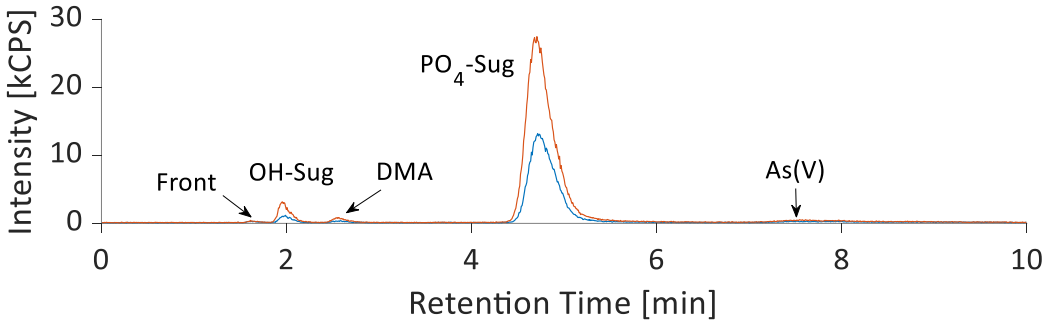


Figure 35: Anion-exchange chromatograms of Wakame extracted with 10 wt% [Ch][Lys] at 60°C for 2 h(blue) and the same extract spiked with approximately 145 µg/L PO_4-Sugar standard (red); Conditions: PRP-X100 column (150 x 4.6 mm; 5 µm, stainless steel) at 30 °C, 20 mM $NH_4H_2PO_4$ buffer at pH = 6.0, flow rate = 1.0 mL/min and 10 µL injection volume

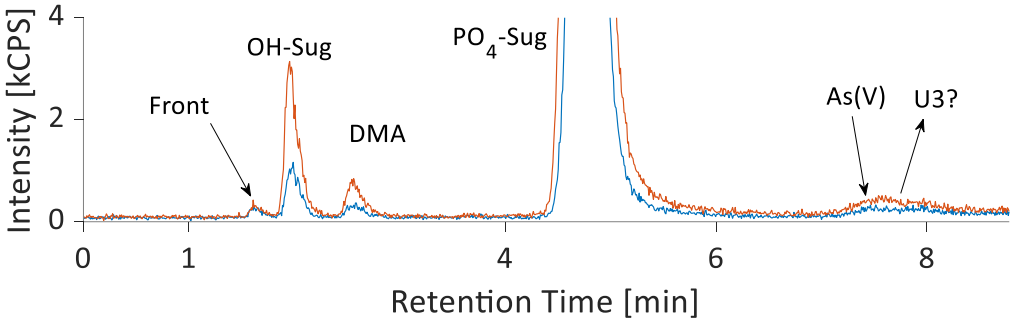


Figure 36: Closeup of figure 35

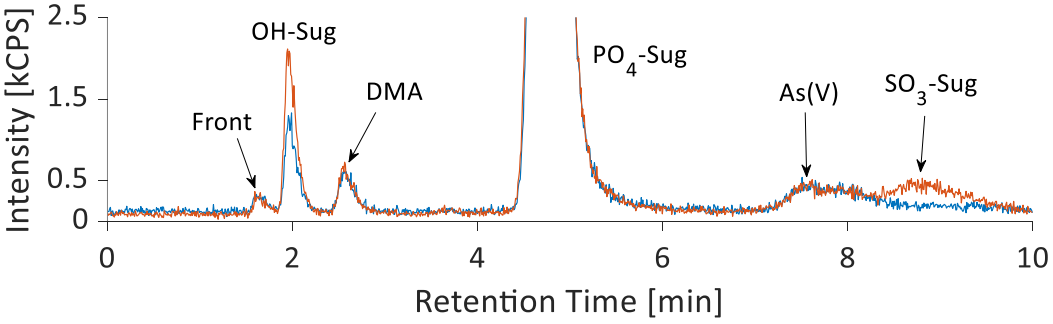


Figure 37: Anion-exchange chromatograms of Wakame extracted with 10 wt% [Ch][Lys] at 80°C for 2 h (blue) and the same extract spiked with approximately 3.4 µg/L OH-Sugar standard and 6.8 µg/L SO_3-Sugar standard (red); Conditions: PRP-X100 column (150 x 4.6 mm; 5 µm, stainless steel) held at 30 °C, 20 mM $NH_4H_2PO_4$ buffer at pH = 6.0, flow rate = 1.0 mL/min and 10 µL injection volume

After cleaning the PRP-X100 column with MeOH in both directions, as well as with 1 % 6 N HNO_3, and prior analysis with $NH_4H_2PO_4$ buffer at pH = 6.0 in both directions, the

retention behaviour of arsenate improved a lot (shorter retention and sharper peak). Surprisingly, the SO_3-Sugar peak eluting after As(V) split into two peaks. Whether there is a second unknown compound is not clear yet. For clarification, a spike experiment with SO_3-Sugar would be necessary. The following Figure 38 highlights the two unknown peaks and their baseline separation from As(V)

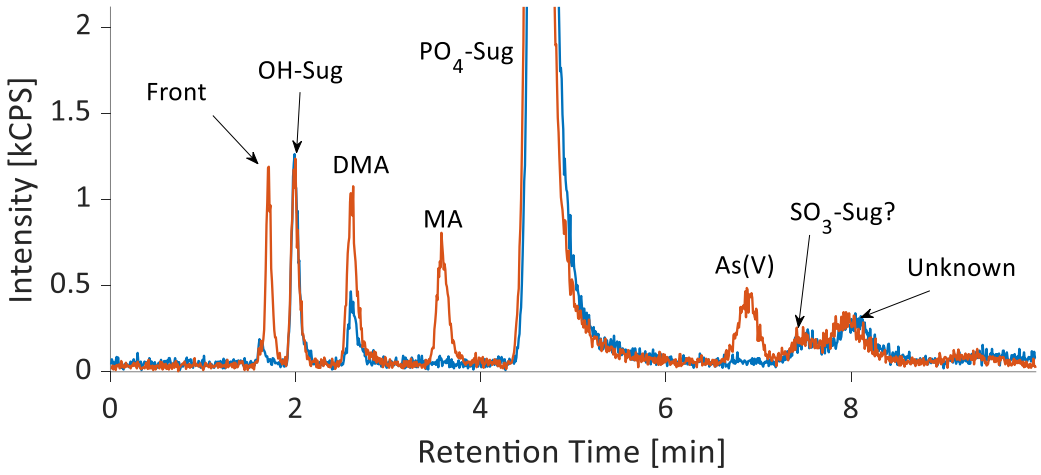


Figure 38: Anion-exchange chromatograms of Wakame extracted with 10 wt% [Ch][Lys] at 80°C for 2 h(blue) and the same extract spiked with 10 µg/L AB, DMA, MA and As(V); Conditions: PRP-X100 column (150 x 4.6 mm; 5 µm, stainless steel) at 30 °C, 20 mM $NH_4H_2PO_4$ buffer at pH = 6.0, flow rate = 1.0 mL/min and 10 µL injection volume

The proof of PO_4-Sugar being the main arsenic species present in the Wakame algae rises some questions. Why is it so poorly extractable with ultrapure water or methanol? Is the PO_4-Sugar irreversibly bond to the matrix of the algae? Does the high pH value of the [Ch][Lys] solution cause a hydrolytic cleavage of the PO_4-Sugar from the cellular structure? Or is the PO_4-Sugar cleaved of from an arsenophospholipids? In which form does it exist (different constitutional isomers of arsenophospholipids – refer to Figure 39)? R_1 and R_2 could be differently branched aliphatic hydrocarbons with different chain lengths, and they may even contain double bonds on different positions and different geometrical orientation (*cis* or *trans*).

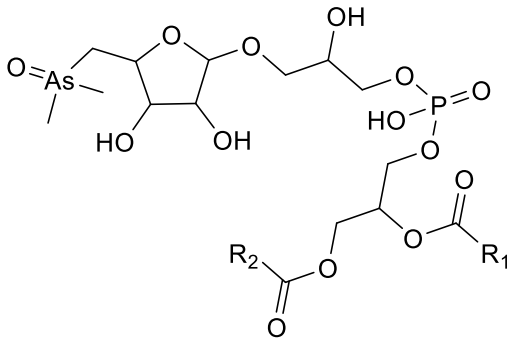


Figure 39: Chemical structure of an arsenophospholipid (R_1 an R_2 are different aliphatic substituents, usually with up to 20 carbon chain length)

Is this the reason why the majority of arsenic species from the Wakame samples are not extractable with ultrapure water? It could easily be the case, that the lipid moiety is cleaved of by the ionic liquid thus releasing the PO_4-Sugar into aqueous solution. This research question is however difficult to answer, and further experiments would be necessary, first to characterise the arsenolipids extracted with chloroform/methanol mixtures and confirm whether they are phospholipids. If yes, why is the extraction efficiency of As with $CHCl_3$/MeOH also so low? Even Morita et. al extracted only 25 % of the total arsenic from fresh Wakame as lipophilic As fraction [81]. What is the form of the remaining arsenic? Is it even feasible to extract all of arsenic or is there a dead end to the story?

3.4.3.1 Quantitative results on arsenic species in Wakame extracts

Continuing with the quantitative results of arsenic species found in the extracts it is straightforward that similarly to the rise of extraction efficiencies with rising temperature the OH-Sugar, DMA, as well as the PO_4-Sugar concentrations increase (refer to Table 22 in the appendix).

Furthermore, H_2O does not yield high arsenosugar concentrations, however it is the only solvent that yielded a small amount of MA in the extract. As for the unknown compounds U3 (t_R = ~7.5 min) and U4 (t_R = ~8.0 min) they do only occur in the extracts that were stored for a longer period - Figure 38. Similarly, the unknown peaks occur in the sodium hydroxide extracts as well as in the 10 wt% [Ch][Lys], stored for a longer period, but not in the extracts measured the same week they were extracted. This might be a consequence of the PO_4-Sugar degradation under this storage conditions (4°C), which is prompt to hydrolytic cleavage of the phosphate moiety.

To showcase the instability of the latter in the [Ch][Lys] solution, the following Figure 40 shows that the PO_4-Sugar decomposes with time to other arsenic species. Since the concentration of the OH-Sugar concentration, as well as the occurrence of U3 (t_R = ~7.5 min) and U4 (t_R = ~8.0 min) is peculiar, it can be concluded that it degrades to those species (DMA concentration stays constant). However, to prove this, a longer period time study should be conducted with measuring extracts on their arsenic species content in one-week frequency. Furthermore, the extracts should be stored at different conditions (-18°C, 4°C and room temperature).

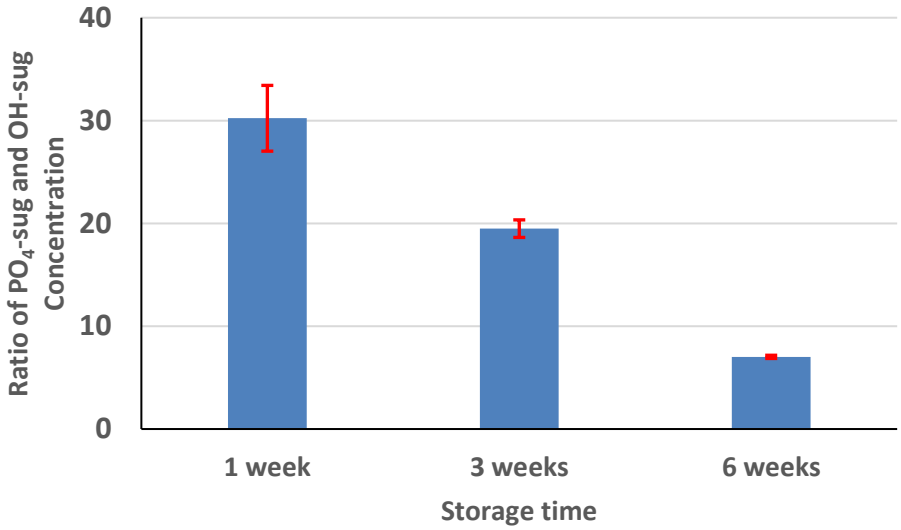


Figure 40: PO_4-Sugar stability as a function of storage time of Wakame extracts extracted with 10 wt% [Ch][Lys] at 80°C for 2 h in a shaking water bath; Storage conditions: 4°C

In the case of 10 wt% [Ch][Lys] extracts the column recoveries are decent (above 95 %), however in the case of water extracts as well as sodium hydroxide extracts the column recoveries are not quantitative (below 75 % and 86 % respectively). The retained species might have longer elution times than the acquisition time of the method, however blank samples were injected afterwards, and no peaks were observed. Therefore, the unretained arsenic species are of unknown nature. In the case of [EMIm][OAc] extracts (column recovery below 40 %), the unretained arsenicals might be even arsenolipids. Whether this is true needs to be investigated with other analytical methods.

For a thorough result set on the arsenic species concentration together with the column recoveries please refer to Table 22 in the appendix.

Additionally, looking into Wakame samples from literature, Wakame extracts were mostly analysed on anion-exchange columns, rarely also on reversed-phase C18 columns. The detection method was mostly ICPMS and in some cases electrospray ionization tandem mass spectrometry (ESI MS/MS). For instance, Garcia-Salgado et al. extracted arsenic species from Wakame samples with ultrapure water with a microwave-assisted extraction method (three-times 90 °C for 5 minutes each) and analysed the extracts with HPLC coupled to hydride generation atomic fluorescence spectroscopy (HG-AFS) [21]. Although extraction efficiencies were high (49 ± 8 % for Wakame samples from Japan and 74 ± 9 % for Spanish samples), speciation results were not ideal. No baseline separation between DMA and PO_4-Sugar was achieved and furthermore the front peak was assigned as the OH-Sugar which should not be assigned as an arsenic species, but as unretained arsenic species. Looking further into quantitation data, one can immediately see that arsenate concentrations may be overestimated (4.5 ± 0.3 mg As/kg dm for Wakame samples harvested in Japan). Additionally, PO_4-Sugar/OH-Sugar ratios are way lower than investigated in our study, indicating that the extraction procedure used was too harsh and might have even more promoted PO_4-Sugar hydrolysis than the extraction with [Ch][Lys] [21].

Furthermore, a simple but long (16 h) mild aqueous extraction method was applied to Wakame samples by Llorente-Mirandes et. al. Extraction efficiency for As did not exceed 30 %, but even so, that is more than 6-times higher than in our work, might indicate, that even water is strong enough to impose leaching of AsPLs from the matrix. Furthermore, speciation analysis showed again higher OH-Sugar concentration than of PO_4-Sugar. Additionally, column recovery was only 13 %, which indicates that most of the arsenic species is unretained. For what reason it is unclear. Whether the AsPLs are released into solution and only part of them decomposes to the PO_4-Sugar is a possibility, but lipids are generally immiscible with water [83].

Again, we see the huge necessity to isolate and characterise AsPL standards, to perform spike recovery experiments during extraction, which would give clear indication on the stability and possible decomposition to the PO_4-Sugar and further on to the OH-Sugar.

Interestingly other authors which report high extraction yield for As from Wakames samples run into the same problem of high OH-Sugar concentrations in comparison to the PO_4-Sugar [84, 85].

Thus, one may propose that the harsh extraction procedures used may destroy the AsPLs and therefore falsely PO4-Sugar is detected and quantified, and even more severe, the PO_4-Sugar might hydrolyse to its OH-Sugar analogue during extraction, again changing the speciation.

3.5 Results on the spike experiments

Spike recoveries have been calculated as follows:

$$Spike\ Recovery\ [\%] = \frac{c_{spiked\ sample} - c_{sample}}{c_{spike\ added}} \times 100 \qquad 6$$

Looking into the results on spike experiments conducted prior extraction to check whether the spiked arsenic species does get lost during the process, Figure 41 shows that in case of an arsenate spike biosorption occurs, but only in the case of sodium hydroxide and 10 wt% [Ch][Lys]. Spiking the aqueous extraction of Wakame seaweed does yield a spike recovery of 105 ± 14 %, which indicates that As(V) stays in solution. Since NaOH does yield a spike recovery of 76 ± 3 %, and its pH is 12.0, likewise the pH of the aqueous IL extractant, the arsenic biosorption is not only pH dependent. At this pH the As(V) is present as the AsO_4^{3-} anion, but the counter ions in the case of [Ch][Lys] are not solely Na^+ cations and water molecules but also the Cholinium cation. Whether the $[Ch]^+$ forms an ion pair with AsO_4^{3-} thus allowing it to interact with the algal surface and entrap it is unclear. In the case of NaOH it would be only an interaction with the positively charged surface (lots of Mg, Na and Ca cations), but in the case of the IL apart from ionic interactions, other type of interactions could also occur (for instance van der Waals interactions). Nevertheless, digestion of the algal residue and the spiked algal residue showed that almost all the arsenate is entrapped by the algae (recovery of digest: 98 ± 10 %). Furthermore, the spiked blanks (10 wt% [Ch][Lys] solution at pH = 12.0 spiked with 5 µg arsenate) did not affect the arsenate (spike recoveries above 98 %).

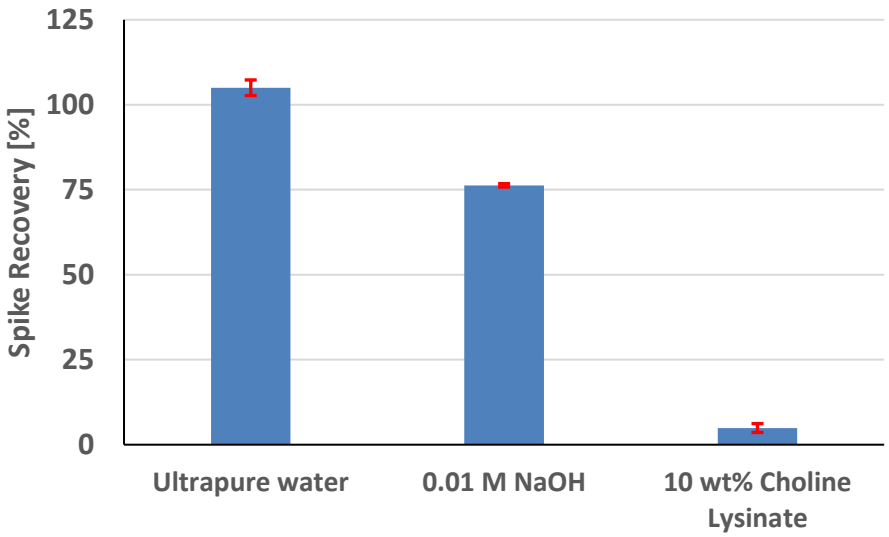


Figure 41: Spike recovery of As(V) during arsenic extraction from Wakame utilizing different extracting solvents used; Conditions: shaking water bath, 2 h, 80°C

In case of the simultaneous spiking experiments of the 10 wt% [Ch][Lys] and Wakame mixtures with DMA, MA and As(V) the pH adjusted extractants showed decent spike recoveries (above 92 %), whereas the spike recovery at pH = 12.0 was only 53 ± 5 % (for the total arsenic content in the extracts determined by ICPMS). This is a little less than was expected (66 %), thus one may conclude that not only arsenate is adsorbed but also part of MA and maybe even DMA. To verify this assumption speciation analysis was also conducted.

Speciation study of the spiked extracts showed that at pH = 12.0 most of the arsenate was adsorbed, as well as more than 12 % of MA. These results are in accordance with the fact that the more negative charges the arsenic species bears the easier it gets adsorbed to the algal surface in presence of [Ch][Lys]. Therefore, MA present as CH_3-AsO_3^{2-} was adsorbed to some extent, whereas DMA present as $(CH_3)_2$-AsO_2^- was not (Figure 42).

Finally, the spike recoveries calculated once as the sum of DMA, MA and As(V) both for the samples and the spiked samples and once from the total arsenic concentration in the extracts and spiked extracts are in good agreement (Figure 43).

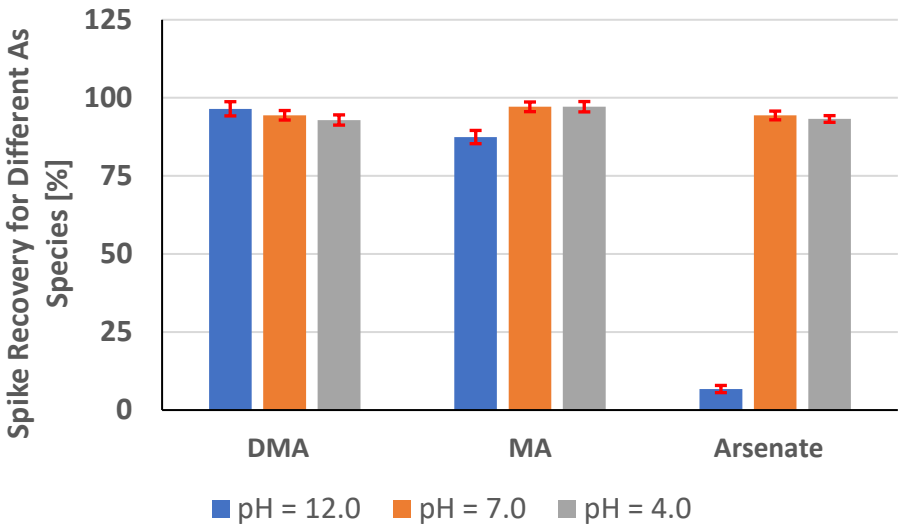


Figure 42: Spike recoveries for DMA, MA and As(V) during arsenic extraction from Wakame depending on the extracting solvent used (pH adjusted 10 %wt [Ch][Lys]), Conditions: shaking water bath, 2 h, 80°C

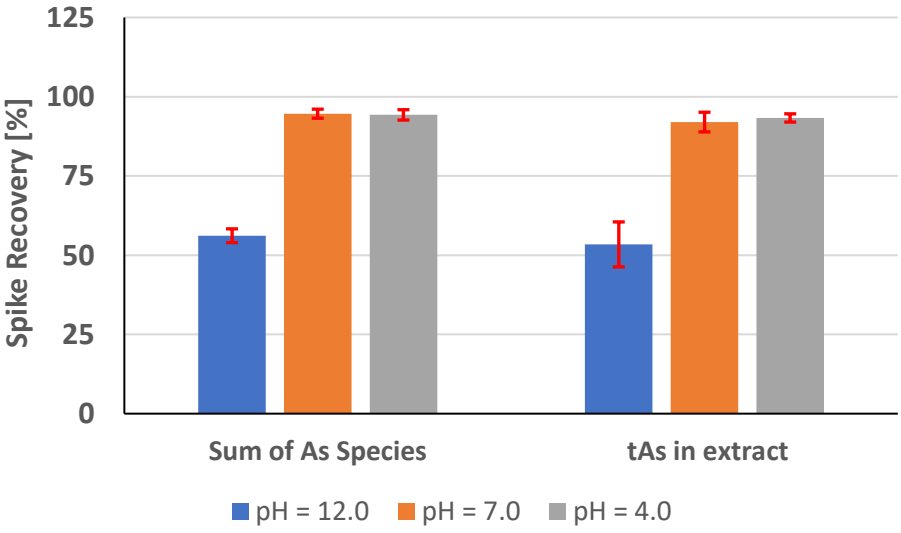


Figure 43: Spike recoveries for the sum of arsenic species (speciation study) and total arsenic determination (elemental study) during arsenic extraction from Wakame extracts, depending on the extracting solvent used (pH adjusted 10 %wt [Ch][Lys]), Conditions: shaking water bath, 2 h, 80°C

4 Concluding remarks and outlook

A clear improvement in terms of improving the extraction yield of arsenic from the "difficult" Wakame sample was achieved, even at low temperatures and with little [Ch][Lys] added as modifier to the water. This makes the process economic and potentially suitable for all kinds of environmental samples. Furthermore, the extraction method is robust and repeatable. Therefore, it would be interesting to perform extractions on other samples which yield poor extraction yield for arsenic as well, for instance the black moss species [33] or the inedible fungi *Thelephora terrestris* [86], because also these two biological samples are poorly extractable for arsenic.

The quantitative extraction however was compromised by the fact that the major extracted arsenic species is prompt to degradation in the aqueous [Ch][Lys] during longer storage times. Furthermore, speciation may change already during extraction, namely the PO_4-Sugar could be formed by hydrolytic cleavage of the fatty acid groups from an arsenosugar containing phospholipid (AsPL) [81], [87].

To evaluate whether the process conditions change the speciation during arsenic extraction from Wakame samples, spike recovery tests with AsPL standards and with arsenosugars standards are necessary. While even the latter is difficult to obtain (synthesized or isolated standards), AsPL standards are not commercially available. Also, the extraction blanks consisting of [Ch][Lys] and ultrapure water should be spiked to evaluate whether the [Ch][Lys] is responsible for the degradation of the AsPL and more importantly is it even miscible with aqueous [Ch][Lys]? Does the IL bring the lipid molecules into the polar phase by cleaving of the fatty acids and release the arsenosugar?

Since the arsenosugar species were identified by spiking experiments with suitable arsenosugar standards, one cannot investigate the accuracy of the performed extraction method. Consequently, for quality control the usage of a CRM which is certified for arsenosugars would be preferable. These are however rare. One example would be the NMIJ CRM 7405-b (Trace Elements and Arsenic Compounds in Seaweed (Hijiki)) with certified concentrations for the OH-Sugar (0.44 ± 0.02 mg/kg dm) and the SO_4-Sugar (1.41 ± 0.04 mg/kg dm) [15].

Furthermore, a kinetic study of a PO_4-Sugar standard decomposition in aqueous [Ch][Lys] would be interesting. To which compound(s) does it degrade? Could we avoid degradation by freezing the mixtures at -80°C? What about the algal extracts, could we avoid decomposition if the solvents were removed, and the material would be stored as the dried extract in pure [Ch][Lys]?

As for the nonpolar extractions, could we exploit the ionic liquids in similar manner than in the case of aqueous [Ch][Lys]? Preliminary tests with [EMIm][OAc] showed little improvement in the extraction efficiency of nonpolar arsenic (~6 %) that is similar to the yields found in the literature [87]. But how can we maximise the yield of arsenolipids without hydrolysis by the ILs? A good access point would be to study different [Ch][Lys] organic solvent mixtures at different temperatures and extraction times. If the results would be satisfying, [Ch][Lys] organic solvent ratio could be reduced to use as little IL as possible. Furthermore, more cycles of extraction and combining the supernatants might produce the desired yields.

Could we possibly get 100 % extraction efficiency combining the nonpolar extraction with a subsequent polar extraction of the algal residue? Since many analytical research questions focus separately on the polar fraction and separately on the nonpolar fraction, many researchers might miss the link between both. This might lead to false reports on arsenosugar concentrations in marine samples, because the author did not consider a look on the broader picture.

Lastly, an arsenic speciation study of urine samples excreted from volunteers on a Wakame diet would help to understand whether the arsenicals in Wakame are bioavailable and pose a risk to humans.

5 Literature

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6 Appendix

6.1 Standard Reference Material 1640a, Trace Elements in Natural Water

Table 11: Certified values in the concentration range of µg/L

Element	Mass Fraction (µg/kg)			*k*	*df*
Aluminum	52.6	±	1.8	2.069	23
Antimony	5.064	±	0.045	2.365	7
Arsenic	8.010	±	0.067	1.980	120
Barium	150.60	±	0.74	1.984	98
Beryllium	3.002	±	0.027	2.060	25
Boron	300.7	±	3.1	2.365	7
Cadmium	3.961	±	0.072	2.365	7
Chromium	40.22	±	0.28	2.021	40
Cobalt	20.08	±	0.24	2.447	6
Copper	85.07	±	0.48	2.228	10
Iron	36.5	±	1.7	2.447	6
Lead	12.005	±	0.040	1.970	227
Manganese	40.07	±	0.35	2.201	11
Molybdenum	45.24	±	0.59	2.017	43
Nickel	25.12	±	0.12	2.026	37
Selenium	19.97	±	0.16	2.228	10
Silver	8.017	±	0.042	2.086	20
Strontium	125.03	±	0.86	2.179	12
Thallium	1.606	±	0.015	2.365	7
Uranium	25.15	±	0.26	2.145	14
Vanadium	14.93	±	0.21	2.447	6
Zinc	55.20	±	0.32	2.010	49

Table 12: Certified values in the concentration range of mg/L except for Li and Rb which are in µg/L

Element	Mass Concentration [b] (mg/L)			*k*	*df*
Calcium	5.615	±	0.021	2.005	54
Magnesium	1.0586	±	0.0041	2.045	29
Potassium	0.5799	±	0.0023	2.040	31
Silicon	5.210	±	0.021	2.005	54
Sodium	3.137	±	0.031	2.571	5
	(µg/L)				
Lithium	0.4066	±	0.0094	2.776	4
Rubidium	1.198	±	0.011	1.961	3657

6.2 Standard Reference Material® 1568b Rice Flour

Table 13: Certified values for elements expressed as mg/kg dry mass

Element	Mass Fraction (mg/kg)		
Aluminum[a,b,c]	4.21	±	0.34
Arsenic[a,d]	0.285	±	0.014
Bromine[b,e]	8.31	±	0.61
Cadmium[f,g]	0.0224	±	0.0013
Calcium[b,h,i]	118.4	±	3.1
Chlorine[b,e]	301.1	±	3.8
Copper[a,e,g,h]	2.35	±	0.16
Iron[b,e,h,j]	7.42	±	0.44
Magnesium[b,e,h,i]	559	±	10
Manganese[b,e]	19.2	±	1.8
Mercury[e,l]	0.00591	±	0.00036
Molybdenum[a,e,k,m]	1.451	±	0.048
Phosphorus[b,i,m,n]	1530	±	40
Potassium[b,e]	1282	±	11
Rubidium[a,b]	6.198	±	0.026
Selenium[a,e,o]	0.365	±	0.029
Sulfur[b,j]	1200	±	10
Zinc[b,e,h]	19.42	±	0.26

Table 14: Certified values for arsenic species expressed as mg As/kg dry mass

Arsenic Species	Mass Fraction (mg/kg, as As)		
Dimethylarsinic acid (DMA)[a,b,c]	0.180	±	0.012
Monomethylarsonic acid (MMA)[a,c]	0.0116	±	0.0035
Inorganic arsenic (iAs)[a,c,d]	0.092	±	0.010

6.3 Standard Reference material IAEA-140/TM, Trace Elements in Seaweed Fucus Spiralis

Table 15: Certified values for elements and methyl mercury expressed as mg/kg dry mass

Analyte	Status	Concentration * dry weight	Unit	Confidence limits** lower	upper
Al	I	1184	mg /kg	919	1449
As	R	44.3	mg /kg	42.2	46.4
Ba	I	20.2	mg /kg	11.4	29.0
Br	R	567	mg /kg	473	661
Ca	R	12.73	g /kg	10.97	14.49
Cd	R	0.537	mg /kg	0.500	0.574
Ce	I	2.05	mg /kg	1.80	2.30
Co	R	0.876	mg /kg	0.746	1.01
Cr	R	10.4	mg /kg	9.6	11.2
Cs	I	0.23	mg /kg	0.205	0.255
Cu	R	5.05	mg /kg	4.77	5.33
Fe	R	1256	mg /kg	1221	1291
Hf	I	0.156	mg /kg	0.125	0.187
Hg	R	38	µg /kg	32	44
Methyl-Hg#	R	0.626	µg /kg	0.519	0.733
K	R	31.1	g /kg	28.5	33.7
Li	R	2.29	mg /kg	1.94	2.64
Mg	R	9.07	g /kg	8.19	9.95
Mn	R	56.1	mg /kg	53.7	58.5
Mo	R	2.65	mg /kg	2.28	3.02
Na	R	32.0	g /kg	25.4	38.6
Ni	R	3.79	mg /kg	3.38	4.20
Pb	R	2.19	mg /kg	1.91	2.47
Rb	R	16.4	mg /kg	14.1	18.7
Sb	R	0.103	mg /kg	0.081	0.125
Sc	I	0.261	mg /kg	0.170	0.352
Se	I	0.079	mg /kg	0.046	0.112

6.4 Certified Reference Material ALVA-PA14/1, Straight Feeding Stuff Oregano, Round Robin

Table 16: Certified values for elements (ALVA-PA14/1 is labelled “Probe 1)

ALVA VIII

Statistische Übersicht der Auswertungen

Nr	Bezeichnung	Einheit	Labors	med	MW	std	VR	Typ1	Typ2
1	Wasser Probe 1	g/kg	19	77,7	78,4	3,67	4,69	0	0
2	Wasser Probe 2	g/kg	20	100,7	96,8	9,37	9,68	0	0
3	Wasser Probe 3	g/kg	19	16,7	16,6	1,88	11,28	0	0
4	Rohprotein (Kjeldahl) Probe 1	g/kg	13	81,9	81,9	1,56	1,91	0	0
5	Rohprotein (Kjeldahl) Probe 2	g/kg	12	232,5	231,9	2,18	0,94	1	0
6	Rohprotein (Verbrennung) Probe 1	g/kg	9	86,8	86,5	3,53	4,08	0	0
7	Rohprotein (Verbrennung) Probe 2	g/kg	8	235,6	235,3	3,06	1,30	1	1
8	Rohfaser Probe 2	g/kg	13	36,8	37,0	3,79	10,24	0	0
9	Rohfett (n. Säurehydrolyse) Probe 2	g/kg	12	73,2	73,4	2,16	2,94	0	0
10	Rohasche Probe 2	g/kg	14	67,6	67,7	1,19	1,76	2	1
11	Salzsäureunl. Asche Probe 2	g/kg	9	1,5	1,5	0,24	15,62	0	1
12	Gesamtzucker(ber. als Sacch.)Probe 2	g/kg	11	57,8	57,1	4,69	8,23	0	0
13	Stärke Probe 2	g/kg	11	404,5	403,1	8,16	2,02	0	1
14	Phosphor Probe 1	g/kg	19	1,64	1,68	0,156	9,31	1	2
15	Phosphor Probe 2	g/kg	19	6,80	6,87	0,570	8,31	0	0
16	Phosphor Probe 3	g/kg	19	51,6	51,9	3,65	7,03	1	1
17	Kalium Probe 1	g/kg	20	13,99	13,85	0,978	7,06	0	0
18	Kalium Probe 2	g/kg	17	12,1	12,1	1,07	8,85	0	1
19	Kalium Probe 3	g/kg	17	2,98	3,06	0,487	15,92	0	1
20	Calcium Probe 1	g/kg	21	31,9	32,2	2,02	6,28	0	0
21	Calcium Probe 2	g/kg	17	12,0	11,8	0,61	5,17	0	2
22	Calcium Probe 3	g/kg	17	217	219	8,7	3,96	0	3
23	Magnesium Probe 1	g/kg	19	3,55	3,56	0,112	3,15	1	2
24	Magnesium Probe 2	g/kg	18	2,38	2,42	0,129	5,31	1	1
25	Magnesium Probe 3	g/kg	18	58,9	59,9	3,49	5,83	2	2
26	Natrium Probe 1	g/kg	17	0,172	0,189	0,0692	36,62	1	1
27	Natrium Probe 2	g/kg	17	1,70	1,75	0,129	7,37	1	2
28	Natrium Probe 3	g/kg	19	70,5	71,0	5,27	7,42	0	0
29	Chlorid Probe 2	g/kg	9	1,84	1,79	0,228	12,70	1	1
30	Chlorid Probe 3	g/kg	7	99,1	100,4	5,18	5,16	1	2
31	Fluorid - EU Methode Probe 1	mg/kg	4	9,1	8,9	1,34	15,02	0	1
32	Fluorid - EU Methode Probe 3	mg/kg	6	296,8	288,1	103,71	36,00	0	0
33	Schwefel Probe 1	mg/kg	8	1770	1777	130,6	7,35	1	2
34	Schwefel Probe 2	mg/kg	8	3169	3100	288,1	9,29	0	2
35	Schwefel Probe 3	mg/kg	8	1666	1699	129,4	7,62	0	2
36	Vanadium Probe 1	mg/kg	8	7,56	7,04	1,122	15,93	0	0
37	Vanadium Probe 2	mg/kg	7	0,539	0,520	0,0541	10,41	2	0
38	Vanadium Probe 3	mg/kg	7	6,89	6,88	0,798	11,59	0	0
39	Eisen Probe 1	mg/kg	20	3453	3486	254,3	7,30	0	1
40	Eisen Probe 2	mg/kg	16	264	266	20,4	7,67	1	2
41	Eisen Probe 3	mg/kg	20	3219	3183	292,3	9,18	0	0
42	Mangan Probe 1	mg/kg	20	115,2	113,9	5,56	4,88	1	1
43	Mangan Probe 2	mg/kg	19	161,8	164,5	13,30	8,08	0	0
44	Mangan Probe 3	mg/kg	20	2524	2532	113,8	4,50	0	0
45	Kupfer Probe 1	mg/kg	19	9,31	9,61	0,917	9,54	0	2
46	Kupfer Probe 2	mg/kg	17	36,2	34,9	2,79	8,00	0	2
47	Kupfer Probe 3	mg/kg	20	1148	1148	83,3	7,26	0	0
48	Zink Probe 1	mg/kg	21	28,61	29,46	2,991	10,15	0	0
49	Zink Probe 2	mg/kg	19	167,2	168,5	11,60	6,89	0	0
50	Zink Probe 3	mg/kg	20	7184	7284	418,4	5,74	0	0
51	Cobalt Probe 1	mg/kg	13	2,22	2,19	0,267	12,18	0	0
52	Cobalt Probe 2	mg/kg	11	1,23	1,23	0,101	8,23	0	1
53	Cobalt Probe 3	mg/kg	13	29,3	29,0	3,23	11,13	0	0
54	Molybdän Probe 1	mg/kg	13	0,527	0,547	0,0706	12,92	1	1
55	Molybdän Probe 2	mg/kg	12	1,14	1,14	0,062	5,45	0	1
56	Molybdän Probe 3	mg/kg	12	2,23	2,28	0,407	17,86	1	0
57	Bor Probe 1	mg/kg	12	36,3	36,7	3,74	10,21	1	1
58	Aluminium Probe 1	mg/kg	8	5175	5256	643,6	12,25	0	1
59	Aluminium Probe 2	mg/kg	8	212,6	213,8	44,60	20,86	0	0
60	Barium Probe 1	mg/kg	5	41,4	41,4	1,12	2,72	0	1
61	Barium Probe 2	mg/kg	6	4,78	4,67	0,694	14,86	0	0
62	Barium Probe 3	mg/kg	6	63,6	64,6	5,53	8,57	1	0
63	Cadmium Probe 1	mg/kg	12	0,0484	0,0614	0,03098	50,44	0	1
64	Cadmium Probe 2	mg/kg	14	0,0622	0,0601	0,01028	17,11	1	0
65	Cadmium Probe 3	mg/kg	13	0,324	0,311	0,0456	14,66	0	1
66	Blei Probe 1	mg/kg	16	2,39	2,32	0,446	19,23	0	0
67	Blei Probe 2	mg/kg	9	0,172	0,177	0,0280	15,81	0	1
68	Blei Probe 3	mg/kg	14	3,57	3,46	1,035	29,88	0	0
69	Chrom Probe 1	mg/kg	13	12,0	13,4	3,51	26,18	0	0

ALVA IX

Nr	Bezeichnung	Einheit	Labors	med	MW	std	VR	Typ1	Typ2
70	Chrom Probe 2	mg/kg	9	0,729	0,764	0,1540	20,14	0	2
71	Chrom Probe 3	mg/kg	10	31,6	32,3	2,37	7,34	0	2
72	Nickel Probe 1	mg/kg	14	23,8	23,2	2,23	9,62	1	0
73	Nickel Probe 2	mg/kg	12	6,33	6,44	0,608	9,44	0	0
74	Nickel Probe 3	mg/kg	11	14,7	14,4	2,61	18,10	0	1
75	Quecksilber Probe 1	mg/kg	9	0,0150	0,0150	0,00220	14,64	0	4
76	Quecksilber Probe 2	mg/kg	7	0,0013	0,0015	0,00081	52,06	1	1
77	Quecksilber Probe 3	mg/kg	7	0,0011	0,0011	0,00616	561,45	0	0
78	Arsen Probe 1	mg/kg	14	1,46	1,46	0,104	7,12	0	1
79	Arsen Probe 2	mg/kg	9	0,076	0,081	0,0173	21,27	0	1
80	Arsen Probe 3	mg/kg	12	1,48	1,58	0,261	16,56	0	1
81	Selen Probe 1	mg/kg	8	0,079	0,075	0,0194	25,82	0	0
82	Selen Probe 2	mg/kg	11	0,556	0,566	0,1061	18,74	0	0
83	Selen Probe 3	mg/kg	12	46,0	46,0	1,82	3,97	0	0
84	Iod - Probe 1	mg/kg	8	0,613	0,566	0,1498	26,45	0	0
85	Iod - Probe 2	mg/kg	7	1,39	1,38	0,178	12,90	0	0
86	Iod - Probe 3	mg/kg	6	98,8	98,0	5,43	5,54	0	2
87	Strontium Probe 1	mg/kg	5	34,5	34,5	2,37	6,87	0	0
88	Strontium Probe 2	mg/kg	5	9,90	9,91	0,626	6,31	1	0
89	Strontium Probe 3	mg/kg	5	320	322	18,9	5,87	0	0
90	Uran Probe 1	mg/kg	5	0,149	0,148	0,0023	1,52	0	0
91	Uran Probe 2	mg/kg	4	0,182	0,189	0,0169	8,93	0	1
92	Uran Probe 3	mg/kg	4	0,761	0,759	0,0294	3,87	1	0
93	Vitamin A Probe 2	IU/kg	8	9611	9716	758,2	7,80	0	2
94	Vitamin D3 Probe 2	IU/kg	10	2186	2212	275,7	12,46	0	0
95	Vitamin E (α-Tocopherolacetat) Pro. 2	mg/kg	10	180,4	174,5	18,80	10,77	0	2
96	Cystin Probe 2	%	9	0,418	0,413	0,0388	9,39	0	2
97	Asparaginsäure Probe 2	%	10	2,32	2,24	0,225	10,03	0	0
98	Threonin Probe 2	%	12	0,875	0,891	0,0799	8,97	0	0
99	Serin Probe 2	%	10	1,141	1,134	0,1139	10,05	0	0
100	Glutaminsäure Probe 2	%	9	4,08	4,05	0,322	7,93	0	1
101	Glycin Probe 2	%	9	0,950	0,934	0,0883	9,45	0	1
102	Alanin Probe 2	%	10	1,079	1,062	0,0906	8,53	0	0
103	Prolin Probe 2	%	9	1,32	1,32	0,086	6,52	0	1
104	Valin Probe 2	%	10	0,997	1,019	0,0777	7,62	0	1
105	Methionin - gesamt Probe 2	%	11	0,359	0,359	0,0190	5,30	1	1
106	Methionin - zugesetzt Probe 2	%	4	0,011	0,010	0,0073	73,34	0	0
107	Isoleucin Probe 2	%	9	0,954	0,958	0,0472	4,93	3	1
108	Leucin Probe 2	%	10	1,89	1,83	0,174	9,53	0	0
109	Tyrosin Probe 2	%	10	0,757	0,746	0,0951	12,74	0	0
110	Phenylalanin Probe 2	%	9	1,120	1,129	0,0355	3,15	1	1
111	Histidin Probe 2	%	11	0,598	0,596	0,0589	9,88	0	0
112	Lysin - gesamt Probe 2	%	11	1,373	1,385	0,0917	6,62	1	1
113	Lysin - zugesetzt Probe 2	%	7	0,112	0,116	0,0221	19,12	1	1
114	Arginin Probe 2	%	10	1,493	1,488	0,1712	11,51	0	0
115	Tryptophan Probe 2	%	7	0,279	0,267	0,0386	14,48	0	0
116	6-Phytase (E 1614) Probe 2	U/kg	9	1353	1324	167,1	12,62	0	0

6.5 Metals and trace elements for water analysis, No M148 and No M149

Table 17: Certified values for elements expressed in µg/L (samples No M148)

Parameter	Probe M148A	Unsicherheit	Probe M148B	Unsicherheit	Einheit
Aluminium	**30.0**	0.3	**15.0**	0.3	µg/l
Chrom	**4.04**	0.03	**0.60**	0.01	µg/l
Mangan	**38.100**	0.2	**2.12**	0.03	µg/l
Eisen	**71.4**	0.3	**18.0**	0.2	µg/l
Nickel	**1.30**	0.02	**3.52**	0.030	µg/l
Kupfer	**1.70**	0.02	**3.20**	0.03	µg/l
Zink	**10.0**	0.8	**28.0**	0.8	µg/l
Arsen	**4.20**	0.03	**1.10**	0.01	µg/l
Selen	**1.00**	0.05	**3.55**	0.06	µg/l
Cadmium	**0.249**	0.003	**0.800**	0.007	µg/l
Quecksilber	**0.95**	0.010	**0.58**	0.01	µg/l
Blei	**0.79**	0.01	**1.98**	0.01	µg/l
Uran	**6.05**	0.04	**3.80**	0.0200	µg/l

Table 18: Certified values for elements expressed in µg/L (samples No M149)

Parameter	Probe M149A	Unsicherheit	Probe M149B	Unsicherheit	Einheit
Aluminium	**72.3**	0.4	**40.3**	0.3	µg/l
Chrom	**6.39**	0.04	**1.71**	0.02	µg/l
Mangan	**21.1**	0.1	**9.25**	0.07	µg/l
Eisen	**52.7**	0.3	**20.8**	0.2	µg/l
Nickel	**3.03**	0.03	**1.83**	0.02	µg/l
Kupfer	**11.2**	0.1	**2.22**	0.03	µg/l
Zink	**13.3**	0.8	**7.56**	0.79	µg/l
Arsen	**6.38**	0.04	**1.87**	0.02	µg/l
Selen	**2.63**	0.06	**0.87**	0.06	µg/l
Cadmium	**2.11**	0.02	**1.00**	0.01	µg/l
Quecksilber	**1.82**	0.02	**0.79**	0.01	µg/l
Blei	**8.03**	0.1	**2.82**	0.02	µg/l
Uran	**2.23**	0.02	**0.80**	0.01	µg/l

6.6 Methodological details for total element concentration measurements with ICPMS

Table 19: Methodological details and determined limits of detection for the second digestion procedure

Monitored isotopes	Integration time/mass	Tune mode	Internal standard	Limit of detection* (µg/L)
^{7}Li	0.3	No gas	^{9}Be	0.003
^{23}Na	0.1	He	^{9}Be	2
^{24}Mg	0.3	He	^{9}Be	5
^{27}Al	0.5	He	^{74}Ge	1
^{39}K	0.3	He	^{74}Ge	5
^{43}Ca	0.1	He	^{74}Ge	25
^{51}V	0.5	He	^{74}Ge	0.06
^{52}Cr	1	He	^{74}Ge	0.06
^{55}Mn	0.3	He	^{74}Ge	0.05
^{56}Fe	0.3	He	^{74}Ge	0.21
^{59}Co	0.3	He	^{74}Ge	0.005
^{60}Ni	1	He	^{74}Ge	0.02
^{65}Cu	0.3	He	^{74}Ge	0.4
^{75}As	1	He	^{74}Ge	0.01
^{78}Se	1	H_2	^{74}Ge	0.005
^{85}Rb	0.3	He	^{74}Ge	0.01
^{88}Sr	0.3	He	^{74}Ge	0.005
^{95}Mo	0.3	He	^{115}In	0.003
^{111}Cd	0.3	No gas	^{115}In	0.005
^{137}Ba	0.3	No gas	^{115}In	4
^{140}Ce	0.3	No gas	^{175}Lu	0.005
^{157}Gd	0.3	No gas	^{175}Lu	0.002
^{201}Hg	1	No gas	^{175}Lu	0.02
^{208}Pb	0.5	No gas	^{175}Lu	0.005
^{209}Bi	0.3	No gas	^{175}Lu	0.005
^{238}U	0.3	No gas	^{175}Lu	0.001

*LODs were determined based on blank determination [88]

Table 20: Methodological details and determined limits of detection for the aqueous algae extracts extracted at room temperature

Monitored isotopes	Integration time/mass	Tune mode	Internal standard	Limit of detection** (µg/L)
^{7}Li	0.3	No gas	^{9}Be	0.1
^{23}Na	0.3	He*	^{74}Ge	1
^{24}Mg	0.3	He*	^{74}Ge	0.3
^{39}K	0.3	He*	^{74}Ge	10
^{43}Ca	0.3	He*	^{74}Ge	8
^{51}V	0.3	He*	^{74}Ge	0.02
^{55}Mn	0.5	He*	^{74}Ge	0.01
^{56}Fe	0.1	He*	^{74}Ge	1
^{59}Co	0.3	He*	^{74}Ge	0.03
^{60}Ni	1.0	He*	^{74}Ge	0.2
^{65}Cu	0.3	He*	^{74}Ge	0.03
^{75}As	1.0	He*	^{74}Ge	0.06
^{78}Se	1.0	H_2	^{74}Ge	0.04
^{85}Rb	0.1	He*	^{74}Ge	0.4
^{88}Sr	0.1	He*	^{74}Ge	0.2
^{137}Ba	0.3	No gas	^{175}Lu	0.1
^{140}Ce	0.1	No gas	^{175}Lu	0.01
^{208}Pb	0.5	No gas	^{175}Lu	0.1
^{238}U	0.3	No gas	^{175}Lu	0.01

*Helium collision mode with the addition of 0.20 L/min CO_2 (1 % (v/v) in Ar) as option gas (pumped and mixed with sample aerosol and nebulizer gas after the Scott-type spray chamber, just before entering the plasma torch); **LODs were determined based on blank determination [88]

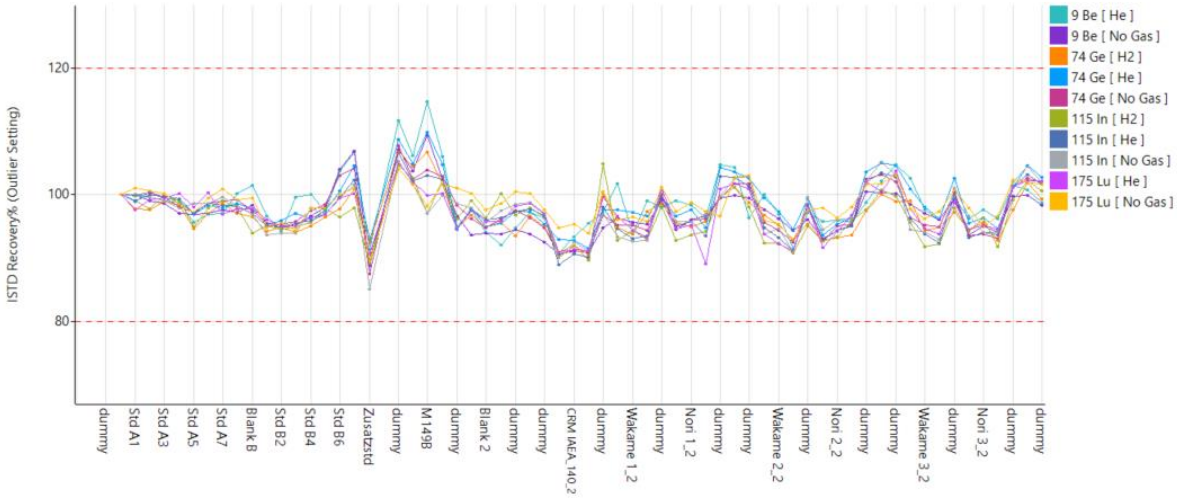

Figure 44: Internal standard recovery during the second digestion experiment acquisition

6.7 Results on total arsenic content in Wakame extracts

Table 21: Summary on the performed extractions with results on total As concentration in extracts and the respective extraction efficiencies

Solvent	Conditions	t [min]	total As [mg/kg dm]	ex [%]
H_2O	rt, sonicator	30	1.7 ± 0.3	4.3 ± 0.9
H_2O	65°C, sonicator	120	4.0	10.2
H_2O	60°C, shaker	30	1.4 ± 0.3	3.5 ± 0.7
H_2O	80°C, shaker	120	2.0 ± 0.1	5.1 ± 0.2
H_2O/MeOH 1+1	rt, sonicator	30	1.4 ± 0.9	3.5 ± 2.3
$NH_4H_2PO_4$, pH=6	65°C, sonicator	120	2.9	7.2
Isopropanol	60°C, shaker	120	0.8 ± 0.1	2.1 ± 0.3
DCM/MeOH	rt, rotary cross	180	1.1 ± 0.1	2.7 ± 0.3
0.01 M NaOH	80°C, shaker	120	5.7 ± 0.2	14.5 ± 0.6
pure [EMIm][OAc]	80°C, beaker	60	24.6	62.2
50 wt% [EMIm][OAc]	90°C, shaker	240	5.8	14.6
50 wt% [EMIm][DEP]	90°C, shaker	240	14.0	35.5
50 wt% [Ch][Lys]	90°C, shaker	240	33.9	85.9
pure [EMIm][OAc]	40°C, heating block	120	1.2 ± 0.2	3.1 ± 0.6
pure [EMIm][OAc]	rt, rotary cross	3600	1.7 ± 0.2	4.2 ± 0.6
[EMIm][OAc]/DCM/MeOH	rt, rotary cross	3600	2.3 ± 0.3	5.9 ± 0.9
10 wt% [Ch][Lys]	40°C, shaker	120	11.4 ± 0.2	29.0 ± 0.6

Solvent	Conditions	t [min]	total As [mg/kg dm]	ex [%]
10 wt% [Ch][Lys] x 2.5 solvent	40°C, shaker	120	8.2 ± 0.5	20.7 ± 1.3
10 wt% [Ch][Lys] 1x not soaked	40°C, shaker	120	9.1	23.0
10 wt% [Ch][Lys]	40°C, shaker	3600	34.8 ± 0.2	88.0 ± 1.1
10 wt% [Ch][Lys]	60°C, shaker	120	22.5 ± 0.4	56.9 ± 1.4
10 wt% [Ch][Lys] Batch 1	80°C, shaker	120	32.4 ± 1.8	81.9 ± 5.2
10 wt% [Ch][Lys] Batch 2	80°C, shaker	120	31.0 ± 2.7	78.4 ± 7.3
10 wt% [Ch][Lys] pH = 12 (Batch 3)	80°C, shaker	120	31.9 ± 0.8	80.7 ± 2.7
10 wt% [Ch][Lys] pH = 7	80°C, shaker	120	4.3 ± 0.1	10.9 ± 0.4
10 wt% [Ch][Lys] pH = 4	80°C, shaker	120	2.8 ± 0.2	7.0 ± 0.7

*blue shaded rows are cases where speciation analysis was conducted (see next chapter)

6.8 Results on arsenic species concentrations in various Wakame extracts

Table 22: Known and unknown arsenic species concentrations [mg/kg dm] found in various Wakame extracts, as well as the corresponding extraction efficiency for total arsenic concentration in the extract and the column recovery of the speciation analysis (both in %)

Conditions	ex [%]	OH-Sugar [mg/kg]	DMA [mg/kg]	MMA [mg/kg]	PO_4-Sugar [mg/kg]	Arsenate [mg/kg]	U3 [mg/kg]	U4 [mg/kg]	Column recovery [%]
H_2O, 80°C, shaker, 120 min	5.1 ± 0.2	0.27 ± 0.02	0.43 ± 0.01	0.021 ± 0.002	0.22 ± 0.01	0.022 ± 0.002	0.02 ± 0.5	0.12 ± 0.01	73 ± 2
0.01 M NaOH, 80°C, shaker, 120 min	14.5 ± 0.6	0.34 ± 0.01	0.32 ± 0.02	< 0.1	2.33 ± 0.13	< 0.1	< 0.1	0.72 ± 0.01	83 ± 3
pure [EMIm] [OAc], 40°C, heating block, 120 min	3.1 ± 0.6	0.10 ± 0.01	0.16 ± 0.01	< 0.02	0.09 ± 0.01	< 0.02	n.d.***	n.d.	33 ± 9**
10 wt% [Ch][Lys], W3, 40°C, shaker, 120 min	25.0 ± 0.2	0.27 ± 0.03	0.11 ± 0.01	< 0.1	8.9 ± 0.3	< 0.1	n.d.	n.d.	98 ± 2
10 wt% [Ch][Lys], 40°C, shaker, 1 day	88.0 ± 1.1	1.42 ± 0.14	0.39 ± 0.08	< 0.2	35.7 ± 1.8	< 0.3	n.d.	n.d.	108 ± 6
10 wt% [Ch][Lys], W2, 60°C, shaker, 120 min	54.6 ± 1.2	0.63 ± 0.03	0.20 ± 0.04	< 0.1	18.7 ± 1.1	0.20 ± 0.02*	n.d.	n.d.	98 ± 6

Conditions	ex [%]	OH-Sugar [mg/kg]	DMA [mg/kg]	MMA [mg/kg]	PO_4-Sugar [mg/kg]	Arsenate [mg/kg]	U3 [mg/kg]	U4 [mg/kg]	Column recovery [%]
10 wt% [Ch][Lys], 80°C, shaker, 120 min	81.9 ± 5.2	0.91 ± 0.04	0.57 ± 0.04	< 0.1	27.5 ± 1.7	0.40 ± 0.10*	n.d.	n.d.	95 ± 11
10 wt% [Ch][Lys], 80°C, shaker, 120 min, 1 month old	78.4 ± 7.3	3.48 ± 0.04	0.48 ± 0.01	< 0.1	24.4 ± 0.3	<0.1	0.90 ± 0.13	1.47 ± 0.09	100 ± 10
10 wt% [Ch][Lys] pH = 12, 80°C, shaker, 120 min	80.7 ± 2.7	1.48 ± 0.05	0.47 ± 0.01	< 0.1	28.8 ± 0.2	< 0.1	0.44 ± 0.07	1.05 ± 0.05	102 ± 4
10 wt% [Ch][Lys] pH = 7, 80°C, shaker, 120 min	10.9 ± 0.4	0.23 ± 0.04	0.39 ± 0.01	< 0.1	0.18 ± 0.02	< 0.1	< 0.1	< 0.1	33 ± 3**
10 wt% [Ch][Lys] pH = 4, 80°C, shaker, 120 min	7.0 ± 0.7	0.49 ± 0.01	0.33 ± 0.02	< 0.1	0.19 ± 0.01	< 0.1	< 0.1	< 0.1	47 ± 7**

*Arsenate coelution with U3 and/or U4 → to high estimation of its concentration

**Low column recoveries indicate the presence of unretained arsenic species, in the case of ILs this could be AsL

***Not determined (n.d.)

6.9 Coelution of Arsenate and SO_3-Sugar in the case of aqueous Cholinium Lysinate extracts of Wakame samples

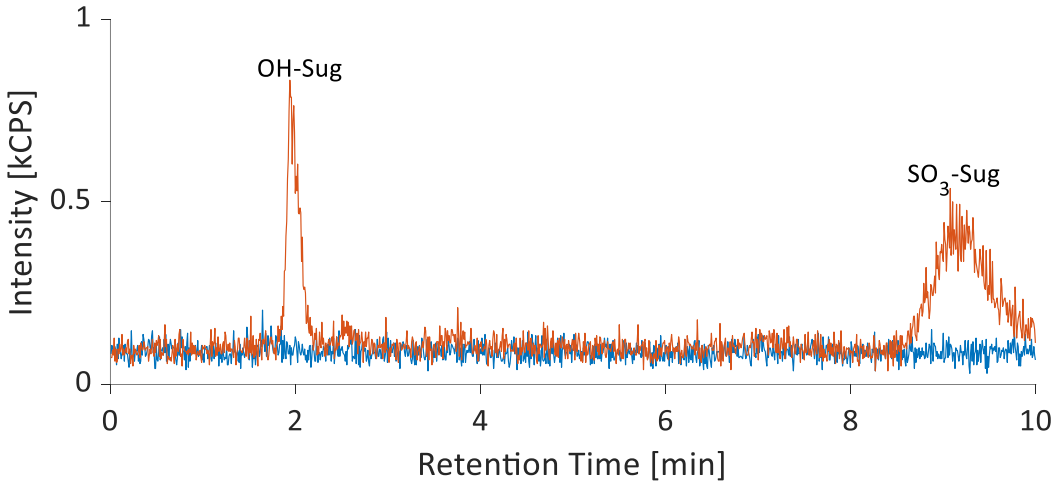

Figure 45: Ultrapure water spiked with approximately 3.4 µg/L OH-Sugar standard and 6.8 µg/L SO_3-Sugar standard (red); Conditions: PRP-X100 column (150 x 4.6 mm; 5 µm, stainless steel) at 30 °C, 20 mM $NH_4H_2PO_4$ buffer at pH = 6.0, flow rate- = 1.0 mL/min and 10 µL injection volume

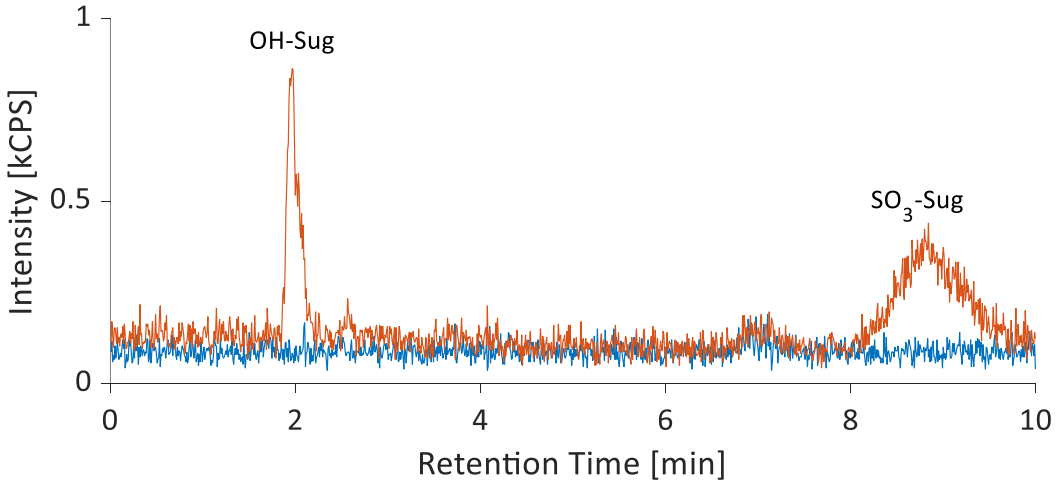

Figure 46: 10 wt% [Ch][Lys] blank sample spiked with approximately 3.4 µg/L OH-Sugar standard and 6.8 µg/L SO_3-Sugar standard (red) – note the retention time shift of SO_3-Sugar (from ~9.2 min to ~8.9 min); Conditions: PRP-X100 column (150 x 4.6 mm; 5 µm, stainless steel) at 30 °C, 20 mM $NH_4H_2PO_4$ buffer at pH = 6.0, flow rate = 1.0 mL/min and 10 µL injection volume

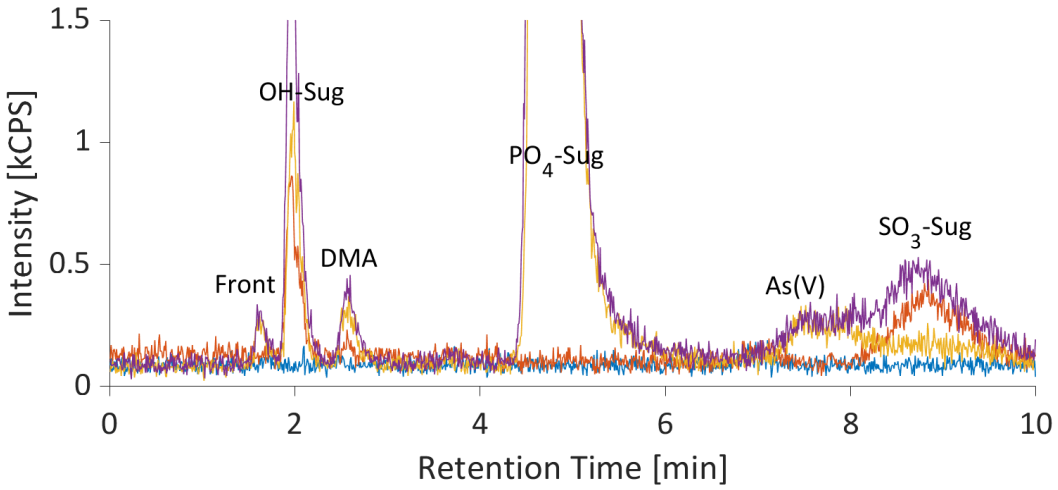

Figure 47: 10 wt% [Ch][Lys] Wakame extract (shaking water bath, 2 h, 80°C) spiked with approximately 3.4 µg/L OH-Sugar standard and 6.8 µg/L SO_3-sugar standard (violet) overlayed with the non-spiked extract (yellow) and the spiked blank sample (red); Conditions: PRP-X100 column (150 x 4.6 mm; 5 µm, stainless steel) at 30 °C, 20 mM $NH_4H_2PO_4$ buffer at pH = 6.0, flow rate = 1.0 mL/min and 10 µL injection volume

6.10 Certificates of analysis for the used ionic liquids

proionic GmbH, Parkring 18 H/1, A-8074 Grambach/Graz
Tel.: +43 316 4009 4200; office@proionic.com

Certificate of Analysis

Product name	***Cholinium L-Lysinate***
PI product number	01334.4000
Purity	techn.
Molecular formula	$C_6H_{13}N_2O_2{*}C_5H_{14}NO$
Molecular weight	249.36 g*mol^{-1}
CAS Number	1361335-94-5
LOT Number	17PI296_3

CHARACTERISTIC	*SPEZIFICATION*	*LOT RESULT*
assay, *$HClO_4$ titration*	≥ 95.0 wt%	97.39 ± 1.57 wt%*
assay free base, *HCl titration*	< 1wt%	not detected, < 0.5wt%
halides, *IC*	< 100 ppm_w	< 10 ppm_w
assay H_2O, *Karl Fischer titration*	< 5 wt%	1.7 ± 0.2 wt%
refractive index, n_D^{20}	1.5147**	1.5058
structure, *^{1}H-NMR*	structure	structure confirmed
aspect, *acc. to Ph. Eur. 5.0*	clear to almost clear	almost clear
color, *acc. to Ph. Eur. 5.0*	yellow	yellow

QC Release Date 07/23/2019

Quality Executive

Quality Assurance

*Short-term available starting material (choline hydroxide solution in water, 48 wt%) contained ~0.5 wt% stabilizer (paraformaldehyde) which stays in the final product. Corrected assay is ~97.9%, which correlates to a water free purity of ~99.6%.

**De Santis, Serena et al., Phys. Chem. Chem. Phys., 2015, 17, 20687. See ESI for refractory index.

proionic GmbH, Parkring 18 H/1, A-8074 Grambach/Graz
Tel.: +43 316 4009 4200; office@proionic.com

Certificate of Analysis

Product name	***1-Ethyl-3-methylimidazolium diethylphosphate***
PI product number	00106.3000
Purity	purum
Molecular formula	$C_{10}H_{21}N_2O_4P$
Molecular weight	264.26 g*mol^{-1}
CAS Number	848641-69-0
LOT Number	17PI231_3

CHARACTERISTIC	***SPEZIFICATION***	***LOT RESULT***
assay $EMIM^+$, *HPLC*	≥ 98.0 %w	99.31 ± 0.29 %w
assay DEP^-, IC	≥ 98.0 %w	99.19 ± 0.48 %w
halides, *IC*	< 50 ppm_w	< 5 ppm_w
assay H_2O, *Karl Fischer titration*	< 0.5 %w	0.13 ± 0.02 %w
assay free base, *HPLC*	< 0.5 %w	0.24 ± 0.01 %w
aspect, *acc. to Ph. Eur. 5.0*	clear liquid	clear liquid
color, *acc. to Ph. Eur. 5.0*	colorless to yellow	yellow

QC Release Date
2018-07-04

Quality Executive

Quality Assurance

proionic GmbH, Parkring 18 H/1, A-8074 Grambach/Graz
Tel.: +43 316 4009 4200; office@proionic.com

Certificate of Analysis

Product name	***1-Ethyl-3-methylimidazolium acetate***
PI product number	00102.3000
Purity	purum
Molecular formula	$C_6H_{14}N_2O_2$
Molecular weight	170.21 g*mol^{-1}
CAS Number	143314-17-4
LOT Number	17PI252_3

CHARACTERISTIC	***SPEZIFICATION***	***LOT RESULT***
assay $EMIM^+$, *HPLC*	≥ 98.0 %w	98.30 ± 0.09 %w
assay OAc^-, *non aqueous titration*	≥ 98.0 %w	98.99 ± 0.18 %w
halides, *IC*	< 50 ppm_w	< 5 ppm_w
assay H_2O, *Karl Fischer titration*	< 0.5 %w	0.06 ± 0.01 %w
assay free base, *HPLC*	< 0.5%w	0.05 ± 0.01 %w
aspect, *acc. to Ph. Eur. 5.0*	clear to almost clear	clear
color, *acc. to Ph. Eur. 5.0*	colorless to slightly yellow	yellow

QC Release Date
2018-07-05

Quality Executive

Quality Assurance

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Used symbols and abbreviations

- AB — Arsenobetaine
- ACHE — Analytical Chemistry for Health and Environment
- As(III) — Arsenite (arsenous acid)
- As(V) — Arsenate (arsenic acid)
- AsL — Arsenolipids
- AsPL — Arsenosugar-phospholipids
- AsSug — Arsenosugars
- [Ch][Lys] — Cholinium Lysinate
- CIC — Compound independent calibration
- cr [%] — Column recovery in precent
- CZE — Capillary zone electrophoresis
- DCM — Dichloromethane
- dm — dry mass
- DMA — Dimethyl arsenate
- [EMIm][DEP] — 1-ethyl-3-methylimidazolium diethyl phosphate
- [EMIm][OAc] — 1-ethyl-3-methylimidazolium acetate
- ex [%] — Extraction efficiency in precent
- GC — Gas chromatography
- HG-AFS — Hydride generation atomic fluorescence spectroscopy
- HPLC — High performance liquid chromatography
- iAs — Inorganic arsenic
- ICPMS — Inductively coupled plasma mass spectrometry
- ICPOES — Inductively coupled plasma optical emission spectroscopy
- *m/z* — Mass to charge ratio
- MA — Monomethyl arsenate
- NIST — National Institute for Standards and Technology
- OH-Sugar — Glycerol arsenosugar
- PO_4-Sugar — Phosphate arsenosugar
- rt — Room temperature (23 °C)

- $scCO_2$ Supercritical carbon dioxide
- SFE Supercritical fluid extraction
- SIMS Secondary ion mass spectrometry
- SO_3-Sugar Sulfonate arsenosugar
- SO_4-Sugar Sulfate arsenosugar
- WHO World Health Organization