Influence of typical cooking processes on the arsenic speciation in common edible mushrooms - determination with HPLC-ICP-MS method

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Influence of typical cooking processes on the arsenic speciation in common edible mushrooms – determination with HPLC-ICPMS

MASTER THESIS

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"Gratitude is the healthiest of all human emotions. The more you express gratitude for what you have, the more likely you will have even more to express gratitude for."

Zig Ziglar

ABSTRACT

The purpose of this research was the investigation of the total arsenic concentration in Sarcosphaera Coronaria and Chantarelle mushrooms and the arsenic speciation analysis of their extracts with HPLC-ICPMS. Additionally, I was studying the influence of typical cooking processes such as frying and boiling in water on the arsenic speciation in these two mushrooms, because this is the normal way of consumption. The influence of vinegar on the arsenic speciation was also studied in Sarcosphaera Coronaria mushrooms because in 1920 people in Switzerland died after consumption of these mushrooms prepared with vinegar. Also, the purpose was learning the use of high performance liquid chromatography (HPLC) coupled with inductively coupled plasma mass spectrometry (ICPMS), process of preparing samples and performing quality control during scientific research.

The results are shown that boiling with vinegar (10 %) does not significantly increase the formation of the toxic As species MA(III) but keeps it steady as opposed to boiling in pure water, causing MA(III) to disappear completely from the mushroom. Probably, MA(III) goes into some other form of arsenic, which is interesting for some future research. Additionally, we investigated the behaviour of other elements during the cooking process in the common edible mushrooms Chantarelle and we confirmed the lose of minerals (Na, Ca, Mg, and Zn), other essential trace elements (Se and Fe) and also toxic elements (As, Pb, and Sr) during typical cooking process. The separation of analytes was performed by high performance liquid chromatography and separated analytes were detected by inductively coupled plasma mass spectrometry. The results of the research have shown that HPLC - ICPMS method is acceptable and satisfactory for this kind of research.

The formation of a new compound during frying needs some further investigations, also it can be interesting to examine the behaviour of arsenic, arsenic species and other element during the thermic process of other edible mushrooms.

Key words: arsenic, arsenic species, edible mushrooms, cooking, high performance liquid chromatography, inductively coupled plasma mass spectrometry, ion-exchange chromatography

SAŽETAK

Cilj ovoga istraživanja bio je odrediti koncentraciju ukupnog arsena u gljivama Sarcosphaera coronaria i Chantarelle te specijaciju arsena iz njihovog ekstrakta HPLC-ICPMS metodom. Također, važan dio ovog rada bio je istražiti utjecaj termičke obrade – tipičnih procesa poput prženja ili kuhanja – na arsen i specijacije arsena, posebice u jestivim gljivama, Chantarellama. Nadalje, cilj je bio ispitati utjecaj vinskog octa na specijacije arsena u gljivama Sarcosphaera coronaria, zbog smrtnog slučaja koji se dogodio u Švicarskoj, 1920. godine, nakon što je ih grupa ljudi konzumirala u pripremi s octom. Točan uzrok njihove smrti do danas nije poznat. Osobna svrha istraživanja bila je učiti o tekućinskoj kromatografiji visoke djelotvornosti (HPLC) povezanoj s induktivno spregnutom plazmom – spektormetrijom masa (ICPMS), postupcima pripreme uzoraka te pravilnom provođenju kontrole kvalitete tijekom znanstvenog istraživanja.

Rezultati ovog istraživanja pokazali su da kuhanjem gljiva uz 10 % octa ne uzrokuje formaciju toksične vrste arsena MA(III), ali on ostaje prisutan u gljivi Sarcosphaera Coronaria i nakon kuhanja. Nasuprot tome, kuhanjem u vodi bez octa, uočeno je da toksična vrsta MA(III) potpuno nestaje iz gljive. Ispitivanjem vode nakon kuhanja potvrđeno je da ne dolazi do ekstrakcije MA(III) iz gljive u vodu pa zaključujemo da MA(III) kuhanjem iz gljive prelazi u neki drugi oblik arsena što ostavlja prostor za daljnja istraživanja. Nadalje, praćena je promjena koncentracija ostalih važnih elemenata u gljivama Chantarelle prije i nakon termičke obrade. Potvrđeno je da termičkom obradom dolazi do gubitka minerala (Na, Ca, Mg i Zn), elementa u tragovima (Se i Fe) i toksičnih elemenata (As, Pb i Sr) tijekom tipičnih procesa obrade. Za separaciju analita korištena je tekućinska kromatografija visoke djelotvornosti a za njihovu detekciju induktivno spregnuta plazma – spektormetrija masa. Rezultati su pokazali da je metoda HPLC-ICPMS prihvatljiva i zadovoljavajuća za ovakvu vrstu istraživanja.

Analizom rezultata uočeno je nastajanje novih komponenata tijekom procesa prženja što treba dodatno istražiti u budućim istraživanjima.

Ključne riječi: arsen, specijacije arsena, gljive, termička obrada, tekućinska kromatografija visoke djelotvornosti, induktivno spregnuta plazma spektormetrije masa, kromatografija ionske izmjene

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1. INTRODUCTION

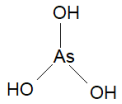
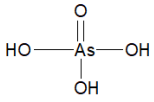
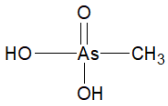
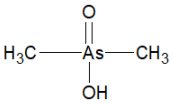
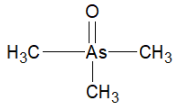
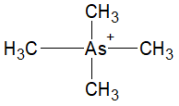
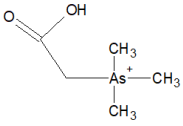
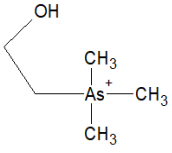
1.1. Arsenic and arsenic species

Arsenic is a crystalline metalloid found in the Earth's crust, but in its free form it is quite rare. Arsenic has a numbers of forms, or allotropes [1]. It is a ubiquitous element with low concentrations in rocks, soil and natural ground water. In the environment arsenic appears in organic (at least one arsenic-carbon bond is in the molecule) and inorganic (no arsenic-carbon bonds in the molecule) forms. The arsenic toxicity is highly dependent of the arsenic species. Inorganic arsenic species are much more toxic than organic arsenic species [2]. Inorganic arsenic compounds include arsenite, As(III) and arsenate, As(V) and they may cause human cancers in many organs, including lung, skin, urinary bladder, and are classified as "carcinogenic to humans" by the International Agency for Research on Cancer (IARC). This was based on the induction of primary skin cancer, as well as the introduction of lung and urinary bladder cancer. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a Provisional Tolerable Weekly Intake (PTWI) for inorganic arsenic of 0.015 mg/kg bodyweight/week in 1988 and in 2011 this PTWI has been withdrawn without replacement. If we consider this, this would mean that an average person of about 70 kg should not consume more than 1 mg of arsenic per week via the food. No new tolerable intake level could be established. Organic arsenic compounds are methylarsonic acid (MA), dimethylarsinic acid (DMA), arsenocholine (AsC), arsenobetaine (AB), trimethylarsine oxide (TMAO), tetramethylarsonium (TETRA), arsenosugars and arsenolipids (AsLp) [2]. The arsenic species, their chemical structures and properties are shown in *Table 1*. The presence of these compounds was detected in marine organisms, some plants and mushrooms. The methylated arsenic species, such as MA and DMA, have been confirmed to be much less toxic, and are classified as „possibly carcinogenic to humans“ by IARC. Arsenobetaine and some other organic arsenic species, which could not be metabolized in the human body, are considered to be nontoxic. Arsenic and its compounds occur in crystalline, powder, amorphous or vitreous forms. They usually also occur in trace quantities in water and air. However, concentrations may be higher in certain areas as a result of weathering and anthropogenic activities including metal mining and smelting, fossil fuel combustion and pesticide use. The toxicity of arsenic species, not only depends on their chemical forms but also their bioavailability. The toxicity of As(III) and As(V) was reported to be related to their high bioavailability, as their absorption rate in the human

gastrointestinal tract was over 80 % [2, 3]. Although arsenic forms species under reducing conditions with the arsenic atom in oxidation state -3 and +3, the most stable arsenic species found under normal environment condition contain the arsenic atom in oxidation state +5 [2].

The determination of arsenic species is still not a routine procedure, and hence clear quality criteria are not yet established. An important criterion is the testing of the method with a reference material certified for arsenic species.

Table 1. The chemical structures and properties of the arsenic species commonly detected in mushrooms

No.	Species	CAS No.	Chemical structure	Chemical formula	Molecular weight (g/mol)	pK_a
1	Arsenous acid (arsenite) As(III)	13464-58-9		$As(OH)_3$	125.94	9.26 13.5 14.0
2	Arsenic acid (arsenate) As(V)	7778-39-4		H_3AsO_4	141.93	2.26 6.76 11.2 9
3	Methylarsonic acid MA(V)	124-58-3		$CH_3AsO(OH)_2$	139.97	3.6 8.2
4	Dimethylarsinic acid DMA (V)	75-60-5		$(CH_3)_2As(O)OH$	137.99	1.28
5	Trimethylarsine oxide TMAO	4964-14-1		$(CH_3)_3AsO$	136.023	3.6
6	Tetramethylarsonium TETRA	27742-38-7		$(CH_3)_4As^+$	135.06	WD
7	Arsenobetaine AB	64436-13-1		$C_5H_{11}AsO_2$	178.06	2.18
8	Arsenocholine AsC	39895-81-3		$C_5H_{14}AsO^+$	165.09	WD

1.2. Human exposure to arsenic and arsenic species

Epidemiological and clinical studies indicate that arsenic is a human carcinogen that does induce cancer in animal models. The majority of humans are chronically exposed to low levels of arsenic, principally through ingestion of food and water and to some extent due to inhalation of arsenic in the ambient air. The clinical manifestations of chronic arsenic poisoning are many but the most commonly observed symptoms include arsenical skin lesions, melanosis, conjunctivitis, keratosis and hyperkeratosis. Cases of gangrene in limbs and malignant neoplasms have been observed. Arsenic is believed to be a progressor, facilitating the transition from benign to malignant tumours in humans, but this is still a theory that is intensely debated. Further studies are required on the mechanism of action in multiple test systems so that the exact mechanism of action of arsenic genotoxicity and carcinogenicity can be understood [4]. According to the World Health Organization (WHO), the provisional total daily intake should not exceed 2 µg of inorganic arsenic per kilogram of body weight (b.w.) [5]. Intake of arsenic and arsenic species from food should always be evaluated on the basis of the product as ingested by the consumer. Otherwise, the risk evaluation may not reflect the real situation of human exposure [6]. According to assumptions by European Food Safety Authority (EFSA) [7], the inorganic arsenic exposure from food and water across 19 European countries, using lower bound and upper bound concentrations, has been estimated to range from 0.13 to 0.56 µg/kg b.w. per day for average consumers, and from 0.37 to 1.22 µg/kg b.w. per day for 95th percentile consumers. Dietary exposure to inorganic arsenic for children under three years of age is in general estimated to be from 2 to 3 fold that of adults. The inorganic forms of arsenic are more toxic as compared to the organic arsenic, but so far most of the occurrence data in food collected in the framework of official food control are still reported as total arsenic without differentiating the various arsenic species. The need for speciation data is evident because several investigations have shown that especially in seafood most of the arsenic is present in organic forms that are less toxic. According to this, a risk assessment not taking into account the different species but considering all arsenic as inorganic arsenic would lead to a considerable overestimation of the health risk related to dietary arsenic exposure. Also, according to research the arsenic content in cooking water seems to be of special importance because it determines

whether the arsenic concentrations in the prepared food may be higher or lower compared to the raw product [6].

1.3. Mushrooms

Mushrooms belong to the kingdom of fungi, which is separated from the kingdoms of plants and animals. Their morphology is shown on *Figure 1*. The biodiversity of mushrooms is large; in Europe more than 15,000 species are recorded [8]. The basic parts of each mushroom are cuticle or cap, tubes, grills, spores, ring, stipe, and mycelium. Mushrooms foraged from the wild are traditional and popular organic food, which is considered rich in macro and microelements and different organic compounds of nutritional and medicinal properties. Dry matter content of fresh mushrooms is around 10 % and is mainly composed of carbohydrates, proteins, fibre and minerals. When considering the chemical composition of mushrooms, it is worthwhile to keep in mind that water content is the parameter that is to some degree, variable for "fresh" mushrooms. This is because changing weather conditions can to some degree influence the moisture content of collected mushrooms bodies. Their bodies, after collection, also lose moisture easily due to evaporation.

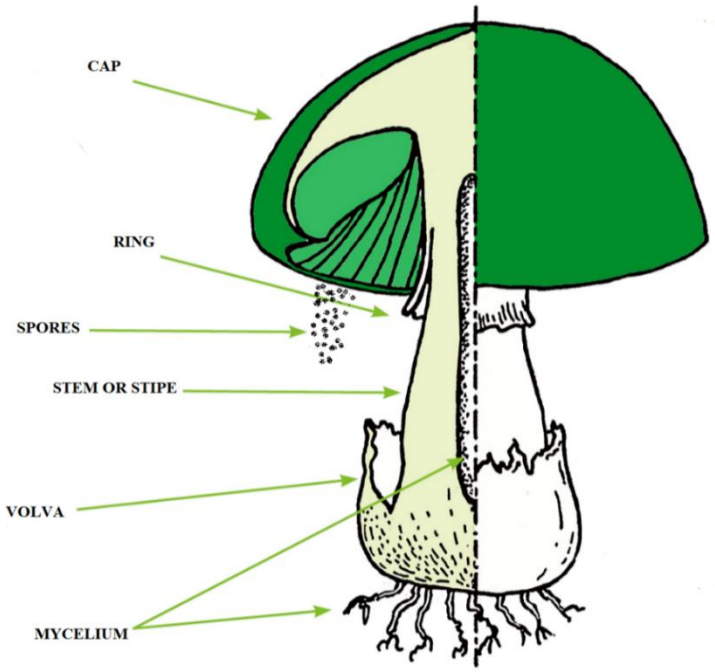


Figure 1. Morphology of mushrooms [9]

There is a consensus that the moisture content of fresh mushrooms bodies is 90 %, and data published on the chemical composition of mushrooms needs to be normalised on dry matter contents [8]. Mushrooms are valued as food for their high protein content, which is between 10 % and 30 % dry mass (d.m.) [10, 11] and high concentrations of carbohydrates. Crude fat (the lipid) content of mushrooms is usually low, between 1 % and 7 %. Often an average water content of 90 % is used for converting concentrations from dry to fresh mass or vice versa [12]. Mushrooms are valued by many people around the world, while accessibility to good foraging areas, gourmet recipes and tradition, and intake per capita highly vary in regions of the world [13]. Due to great diversity, many mushrooms are not studied yet, and vast proportions of mushrooms are without data on organic and inorganic As compounds. Most mushrooms are never eaten raw and this is the reason why it is important to investigate what is happening with mushrooms during the typical cooking processes. Nevertheless, this data are important because it is possible that during the cooking of mushrooms many main As constituents of mushrooms (inorganic As, DMA, TMAO, AB) can be changed.

1.3.1. Chantarelle

Chantarelle commonly known as golden chantarelle and egg mushrooms is a pleasantly aromatic fleshly wild mushrooms, shown on *Figure 2*. They are among the most popular of wild edible mushrooms and can be orange, yellow or white, meaty and funnel-shaped. Their false gills appear as wrinkles that are forked and wavy with blunt edges and run down the stem, the same color as the rest of the chanterelle. The ridges are rounded, blunt, shallow and widely spaced. At the edge of the cap they are forked and interconnected. The chantarelles' aroma is variously described as apricot or peach-like. Chantarelles are common in Europe, Asia, North and Central America and Africa. They tent to grow in clusters in mossy coniferous forests, but are also often found in mountainous birch forests and among grasses and low-growing herbs. In central Europe, the golden chanterelle mushrooms are often found in beech forests among similar species and forms. They can be as large as 12 cm in diameter, but 5 cm is closer to average [14].

Figure 2. Chantarelle [15]

1.3.2. Sarcosphaera coronaria

Sarcophaera coronaria or Sarcophaera crassa commonly known as the pink crown, the violet crown-cup, or the violet star cup is edible fungus according to some authors and once thought to be a good edible but today is not recommended for consumption, after several reports of poisonings causing stomach aches, and in one instance, death. According to some authors the fruit body of Sarcosphaera coronaria are known to bioaccumulate the toxic states that it may concentrate arsenic from the soil in the form of the compound methylarsonic acid. Although less toxic than arsenic trioxide, it is still relatively dangerous [16]. Concentrations higher than 1000 mg/kg dry weight are often reached. In a 2017 Turkish study of 23 wild edible mushrooms species, S. coronaria had the highest concentration of arsenic of 8.8 mg/kg dry weight, while the arsenic concentration of the other tested mushrooms ranged from 0.003 mg/kg to 0.54 mg/kg [17]. Sarcophaera is a cup fungus with largely underground development. Only at maternity the ascocarp, a hollow ball-like structure, breaks through the soil surface and opens. Fruiting body is between 2.0 - 10.0 cm broad, at first hypogeous, then partially emergent, with or without a short base; ascocarp compressed-globose, hollow, opening by a pore, then appearing urn-shaped, at maturity the margin sometimes coarsely

toothed. Outer surface is whitish, typically dingy from adhering soil, inner surface (hymenium) pale-lavender to pinkish-brown. Spores are around 15-20 x 7.5x10 µm, elliptical to oblong, smooth and thin-walled. Sarcosphaera coronaria is shown on *Figure 3*. It is commonly found in the mountains in coniferous woods under humus on the forest floor, and often appears after the show melts in late spring and early summer. It can be found in the Western United States and Europe. This kind of mushrooms are known accumulators of arsenic, where up to 2000 mg/kg dry weight were found [18]. A number of poisoning attributed to this species have been reported from Europe, including one fatal poisoning in the Switzerland, in Jura Mountains area, 1920, following which a warning was issued not to eat it raw or in salads because some people died after they ate this mushrooms like salad [19]. The reason for their death is still unknown.

Figure 3. Sarcosphaera coronaria [20].

1.3.3. Arsenic in mushrooms

Arsenic is an element toxic to humans, and its occurrence in food, water, and so on is strictly regulated and should be controlled and monitored including edible mushrooms. Mushrooms can accumulate various chemical elements and arsenic is an example of a toxic chemical element that is even hyperaccumulated by certain mushrooms [21]. Intake of arsenic species by human occurs

mainly through food digestion, as an outcome of biotransformation and bioaccumulation processes in biota. When we take into account all kinds of food, wild grown mushrooms are, besides of seafood, one of the important sources for arsenic dietary intake [2]. Mushrooms constitute an important food item and the consumption of edible mushrooms has increased considerably worldwide in recent years due to their nutritional properties [22]. The proportions and forms of arsenic varied massively different mushrooms species, and most mushrooms have a higher proportion of organic arsenic and lower inorganic arsenic. Although the total arsenic content in fresh mushrooms is relative high, it does not represent a high risk of dietary exposure [2]. Arsenic can be highly accumulated in mushrooms, with levels commonly reaching tens but rarely also thousands of mg/kg dry weight [21]. The terrestrial mushrooms, which are in symbiosis with plants as well as the saprobes, all take up arsenic from the substrate, where mycelia live. The valued boletus mushrooms (Boletus edulis Bull., Boletus pinophilus Pilat & Dermek, Boletus reticulatus Schaeff.) are rich in selenium but usually also in mercury [13] [23] [24]. However, in wild mushrooms, traces or micro-amounts of chemical elements, such as arsenic, mercury, lead, nickel, chromium and cadmium, are often detected because of the bioconcentration from the natural environment such as soil and water. Differences in their toxicity, biochemical and environmental behaviours require determination of the arsenic species. According to the literature, the most prevalent As compounds were inorganic arsenicals, dimethylarsinic acid, methylaronic acid and arsenobetaine, but the proportions of As compounds varied between species as well as between the batches that grew in compost with different fructification levels [21].

1.4. Arsenic transformation during heat treatment

Before consumption, mushrooms are usually cooked in different ways, such as boiling, griddling, steaming, microwaving, breading and frying but scarce information is available about the changes in nutritional quality after culinary treatments. The ways of a food preparation and cooking in the household have an obvious impact on arsenic content in the prepared foods [2, 25]. According to some studies, the bioavailability of arsenic in edible mushrooms (Lentinula edodes, Agaricus bisporus and Pleurotus ostreatus) was assessed by different cooking process (boiling and griddling). It was demonstrated that the total arsenic content of the mentioned mushrooms is reduced by 53 %, 60 % and 71 % respectively after boiling with double distilled water for 10 minutes. In addition, the effect of artificial gastric and intestinal solutions on the extraction of total arsenic in the same species of mushrooms to simulate arsenic dissolving in the human body was investigated. The results showed that about 74.9 % of the total arsenic were dissolved in gastric solution in various raw, cooked and grilled mushrooms and 86 % to 97 % of the total arsenic were dissolved in the process of digestion with gastric solution and intestinal solution, indicating that the bioavailability of arsenic was very high [26]. In terrestrial systems a variety of arsenic compounds is found in higher fungi (mushrooms). Arsenobetaine (AB), a nontoxic arsenic compound, is the species commonly detected in most studies. Authors have suggested a relationship between taxonomy and arsenic compounds present, the more primitive fungi having predominately more inorganic and fewer methylated species. Marine animals and plants and to a lesser extent mushrooms are an important source of arsenic in the human diet. Although ingestion involves no toxic effects because of the predominance of AB, food treatment might partly decompose AB to more toxic compounds [27, 28]. The effect of cooking temperature on chemical changes in species of organic arsenic was investigated on seafood products. The following transformations were observed: AB into TMAO and TETRA, DMA into MA, and MA into As(III) and As(V). Transformation of AB into TMAO at temperatures of 150 °C or above and the transformation of AB into TETRA at temperatures of 160 °C or above follow first–order kinetics [27, 29]. The problem is transformation of AB into TETRA because TETRA is the most highly methylated species of arsenic, being more toxic than MA and DMA [30]. Also important is that in previously study [30] the presence of TETRA was not observed in any of the raw seafood samples, but the analysis of arsenic species present in the cooked products showed the presence of TETRA in all of the samples. The TETRA contents ranged from 0.38 to 1.28 μg/g dw, representing 4.6 %

(90 °C) to 8.8 % (120 °C) of the total arsenic in the cooked product [30]. With the knowledge that AB is also present in mushrooms, it is likely that TETRA will be produced in the mushrooms processing process as well and this is a good topic for future researches. Many researches of mushrooms was carried out and their thermal treatments. According to all these researches, it was revealed that thermal treatments and the cooking technique clearly influence the nutritional value and the antioxidant activity of mushrooms. Also, some treatment produced more severe losses in protein, ash, and carbohydrates content but increased the fat and energy. Information about transformation of the arsenic species do not exist [31]. Cooking and bioavailability of inorganic arsenic are aspects that must be considered in order to obtain a more reliable estimate of potential human health risks associated with arsenic in food [32].

1.5. Kinetic of arsenic transformation

For the temperature range from 85 °C to 120 °C, transformations of arsenic species were not observed under any of the working conditions described. It is to be expected, therefore, that at the temperatures normally employed in cooking or processing, such as boiling or sterilizing, the arsenic species would remain unaltered. At higher temperatures (150 – 180 °C), AB was transformed generating TMAO and/or TETRA, and the percentage of transformation increased with temperature. Significant transformations at temperatures > 150 °C are only present in AB arsenic speciation and according that, the kinetic study is concentrate on this specie. It's assumed that the thermal transformation takes place as a result of two competing reactions, with AB being the initial substrate for both: a process of decarboxylation generating TETRA or cleavage of the As-CH_2 bond with subsequent oxidation giving rise to TMAO. The process of degradation of AB would be as follows:

$$\mathrm{AB} \xrightarrow{k_1} \mathrm{TMAO} \quad (1)$$

$$\mathrm{AB} \xrightarrow{k_2} \mathrm{TETRA} \quad (2)$$

The kinetic equation in this case would be given by

$$ln([AB]/[AB]_0) = -(k_1 + k_2)t \quad (3)$$

where $[AB]$ is the concentration of AB at time *t*, $[AB]_0$ is the initial concentration of AB, k_1 is the kinetic rate constant for a transformation of AB into TMAO, and k_2 is kinetic rate constant for a transformation of AB into TETRA.

Previous study [30, 31] have found that AB standards are transformed only in TMAO and TETRA, and this is the reason why the concentration of AB at time is obtained from the difference between the initial concentration of AB and the concentration of TMAO and TETRA formed at time *t*.

The concentration of the products, TMAO and TETRA, with time can be expressed by equations:

$$[TMAO] = (k_1[AB]_0/k_1 + k_2)\left(1 - e^{-(k_1+k_2)t}\right) \quad (4)$$

$$[TETRA] = (k_2[AB]_0/k_1 + k_2)\left(1 - e^{-(k_1+k_2)t}\right) \quad (5)$$

By division of these equations, a relationship between the two individual constants in the process can be obtained:

$$[TMAO]/[TETRA] = k_1/k_2 \quad (6)$$

where $[TMAO]$ is the concentration of TMAO formed from AB at a given time and $[TETRA]$ is the concentration of TETRA formed from AB at given time.

From these equations, it is possible to calculate the values of each of the kinetic constants. Previous results show that, at all of the temperatures studied, the transformation of AB into TMAO takes place more rapidly than the transformation of AB into TETRA. At temperatures of 190 °C the difference in the formation of the two products is not as significant as at lower temperatures, at which the difference between the two constants is of an order of magnitude [29].

For each temperature, time, and initial concentration of AB, the values of the constants obtained experimentally make it possible to establish both the concentration of AB not transformed (eq1) and the concentration of TMAO (eq2) and TETRA (eq3) generated.

The activation energy of the reactions is given by the following equation:

$$k = Ae^{-E_a/RT} \quad (7)$$

where A (pre-exponential factor) and E_a (Arrhenius activation energy) are constants characteristic of each reaction and R is the gas constant (8.3145 $Jmol^{-1}K^{-1}$). By applying logarithms, we have

$$\ln k = \ln A - E_a/RT \quad (8)$$

By plotting ln k against $1/T$ for each arsenic species we can obtain the activation energy values for two AB transformation reactions: E_{a1}(TMAO) and E_{a1} (TETRA) [29].

1.6. Methods for arsenic speciation analysis in mushrooms

There are several examples for arsenic speciation analysis by combining analytical techniques to ensure effective separation of species with appropriate detection and quantification. Such related techniques provide a high degree of automation, good reproducibility, and offer application in a variety of areas. The most commonly used ones are inductively coupled plasma - mass spectrometry (ICPMS), inductively coupled plasma - optical emission spectroscopy (ICPOES), atomic absorption spectroscopy (AAS, with or without graphite furnace (GF) or hydride generation (HG)), atomic fluorescence spectroscopy (AFS) and neutron activation analysis (NAA) [33]. The combination of high performance liquid chromatography (HPLC) and inductively coupled plasma mass spectrometry (HPLC-ICPMS) is often used. A large number of studies use a strong anion-exchange column (SAX) and $NH_4H_2PO_4$, NH_4NO_3 or $NaHCO_3$ as a mobile phase. Liquid chromatography coupled to electrospray ionisation (ES) mass spectrometry (HPLC-ESMS) or tandem mass spectrometry (HPLC-ESMS/MS) is suitable for the determination of arsenic compounds in samples containing more complex compounds than inorganic arsenic compounds, but they are not good enough to be suitable for small molecules such as arsenic, arsenite and their methylated compounds. HPLC-ICPMS technique is the most powerful analytical technique for determining arsenic with detection limits of 0.05 μg/l [34]. The advantages of HPLC-ICPMS technique are high elemental selectivity, ability to capture real-time chromatography, ability to separate interference signal from a given point, high linear range. However, the use of ICPMS as a HPLC detector leads to some limitations in the selection of chromatographic conditions relating to the nature and concentration of the mobile phase with respect to the salt concentrations and the presence of organic solvents. Due to its high sensitivity, ICPMS may suffer from polyatomic interference caused by plasma or ion extraction devices. For example, the presence of chlorine in the sample may result in the formation of $^{40}Ar^{35}Cl^+$ which strongly disrupts the monoisotopic $^{75}As^+$ [35].

1.6.1. ICPMS

Inductively coupled plasma mass spectrometry (ICPMS) was developed as a commercial analytical technique in the early 1980s and since then it has been applied to the determination of trace, minor and major elements in almost every analytical field. An ICPMS instrument consists of several distinct parts: sample introduction, ion generation in the ICP, plasma/vacuum interface, ion focusing and ion separation and measurement. The basic instrument components of an ICPMS are shown on *Figure 4* [36-40].

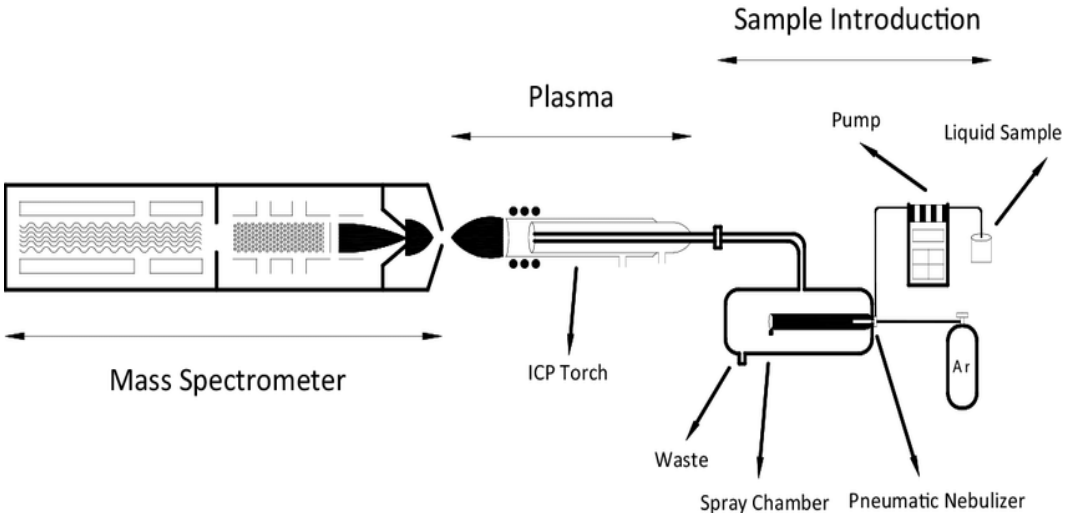


Figure 4. Basic instrument components of an ICPMS [36]

The liquid sample is typically introduced into the ICP by the autosampler needle and gets pumped into simple pneumatic nebulizer. When the liquid sample passes through the nebulizer an aerosol if formed. Lager aerosol droplets (larger than 10 μm) are removed from the gas stream in the spray chamber into the waste, and the remaining smaller droplets are swept into the central channel of the argon plasma, called torch [37-40].

The spray chamber temperature is maintained with a thermoelectric device to prevent signal drift caused by large changes in room temperature and also to reduce solvent loading on the plasma. This reduced solvent loading leads to a higher plasma temperature, reducing oxide interferences, and assisting in matrix decomposition. The sample aerosol is passed into the plasma, which is generated in a stream of argon contained in a quartz tubes or torch. The torch is located in the centre of a cooled cooper coil, through which a high power, high frequency electric current is passed. The torch consists of three quartz tubes, one of them is for the carrier gas, which brings the sample

aerosol from the nebulizer into the plasma. One is the plasma gas which constitutes the plasma and the third gas is the auxiliary gas. In the case of this research, CO2 with Ar was introduced between spray chamber and torch because of carbon enhancement effect of elements (especially of arsenic). The intense magnetic fields created by the electric current causes collisions between free electrons and Ar atoms, producing ions and more electrons, until a stable, high temperature plasma of Ar^+ ions is formed. The high frequency current is produced by a radio frequency (RF) generator operating at power up to 1600 W. Because of the very high temperature of the plasma (up to 10,000 K maximum and around 7,500 K in the central channel) and the fact that most element have a lower first ionization potential than argon (15.75 eV), the introduced aerosol droplets are rapidly dried, decomposed, evaporated, atomized and the ionized by the removal of one electron from each atom. The resulting ions, which are formed within about 10 ms of the original aerosol droplet entering the back of the plasma, are present at the highest level at about 7 mm from the end of the load coil, which is where the spectrometer interface is positioned. The positively charged ions that are produced in the plasma are extracted into the vacuum system, via a pair of interface „cones". The cones are essentially metal plates with central orifices through which the ion pass. Small orifices are used, typically 1 mm diameter or less, to maintain the high vacuum in the mass spectrometer region. Electrostatic lenses keep the ions focused in a compact „ion beam" as they pass through the vacuum system to the final chamber, where the mass spectrometer and detector are housed. The ion lenses perform a second, essential, function of separating the ions from the photons and residual neutral material [37-40].

After the ion lenses the collision or reaction cell is installed. It is used to remove interferences from the ion beam. A collision gas can be any non-reactive gas such as He and it simply collides with the ions (interferences and analyte ions) and reduces their kinetic energy. Due to the larger size of the polyatomic interferences, collisions are more likely to occur and the interferences loose more kinetic energy than the analyte ions. A positive potential after the collision/reaction cell hinders the polyatomic interferences to enter the quadrupole. Collision gas mode also plays an important role when analysing arsenic in the presence of chloride. To avoid the $^{40}Ar^{35}O^+$ interference at m/z 75 He was used as collision gas. Three different types of mass spectrometers have been used with the inductively coupled plasma as ion source: quadrupole, magnetic sector, and time-of-flight mass analysers. By far the most common mass analyser used in ICPMS is the quadrupole, which was also used during this work. The quadrupole is made up by 4 rods that are arranged in a square and it used a combination of DC (direct current) and AC (alternating current) electrical fields to

separate ions based on their mass to charge ratio (*m/z*). Since the plasma produces almost exclusively single-charged ions, the mass/charge ration is equal to the mass of the ion, making the spectrum very simple to interpret. The ratio of the DC and AC electrical fields is fixed but the voltages can be changed. For a given voltage setting, only one *m/z* is stable and the quadrupole scans rapidly across the mass range, passing each mass of interest sequentially to the electron multiplier detector. The electron multiplier detects each ion as it passes the quadrupole. The detector electronics count and store the total signal for each mass (*m/z*), creating a mass spectrum. The spectrum that is produced provides a simple and accurate qualitative representation of the sample. The magnitude of each peak is directly proportional to the concentration of an element in a sample; quantitative results are produced by comparing signal intensities to those generated by calibration standards [37-40].

ICPMS allows the determination of almost all elements of the elements of the periodic table. A wide linear range, excellent limits of detections (as low as < 1 ng/l) and isotopic information, make this method great for application for many different analytical questions [37-40].

1.6.1.1. Spectral interferences in ICPMS

Although the ICPMS is known to be a powerful multi-element analytical technique, it suffers from some spectral and non-spectral interferences. The most common source of spectral interferences are direct overlap from a different element with an isotope at the same nominal mass – known as an isobaric interference (e.g. ^{114}Sn overlaps with ^{114}Cd, ^{204}Pb and ^{204}Hg). Further, polyatomic ions, which give rise to non-analyte peaks in the mass spectrum, are one of the main sources of spectral interferences in ICPMS. Consequently, plasma conditions can have a major impact on the occurrence of polyatomic ions in the mass spectrum. Polyatomic interferences are result from the combinations of two isotopes from different elements, which usually occur in the plasma, and the elements that form the polyatomic interferences usually results from the sample matrix, sample diluent, and argon itself. A common polyatomic interference is for example $^{40}Ar^{35}Cl^{+}$ and $^{75}As^{+}$, both of them occur at *m/z* 75. The $ArCl^{+}$ forms a combination of Ar from the plasma gas and Cl from the sample matrix or diluent. This is important because in this thesis biological samples are analysed. It is known that biological samples can accumulate a lot of chlorine, and the above described interference may appear. Without chloride in the samples this polyatomic interference

will not form, and the arsenic signal is not influenced by this interference. Generally, if a high plasma temperature is maintained, most potential polyatomic interferences will be reduced, often to levels where, in practice, they become negligible. The level of polyatomic interferences can be monitored using the production of refractory oxide ions of specific elements. Cerium is an element commonly used for this purpose as it forms a strong oxide bond and therefore has one of the highest oxide formation rates. The M-O decomposition efficiency is typically expressed as the $\%MO^+$, relative to the parent M^+ ion, the $^{140}Ce^{16}O^+/^{156}Ce^+$ ratio. Most ICPMS systems operate at $^{140}Ce^{16}O^+/^{140}Ce^+$ ratios of 2-3 %, whereas a well-designed ICPMS can achieve a $^{140}Ce^{16}O^+/^{140}Ce^+$ratio of 0.3-0.5 % – about 5-10 times lower. This translates into 5-10x lower levels for many interferences, such as those based on matrices containing chloride and sulphate [37].

1.6.1.2. Matrix interference in ICPMS

When analysing carbon containing samples with ICPMS the carbon enhancement effect might occur; especially for elements P, As, Se and Te an enhancement which can reach between 150-600 % depending on the instrumental settings can be observed. The effect is therefore linked to the presence of carbon in the matrix [41]. Until now, no clear explanation is available why this happens in the plasma. By adding CO_2, a continuously high carbon background is created which compensates for possible carbon concentration differences in the samples. Additionally, one get a ~5x better sensitivity. Matrix effects induced by the presence of carbon consist of a signal enhancement might be caused by (a) charge transfer reaction from C^+ species to analyte atoms, (b) improvement in the nebulisation of the sample, and (c) shift of the zone of maximum ion density in the plasma. Mechanism (a) operates for hard-to-ionize elements like As and Se having ionization potentials (9.82 and 9.75 eV, respectively) lower than carbon (11.36 eV).

1.6.2. High performance liquid chromatography [42]

High performance liquid chromatography is a common technique for the separation of different chemical components in a mixture, for identification and quantification each component and also

applicable in many industries. The main parts of an HPLC system are shown on *Figure 5.*

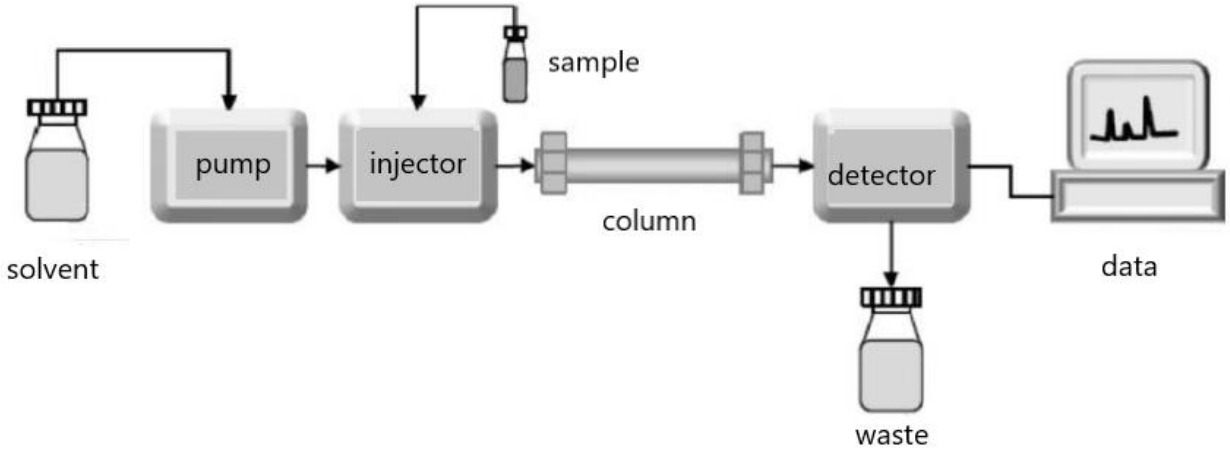


Figure 5. Scheme of an HPLC system [43]

The solvent is the mobile phase and the sample must be dissolvable in it. The mobile phase is the liquid that flows through the chromatography system and moving the sample through the HPLC system. There are many different liquids to choose for the mobile phase, depending on the analytes to be separated. These different liquids can interact with the analytes and have effect on the retention time. A pump is needed to transport the mobile phase through the column. The pump moves the solvent continuously from the flask through the column to the detector and finally it ends up in the waste can. To measure samples, an injection system is needed to inject the samples into the mobile phase. When the samples reaches to the column, it leaves the mobile phase and enters the chromatographic column which is filled with the stationary phase. The analytes are now interacting with the stationary phase and leave the column at different times depending on the properties of the molecules. The identification and quantification of the analytes happens after the column with the detector, often by comparing the retention times with known standards. The detector sends the signals to the software on the computer which computes the electric signal into a chromatogram.

1.6.1.2. Ion-exchange chromatography

Ion chromatography is the analytical method which was first introduces by Small, Stevens and Baumann. Ion chromatography has evolved rapidly in a modern technique for determining a many

numbers of cations and anions [44]. The chromatography of ions as practiced today is a result of the merging of two major areas of development, chromatography and ion-exchange. Ion-exchange chromatography is utilized for the separation and analysis of small ionic molecules such as inorganic anions and cations, for analysis of ionic organic molecules such as organic acids and amino acids, for analysis of some neutral molecules such as carbohydrates and alcohol, as well as for analysis of polymeric ionic molecules such as proteins and nucleic acids [45]. Separation in ion-exchange chromatography is based on reversible adsorption of charged solute molecules to immobilized functional groups of opposite charge. Ion-exchange chromatography is further subdivided into cation-exchange and anion-exchange chromatography. Their stationary phases are classified as strong or weak, depending on how much the ionization state of the functional groups vary with pH.

Anion-exchange chromatography is practiced with either a strong or a weak anion-exchange column, containing a quaternary ammonium ion, or with a weak anion-exchanger, having either a tertiary or secondary amine functional group, such as DEAE (diethylaminoethyl). Ionic compounds in the mobile phase, a counter ion, often Cl^-, maintains electro-neutrality and often are interacting with the ions on the stationarity phase and form ionic bonds of different strength. In anion-exchange chromatography columns have a positively charged stationarity phase and used to separate anionic compounds and the pH of the mobile phase buffer must be between pK_a of the charged molecule and the pK_a of the charged groups on the solid support.

Cation-exchange chromatography is practiced with either a strong or a weak cation-exchange column, containing a sulfonium ion, or with a weak cation-exchanger, having usually a carboxymethyl (CM) functional group. A counter ion, often Na^+, maintains electro-neutrality. For cation-exchange chromatography it is used a negatively charged stationary phase to separate cations in the mobile phase [27].

1.6.3. HPLC-ICPMS [42, 46]

To know which forms of an element are present, they must be separated and detected individually, which is accomplished most easily by separating the species with high performance liquid chromatography (HPLC) and detecting them with inductively coupled plasma mass spectrometry

(ICPMS). The coupling of a chromatographic unit with an inductively coupled plasma mass spectrometer has clear advantages in measuring analytes across the periodic table of elements. This combined method of analysis preserves crucial information about the chemical form. In conventional ICPMS analysis, harsh sample preparation and harsh conditions inside the plasma result in the loss of all information on the elemental species, binding sites, oxidation states and only quantified total results of the measured elements are obtained. Yet, there are countless examples where the chemical form of an element is the analytically interesting trait because the form largely governs key attributes - for instance, bioavability and toxicity. A common example is the highly toxic inorganic arsenic species As(III) which disrupts biochemical processes due to its affinity to thiol groups, compared to the not so readily bioavailable organic arsenic species commonly found in plant or marine species. Coupling an HPLC to an ICPMS allows the chromatographic separation prior to the ICP and thus allowing time-resolved detection of the different analyte species. This approach can provide further information on the chemical nature of the elements in a sample while retaining the ability to detect most elements and/or certain isotopes in one analytical run. This methodology has low detection limits in a wide concentration range of up to 10 orders of magnitude (sub ng/l to mg/l range) and multi-element capacity. Also, without the installed column, this can be used for flow injection to reduce sample consumption.

1.7. Sample preparation for ICPMS

Many analytical instruments, such as inductively coupled plasma - mass spectrometry require that a sample be converted into a liquid for introduction into the analyser. Some samples will readily dissolve in water or in inorganic solvent, but some samples are stubborn. Using a combination of strong chemicals, high temperature and high pressure any biological sample can be dissolved. During the heating of the organic material in chemical reagent (nitric acid) with the use of microwave energy high temperatures (typically 250 °C are employed) and pressures of up to 110 bars are obtained. In this way the organic matrix is destroyed and converted into CO_2 and H_2O. Where a reagent is used, its nature will depend on that of the matrix. The amount of reagent used is dictated by sample size which, in turn, depends on the sensitivity of the method of determination. The majority of wet digestion methods involves the use of some combination of oxidizing acid (hot conc. HNO_3, hot conc. $HClO_4$ and H_2SO_4), non-oxidizing acid (HCl, HF, H_3PO_4, dilute H_2SO_4,

and dilute $HClO_4$), and hydrogen peroxide. Wet digestion has the advantage of being effective on both inorganic and organic materials. It often destroys or removes the sample matrix, thus helping to reduce or eliminate some types of interference. Very important in this respect is the nature of the material. The suitability of materials may be estimated according to the following criteria: heat resistance to acids and alkalis, surface properties, reactivity and contamination, whereby the specific characteristics of the organic and inorganic must also be given special consideration. Nitric acid is an almost universal digestion reagent and the most widely used primary oxidant for the decomposition of organic matter, because it does not interfere with most determinations and it is available commercially in sufficient purity. During the last few years, methods of wet sample preparation using closed vessels have become widely applied. Closed systems offer the advantage that the operation is essentially isolated from the laboratory atmosphere, thereby minimizing contamination and loses. These techniques are generally much more efficient than conventional wet digestion in open system, the loss of volatile elements is avoided any contribution to blank values may be reduced and the digestion of more difficult samples is possible. Closed system digestion is particularly suitable for trace and ultratrace analysis, especially when the amount of sample is limited [46]. In this thesis we used pure concentrated and subboiled HNO_3 in a closed system under elevated temperatures and pressure to digest the samples.

1.8. Freeze-drying process

Freeze-drying process is the removal of ice or other frozen solvents from a material through the process of sublimation and the removal of bound water molecules through the process of desorption. Lyophilisation and freeze-drying are terms that are used interchangeably on the industry. Controlled freeze-drying keeps the product temperature low enough during the process to avoid changes in the dried product appearance and characteristic. It is an excellent method for preserving a wide variety of heat-sensitive materials such as protein, microbes, pharmaceuticals, tissues and plasma. The key building block to gaining knowledge of freeze-drying is thoroughly understanding the concept of sublimation. Sublimation is when a solid (ice) changes directly to a vapour without first going through a liquid (water) phase. Low pressures are required for sublimation to take place as we can see on the phase diagram for water showed on *Figure 6.*

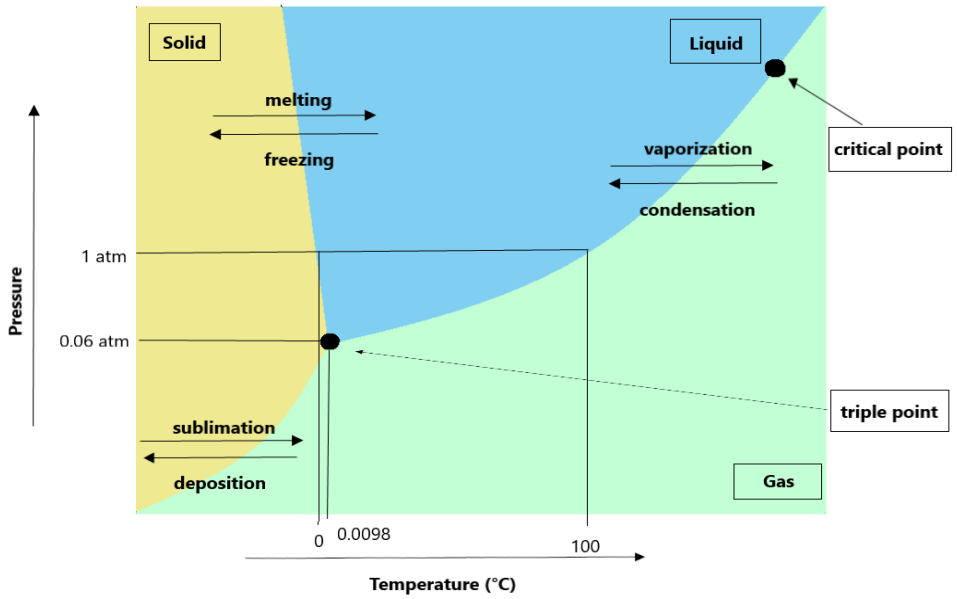


Figure 6. The phase diagram for water

Sublimation is a phase change and heat energy must be added to the frozen product for it to occur. Sublimation in the freeze-drying process can be described simply in three steps:

1. Freeze – the product is completely frozen, usually in a vial, flask or tray. It is extremely important that the sample be fully and completely frozen prior to pulling a vacuum and starting the drying process.
2. Vacuum – the product is then placed under a deep vacuum, well below the triple point of water
3. Dry – causing the ice to sublime [47].

2. EXPERIMENTAL PART

2.1. Materials

2.1.1. Chemicals

2.1.1.1. Single-element ICP standard solutions

Information about the single-element ICP standard solutions and the concentrations of prepared solutions are shown in *Table 2.* Prepared solutions were used for preparing calibration solutions.

Table 2. Single-element ICP standard solutions

Element	Concentration	Starting Reagent	Purity (%)	Matrix
Ag	1000 mg/L ± 0,2 %	$AgNO_3$	99.999	2 % HNO_3
Al	1000 mg/L	Al	99.995	2 % HNO_3
As	1000 mg/L	As_2O_3	99.995	-
Ba	1000 mg/L	$Ba(NO_3)_2$	99.999	1 % HNO_3
B	1000 mg/L ± 0,2 %	H_3BO_3	99.999	H_2O
Bi	1000 mg/L	Bi	99.999	3 % HNO_3
Cd	1000 mg/L ± 0,2 %	Cd	99.999	2 % HNO_3
Cl	1000 mg/L ± 0,2 %	NaCl	99.999	H_2O
Co	1000 mg/L	$Co(NO_3)_2$	99.999	2 % HNO_3
Cr	1000 mg/L ± 0,2 %	$Cr(NO_3)_2$	99.999	2 % HNO_3
Cu	1000 mg/L ± 0,2 %	Cu	99.99	2 % HNO_3
Fe	1000 mg/L	Fe	99.99	10 % HNO_3
Hg	1000 mg/L	Hg	99.999	2 % HNO_3
Gd	1000 mg/L ± 0,2 %	Gd_2O_3	99.999	2 % HNO_3
Li	1000 mg/L ± 0,2 %	Li_2CO_3	99.999	2 % HNO_3
Mn	1000 mg/L	Mn	99.95	2 % HNO_3
Mo	1000 mg/L	$(NH_4)_2MoO_4$	99.999	4 % NH_3
Ni	1000 mg/L ± 0,2 %	Ni	99.998	2 % HNO_3
Pb	1000 mg/L	$Pb(NO_3)_2$	99.999	-
Rb	1000 mg/L ± 0,2 %	$RbNO_3$	99.999	2 % HNO_3
Sc	1000 mg/L ± 0,2 %	Sc_2O_3	99.99	2 % HNO_3

Element	Concentration	Starting Reagent	Purity (%)	Matrix
Sn	1000 mg/L ± 0,2 %	Sn	99.999	10 % HCl
Sr	1000 mg/L ± 0,2 %	$Sr(NO_3)_2$	99.999	2 % HNO_3
Sb	1000 mg/L ± 0,2 %	Sb	99.999	20 % HCl
Te	1000 mg/L ± 0,2 %	Te	99.999	2 % HNO_3
Tl	1000 mg/L ± 0,2 %	$TlNO_3$	99.999	2 % HNO_3
U	1000 mg/L ± 0,3 %	U	99.99	2 % HNO_3
V	1000 mg/L	V_2O_5	99.99	2 % HNO_3
Zn	1000 mg/L	Zn	99.999	2 % HNO_3
Ca	10 000 mg/L	$CaCO_3$	99.994	2 % HNO_3
Mg	10 000 mg/L	$Mg(NO_3)_2$	99.999	2 % HNO_3
Na	10 000 mg/L	$NaCO_3$	99.999	2 % HNO_3
K	10 000 mg/L ± 0,2 %	KNO_3	99.999	2 % HNO_3
P	10 000 mg/L	$NH_4H_2PO_4$	99.999	H_2O
S	10 000 mg/L	$(NH_4)_2\ SO_4$	99.999	H_2O

The above chemicals, single-element ICP standard solutions are from company Carl Roth GmBH + Co. KG, Karlsruhe, Germany, except single-element standard of uranium which is from Peak Performance, Single Element Standard, Analytical Science and Technology company from Santa Rosa, USA.

2.1.1.2. Solutions of arsenic species

Quantification and identification of the arsenic species were achieved via external calibration (0.05 – 100 µg As^{-1} for each compound). The calibration solutions were prepared in 15 ml polypropylene tubes (PP-tubes) with ultrapure water and aliquots of standard solutions of the different arsenic species. These standard solutions (1000 mg As L^{-1} each) were prepared as follows. As(V) was prepared from $Na_2HAsO_4{\cdot}7H_2O$, purchased from Merck KGaA (Darmstadt, Germany). Methylarsonic acid (MA) was synthesized from $NaAsO_2$ (purchased from Merck) and Mel (Meyers reaction). DMA was prepared from sodium dimethylarsinate (Fluka, Buchs, Switzerland). MA(III) was prepared by dissolving diiodomethylarsine in water with 5% *v/v* methanol. AB, TMAO, AC and TETRA were synthesized according to literature [48-51].

2.1.1.3. Other used chemicals

- HNO_3 (nitric acid) – 65 % p.a., Carl Roth GmBH + Co. KG, Karlsruhe, Germany, further purified via sub-boiling (1 x subboiled)
- HCl (hydrochloric acid) – 32 % p.a., VWR Chemicals, Austria (2 x subboiled)
- Ultrapure (MilliQ) water (an ultrapure laboratory grade water that has been filtered and purified by reverse osmosis), resistance: 18.2 MΩ cm
- H_2SO_4 (sulfuric acid) – 99,9 %, Sigma Aldrich, Germany
- H_2O_2 (hydrogene peroxide), Rotipuran® 30 % p.a., stabilized, Carl Roth GmBH + Co. KG., Germany
- Pyridin, ≥ 99.5 %, pro analysis, EMD Millipore Corporation, USA
- Ammonia solution: Rotipuran®,≥ 25 %, p.a., Carl Roth GmBH + Co. KG., Germany
- Tuning solution - prepared from single-element solutions concentration of 1 mg/L of each element; Li, Y, Tl, As, Fe, Se, Co, Ce, with 2 % HNO_3
- Certified standard reference materials (SRM):
  - Standard Reference Material 1640a, Trace elements in Natural water, National Institute of Standard and Technology, U. S. Department of Commerce, Gaithersburg
  - Standard Reference Material IPE 120, (arsenic species; AB, TMAO, TETRA, MA(III), DMA, MA(V), As(V)), Mushrooms/Agaricus bisporus, Wepal, Wageningen Evoluating Programs for Analytical Laboratories, The Netherlands
  - Standard Reference Material Tomato Leaves 1573a, National Institute of Standard and Technology, U.S. Department of Commerce, Gaithersburg
  - Standard Reference Material M144A and M144B (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Cd, Hg, U)

SRM are intended to check the accuracy of the results.

2.1.2. Foodstuffs for typical cooking processes

- Oil – 100 % Corn oil / S Budget Spar, Graz, Austria
- Vinegar - with 5 % acid / S Budget Spar, Graz, Austria

2.1.3. Sampling

2.1.3.1. Chantarelle

Fresh samples of Chantarelle mushrooms were bought in Billa Market, Austria, in July. Mushrooms were cleaned, weighed and stored in PP-tubes at 4 °C until analysis.

2.1.3.2. Sarcosphaera coronaria

Fresh samples of Sarcosphaera coronaria mushrooms were collected in Austria, in June in the mountainous area near Graz city. Mushrooms were cleaned, weighed and stored in PP-tubes at -20 °C until analysis.

2.2. Instruments

2.2.1. Freeze dryer

To remove the water from the samples through the process of sublimation freeze dryer Gamma 1-16 LSC instrument by Martin Christ Company is used. The instrument is shown on *Figure 7.*

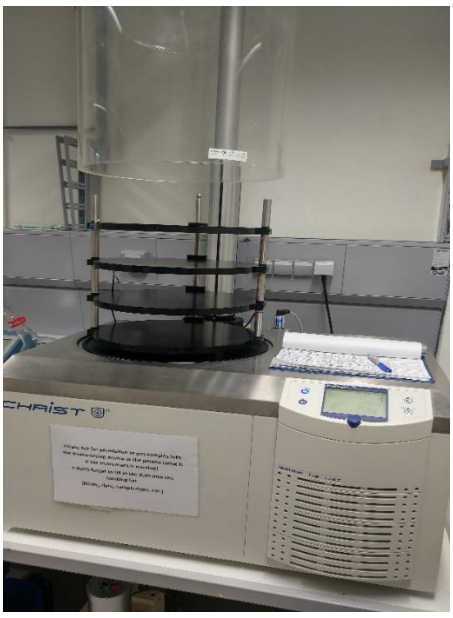


Figure 7. Freeze dryer Gamma 1-16 LSC

Features and specifications of Gamma 1-16 LSC freeze dryer:

- Number of shelves: 4
- Method: double-chamber
- Vacuum control
- Defrost function
- Time duration: 30 h

2.2.2. Mixer

Samples were homogenized with instrument Mixer Mill MM 400 showed on *Figure 8* purchased from Retsch Company. This is a ball mill where the grinding jars perform radial oscillations in a horizontal position. The inertia of the grinding balls causes them to impact with high energy on the sample material at the rounded ends of the grinding jars and pulverizes it. The degree of mixing can be increased even further by using several smaller balls.

Figure 8. Mixer Mill MM 400

Features of Mixer Mill MM 400:

- Material feed size: ≤ 8 mm
- No. of grinding stations: 2
- Grinding jar sizes: 5 ml
- Batch size / feed quantity: max. 2 x 20 ml
- Setting of vibrational frequency: 25 Hz (1500 min^{-1})
- Setting of grinding time: 30 s

2.2.3. High performance microwave reactor

Microwave assisted acid digestion is made with UltraCLAVE III high performance microwave reactor by MLS GmbH Mikrowellen-Labor-Systeme Company (Leutkirch, Germany) shown on *Figure 9.*

Figure 9. Ultra Clave III high performance microwave reactor

Features and specifications of Ultra Clave III high performance microwave reactor:

- Cross-contamination is non-existent and extremely high throughput can be achieved
- Reduced acid usage
- Highest sample amount (40 samples), highest sample throughput
- Same conditions for all samples
- High pressure stainless steel reactor with 3.5 liters TFM liner
- Cover lift mechanism: auto-lift and clamps controlled by electronic position sensors
- Vessel cooling: closed-loop cooling system
- Microwave power: fully programmable from 0 to 100 Watt at 2450 Hz frequency

- Microwave coupling: directly to pressure reactor
- Maximum temperature: over 300 °C depending on container material limits
- Maximum pressure: more than 200 bar
- Power: 230 V/50 Hz; 2,4 kW
- Operating software: Via external WindowsTM-based computer through dedicated EasyCLAVE 5.25 software

2.2.4. Ultrasonic bath

The ultrasonic bath has many applications in chemistry and biotechnology. Ultrasonic is effective in improving the reaction (higher yields, better quality) but also has an influence on the duration of the chemical reaction. In this research ultrasound bath, showed on *Figure 10* is used to extract arsenic and other metals from fungi material.

Figure 10. Ultrasonic bath Elma 70/H

This ultrasonic bath has the capability to regulate temperatures from room temperature to temperature of 90 ºC.

2.2.5. Centrifuge

For centrifugation, an instrument Rotina 420 R, Hettich Lab Techology (Tuttlingen, Germany) was used *(Figure 11).*

Figure 11. The Rotina 420 R centrifuge

Features and specifications of instrument:

- Max. capacity: in a swing-out rotor: 4 x 600 ml
  : in a angle rotor: 4 x 250 ml
- Max. speed (RPM): 15,000 min^{-1}
- Max. RCF: 24,400
- Dimensions: 423 x 506 x 650 mm / 423 x 713 x 654 mm
- Refrigeration: air cooling / infinitely variable from 20 °C to 40 °C

2.2.6. Inductively coupled plasma - mass spectrometry

For arsenic speciation analysis an inductively coupled plasma triple quadrupole mass spectrometer (8800 ICP-QQQ-MS), Agilent Technologies, Waldbronn, Germany which is shown on *Figure 12* was used. For total element analysis from the digested samples and the extracts a single quadrupole instrument ICPMS 7700x system from Agilent Technologies was used. These instruments are equipped with autosampler, a MicroMist nebulizer, Scott-type spray chamber and a collision/reaction cell.

Figure 12. ICP-QQQ coupled with HPLC

The difference between ICPMS 7700x and (ICP-QQQ-MS) is higher sensitivity of the ICP-QQQ-MS and lower backgrounds. The ICPMS MassHunter Workstation software version 4.5 was used to control both instruments and for the calculation of the results

To reduce interferences different measurement modes were applied for the mulita-element determinations. Some elements were measured in no gas mode, while others had to be analysed with helium collision gas. Selenium was the only element that was measured in reaction gas mode with hydrogen as reaction gas (*Table 3.).*

Table 3. Settings for ICPMS measurements of different elements (*m/z* of internal standards: Be= 9, Ge = 74, In = 115, Lu = 175)

Element	**Isotope** ***m/z***	**Tune mode**	**Internal standard**
Li	7	No gas	Be
B	11	No gas	Be
Na	23	He	Be
Mg	24	He	Be
Al	27	No gas	Be
P	31	He	Be
S	34	He	Be
K	39	He	Be

Element	Isotope *m/z*	Tune mode	Internal standard
V	51	He	Be
Ca	43	He	Ge
Cr	52	He	Ge
Mn	55	He	Ge
Fe	56	He	Ge
Co	59	He	Ge
Ni	60	He	Ge
Cu	65	He	Ge
Zn	66	He	Ge
As	75	He	Ge
Se	78	H_2	Ge
Rb	85	He	Ge
Sr	88	He	Ge
Mo	98	No gas	In
Ag	107	No gas	In
Cd	111	No gas	In
Sn	118	No gas	In
Sb	121	No gas	In
Te	125	No gas	In
Cs	133	No gas	In
Ba	137	No gas	Lu
Gd	157	No gas	Lu
Hg	201	No gas	Lu
Tl	205	No gas	Lu
Pb	208	No gas	Lu
Bi	209	No gas	Lu
U	238	No gas	Lu

2.2.7. High performance liquid chromatrography

In this research is used 1260 Infinity HPLC by Agilent Technologies, coupled with ICPMS 7700x, equipped with a degasser, a binary pump, thermostatted autosampler and thermostatted column compartment. An HPLC 1200 series from Agilent Technologies was coupled with the ICPMS/MS.

2.2.8. HPLC columns and vials

- Hamilton PRP-X100 column, dimensions 150 x 4,6 mm, particle size 5 µm
- Maisch ReproSil-XR 300 SCX, dimensions 150 x 4,6 mm, particle size 5µm
- Microvials 0.3 ml and 0-7 ml PP, with snap ring, 32 x 11 mm, 6 mm, transparent, Brucker Analysentechnik, Linz, Austria.
- HPLC-caps: 11 mm shutter, PE snap ring cap, red orange rubber/ TEF transparent, 60° shore A, 1.0 mm, Brucker Analysentechnik, Linz, Austria.

2.2.9. Other equipment

- Balances (Denver Instrument® SI-234 Summit Series Analytical Balance, 230 g x 0.1 mg, with Internal Calibration, Geottingen, Germany)
- Test tubes (Greiner bio-one, Germany, 15 mL and 50 mL, polypropylene, 17/120 mm, CELLSTAR®, blue screw cap, natural, graduated, writing area, conical bottom, blue screw cap, sterile)
- Pipettes tips (Greiner bio-one, Germany, tips of 1250 µl, PP, unfiled, blue, natural lid, non sterile and tips of 200 µL PP, unfiled, yellow, natural lid, non sterile)
- polyamide syringe filters (0.2 µm Chromafil® Xtra PA-20/13, Macherey-Nagel GmbH & Co. KG, Düren, Germany and 25 mm, Nylon 66, 0.22µm, BGB®, China.)
- Pipettes (Socorex Acura 825)
- Pan (material: $F_2C = CF_2$, polyethylenterephthalat, high temperature resistance)
- Laboratory electric cooker
- pH-meter: Orion 5 Star, Thermo Scientific (Cambridgeshire, United Kingdom)

- Syringes (Norm-Ject®, single use, sterile, 2 ml (3 ml), 5 ml (6 ml), Tuttlingen, Germany)

2.3. Methods of work

2.3.1. Sarcosphaera coronaria sample preparation

First of all, 5 mushrooms from all collected mushrooms of Sarcosphaera coronaria are subjected to total analysis determination and then from one big mushroom five subsamples were taken and analyzed directly. Those five samples are marked with blue colour on *Figure 13.* Afterwards, five parts from the same mushrooms were taken and cut each in three equal parts (green, yellow and red colours on *Figure 13*.) and exposed to different cooking process. From these 15 samples, five samples were fried on 15 ml of corn oil for 6 minutes. Oil was weighted before and after frying, so it is known how much oil is consumed during frying process for every sample. Five samples were boiled in 50 ml pure water from tap, and 5 samples were boiled in 50 ml water with 10 % vinegar. The boiling process lasted for 10 minutes for each samples and mass of water was weighted before and after boiling for every sample. Every mushroom was weighted before and after cooking process also. The samples colored in purple (*Figure 13.*) were used for total arsenic analysis in the extract.

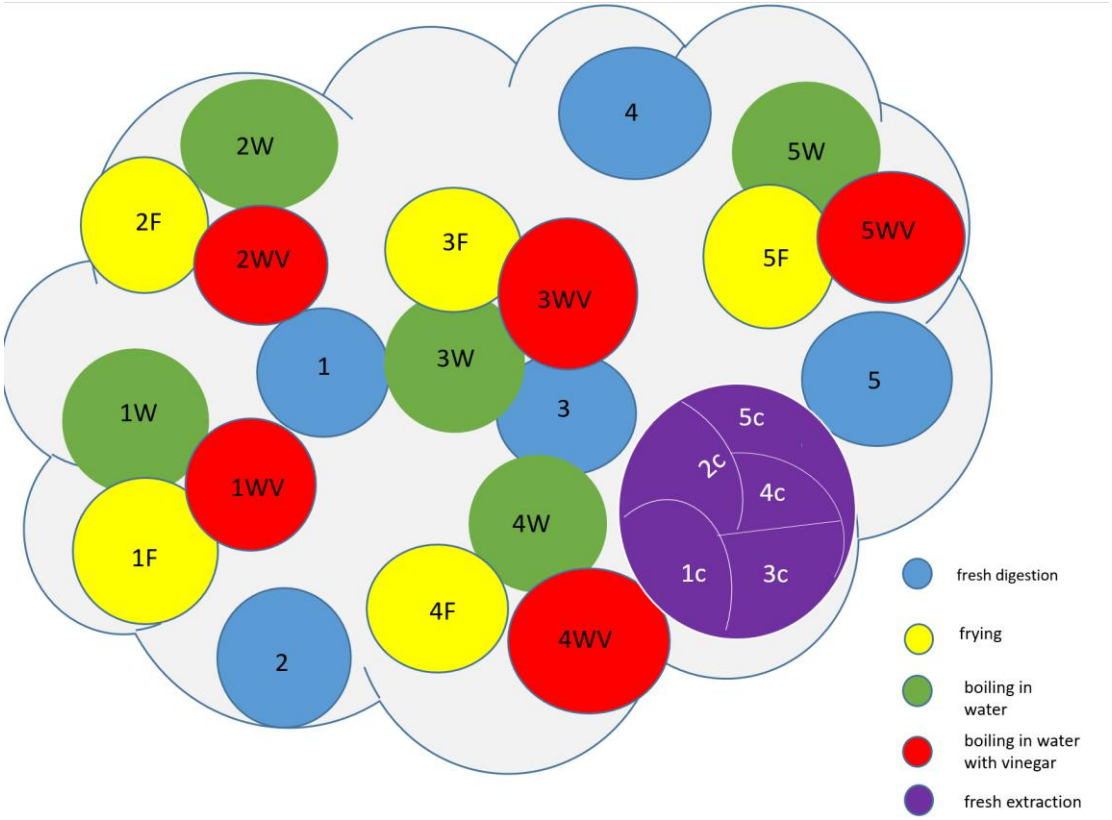


Figure 13. Sampling Sarcosphaera coronaria

All preparations were done on laboratory electric cooker and regular kitchen pan. Before analysis samples were homogenized with Mixer Mill MM 400 and stored at -20 °C until analysis. For homogenization with MM 400 was used liquid nitrogen gas because samples were elastic and it was hard for homogenization. Therefore, the samples were frozen in liquid nitrogen before the homogenization process.

2.3.2. Chantarelle sample preparation

Each fungus is cut into three equally parts, shown on *Figure 14.* One of these parts is exposed to boiling process for 15 min in tap water, one to frying on 15 mL of corn oil for 10 minutes and one part was analyzed fresh. Before analysis samples were homogenized with Mixer Mill MM 400, placed in plastic tubes and stored at -20 °C until analysis. All preparations were done on a

laboratory electric cooker and regular kitchen pan. For boiling process plastic tubes in glass beaker was used.

Figure 14. Sampling Chantarelle

2.3.3. Samples of water and oil used for cooking

The water and oil used for cooking of mushrooms were weighted before and after cooking and stored after cooking. Oil was not analyzed. Water was subjected to total trace elements determination and to arsenic species determination with anion- and cation-exchange chromatography. Samples for total trace elements determination were prepared by pipetting 4.5 mL water (filtered previously) and 0.5 mL HNO_3 in the plastic tubes. Samples for arsenic species determination were prepared by pipetting 0.5 mL H_2O in vials or with 0.1 mL H_2O and 10 µL H_2O_2 in vials.

2.3.4. Total trace elements determination – digestion process

For the total element determination of Ag, As, Bi, Cd, Ce, Co, Se, Cs, Gd, Li, Pb, Sn, Te, Tl, U, V, B, Ba, Cr, Ni, Al, Cu, Mn, Mo, Rb, Sr, Zn, Fe, Hg, Sb, Ca, Mg, Na, K, P and S, the digested samples were analyzed using ICPMS/MS. For digestion, crushed and homogenized samples of mushrooms were weighted with a mass between 0.1 – 0.6 g in quartz tubes that had been cleaned three times and digested with 5 ml HNO_3 in Ultra Clave III high performance microwave reactor with method developed for digestion vegetables and mushrooms samples. Quartz tubes were closed

with PTFE caps and put into the microwave. Absorbing solution consisted of 300 ml ultrapure H_2O and 3 ml H_2SO_4 was added to digestion bowl. After placing the sample racke in the microwave autoclave it was closed, filled with 40 bar argon and heating was started with the programme shown in *Table 4.* The samples were heated up to 250 °C and the operating power was 1000 W. After completion of the heating and cooling down to less than 80 °C, digested samples were poured in PP-tubes, quartz tubes were rinsed three times with ultrapure water and then digested samples were diluted with ultrapure water to 50.00 ml. The elements were quantified with inductively coupled plasma triple quadrupole mass spectrometry ICPMS/MS via external calibration.

Table 4. Process parameters for microwave digestion of mushrooms

	t (min)	**E (W)**	**T_1 (°C)**	**T_2 (°C)**	**p (bar)**
0	0	0	25	25	0
1	0 – 10	1000	80	60	140
2	10 – 30	1000	150	60	140
3	30 – 50	1000	250	60	140
4	50 -110	1000	250	60	140
5	110 – 130	cooling down			

For checking the accuracy of the analyses standard reference materials were prepared in the same way and subjected to the same procedure of digestion and analysis. Every digestion also included three digestion blanks. These digestion blanks consisted of the same amount of nitric acid as the samples, but without any sample.

2.3.5. Preparation of calibration solutions

For the total trace elements determination, calibration solutions were prepared in different ranges in PP-tubes of 10 ml according *Table 5*.

Table 5. Different ranges for calibration solutions – total analysis

Original standard solution:	
1 000 mg/L	Ag, As, Bi, Cd, Ce, Co, Se, Cs, Gd, Li, Pb, Sn, Te, Tl, U, V, B, Ba, Cr, Ni, Al, Cu, Mn, Mo, Rb, Sr, Zn, Fe, Hg, Sb
10 000 mg/L	Ca, Mg, Na, K, P, S

CALIBRATION 1:	**Calibration range**	**10 % HNO_3**
Group 1	10 ng/L - 10 µg/L	Bi, Gd, Mo, Sb, Te, Tl, U, Ag, B, Ba, Co, Li, Ni, Pb, Se, Sr, Sn, V
Group 2	100 ng/L - 100 µg/L	As, Cs, Mn, Cr
Group 3	1 µg/L - 1 mg/L	Al, Cu, Fe, Rb, Zn

CALIBRATION 2:		**10 % HNO_3, 2 % HCl**
Group 1	10 ng/L - 10 µg/L	Hg
Group 2	10 µg/L - 10 mg/L	Ca, Mg, Na, S, P
Group 3	100 µg/L – 100 mg/L	K

2.3.6. Total metal analysis in mushrooms extract

2.3.6.1. Sarcosphaera coronaria

For total metal analysis in Sarcosphaera coronaria, mushroom extract was prepared according the following procedure. Previously prepared mushroom samples were weighted (0.1 – 0.2 g) and extracted with 10 ml ultrapure water. Afterwards, they were sonicated in an ultrasonic bath for 15 min and centrifuged. After 15 min centrifugation and filtration through 0.2 µm polyamide syringe filters, 100 µl of extract was mixed with 9.9 ml H_2O. One ml of this dilution is added to 9 ml H_2O with 10 % HNO_3 acid and this solution was used for determination of metals in mushrooms extract.

2.3.6.2. Chantarelle

For total metal analysis in Chantarelle extract, samples of these mushrooms were weighed (around 1 g) into 15 ml polypropylene tubes and extracted with 3 ml of ultrapure water. Afterwards, they were sonicated in an ultrasonic bath for 15 min and then centrifuged. After 15 min of centrifugation and filtration through 0.2 µm polyamide syringe filters, 0.5 ml aliquot of each mushroom samples extract was taken and analysed by ICPMS. In every 0.5 ml extract 0.5 ml HNO_3 and 4 ml MilliQ water was added before analysis.

All samples were analysed using ICPMS. Based on the results obtained through this procedure and digestion process, the extraction efficiency (% *ee*) was calculated according to following with formula:

$$\%ee = \frac{c(X)_{EXTRACTION}}{c(X)_{DIGESTION}} \cdot 100 \qquad (9)$$

where *c(x)* is mass concentration (µg/kg) of each elements given by digestion/extraction.

2.3.7. Total arsenic species analysis

For the determination of the arsenic species in Chantarelle and Sarcosphaera coronaria different extract are subjected to HPLC coupled to ICPMS (same instrument as for a total determination). The analysis was done with cation-exchange and anion-exchange chromatography. The temperature of HPLC autosampler was always set on 4 °C. Arsenobetaine (AB), trimethylarsine oxide (TMAO), arsenocholine (AC) and the tetramethlyarsonium ion (TETRA) were performed by cation-exchange chromatography while dimethylarsinic acid (DMA), methylarsonic acid (MA), arsenate As(V) were separated via anion-exchange chromatography. The cation-exchange chromatographic column was a ReproSil-XR 300 SCX (150 x 4.6 mm, particle size 5 µm) purchased from Maisch company in Germany. The mobile phase was 10 mmol/L aqueous pyridine buffer pH 2.3 (*Table 6.*). This buffer was prepared by diluting 800 mg pyridine in 1 L ultrapure water and adjusted pH with formic acid to 2.3.

Table 6. Experimental conditions for the cation-exchange chromatography analysis

COLUMN	ReproSil-XR 300 SCX (150x4.6 mm, particle size 5 µm)
MOBILE PHASE	10 mmol/L aqueous pyridine buffer pH 2.3
FLOW RATE	1.3 ml/min
INJECTION VOLUME	20 µl
TEMPERTATURE	30 °C
PRESSURE	cca 130 bar

Anion-exchange chromatography was carried out with Hamilton PRP-X100 column with the following dimensions 150 x 4,6 mm, particle size 5 µm. The mobile phase was a 20 mmol/L aqueous ammonium phosphate pH 6.0. It was prepared by diluting 2.31 g orthophosphoric acid in 1 L ultrapure water. The pH was adjusted with aqueous NH_3 to 6.0.

An isocratic elution was performed, the column temperature was maintained at 30 °C, the flow rate was set to 1.3 mL/min. Under these conditions we observed a backpressure around 130 bar (*Table 7.*).

Table 7. Experimental conditions for the anion-exchange chromatography

COLUMN	Hamilton PRP-X100 (150x4.6 mm, particle size 5 µm)
MOBILE PHASE	20 mmol/L aqueous ammonium phosphate pH 6.0
FLOW RATE	1.3 ml/min
INJECTION VOLUME	20 µl
TEMPERTATURE	30 °C
PRESSURE	cca 130 bar

Every sample extract of Chantarelle and Sarcosphaera Coronaria was prepared with and without 10 % hydrogen peroxide. Samples without hydrogen peroxide were prepared with only 0.5 ml extract in HPLC-vials. For the samples with hydrogen peroxide, we took 100 µl extract and added 10 µl H_2O_2 to the same vial. These samples were then heated at temperature 45 °C for one hour before analysis by HPLC-ICPMS. With this procedure trivalent- and thio-arsenicals are oxidized with H_2O_2 to their pentavalent and/or oxygenated forms. The samples of Sarcosphaera Coronaria were further 100-fold diluted because of their high arsenic concentrations in them.

For both samples (non-oxidised and oxidised samples) the anion-exchange and cation-exchange chromatographic conditions were identical.

For quality control, the certified reference materials were prepared in the same way as the samples, with and without 10 % hydrogen peroxide.

For the determination of the arsenic species in samples Sarcosphaera Coronaria the same column for anion-and cation-exchange chromatography was used like previously, but the ICPMS/MS was used as element selective detector. Arsenic was measured in oxygen reaction mode at the transition *m/z* 75 – 91.

2.2.8. Preparation of calibration solutions for anion- and cation-exchange chromatography

For the determination of arsenic species by ion chromatography, calibration solutions were prepared in different concentration ranges in tubes of 10 ml according to *Table 8*.

Table 8. Calibration solutions for anion and cation-exchange chromatography analysis

Original standard solution:		
1 000 mg/L		AB, DMA, MA, As(V), TETRA, TMAO, AC
Calibration ranges:		
Anion-exchange	0.05 – 100 µg/l	AB, DMA, MA, As(V)
Cation-exchange	0.05 – 100 µg/l	AB, TETRA, TMAO, AC

For checking the accuracy of the anion- and cation-exchange chromatography results IPE 120 Mushrooms/Agaricus bisporus was used. Therefore, an aliquot of this reference material was weighed (around 0.15 g) and dissolved in 3 ml H_2O. Extraction and analysis was the same like as previously described in total metal analysis from mushrooms extract.

3. RESULTS AND DISCUSSION

3.1. Performance of ICPMS instruments

Before every measurement the performance (sensitivity and interferences of the ICPMS instruments was checked. Average performance data for all measurements done in this research are shown in *Table 9.*

Table 9. Performance of instrument ICPMS 7700x (sensitivity and interferences), obtained for a tuning solution at a concentration of 1 µg/L elements (Li, Y, Tl, Co, Se).

Tune mood	**Pressure (Pa)**	**Li (Kcps)**	**Y (Kcps)**	**Tl (Kcps)**		**Oxide ratio**	**Doubly charged ratio**	**RSD %**
		***m/z* = 7**	***m/z*=89**	***m/z*=205**		**156/140**	**70/140**	
No gas	2.02E-04	145	122	80.8		1.2	1.2	2.4
		Co (Kcps)	**Y (Kcps)**	**Tl (Kcps)**				
He	3.27E-04	53.2	36.6	85.9		0.49	1.8	2.6
		Co (Kcps)	**Y (Kcps)**	**Tl (Kcps)**	**Se (Kcps)**			
H_2	2.59E-03	41.0	24.3	18.4	1.13	0.80	1.7	2.8

3.2. Quality control

Quality control of the measurements for total element analysis samples after digestion process was checked with the certified standard reference material (SRM) Tomato Leaves 1573a. This SRM is intended primarily for use in evaluating the reliability of an analytical method for the determination of major, minor, and trace elements in botanical material, agricultural food products and materials of similar matrix. The certified values were compared with the obtained values in *Table 10.* and are shown on *Figure 10.*

Table 10. Certified and noncertified values for SRM Tomato leaves in comparison with the results of total element analysis after digestion process

Element	Certified mass fraction (w_B) (mg/kg)	Mass obtained by measuring (mg/kg)	%
B	33.3 ± 0.7	25.7 ± 2.8	**77.1**
Na	136 ± 4	112 ±15.5	**82.0**
Al	598 ±12	411 ±27.9	**68.8**
P	2160 ± 0.05	2280 ± 298	**105.3**
K	27000 ± 0.05	26140 ±980	**96.8**
Ca	50500 ± 0.09	52100 ±6630	**103**
V	0.835 ± 0.010	0.63 ±0.040	**75.0**
Cr	1.99 ± 0.06	1.53 ±0.11	**76.6**
Mn	246 ± 8	204 ±10.8	**83.9**
Fe	368 ± 7	299 ±10.4	**81.2**
Co	0.57 ± 0.02	0.43 ±0.02	**75.7**
Ni	1.59 ± 0.07	1.09 ±1.30	**68.7**
Cu	4.70 ± 0.14	3.74 ±0.18	**79.5**
Zn	30.9 ± 0.7	26.3 ±2.4	**85.0**
As	0.112 ± 0.004	0.13 ±0.030	**117**
Se	0.054 ± 0.003	0.06 ±0.006	**113**
Rb	14.89 ± 0.27	12.6 ±0.45	**84.8**
Cd	1.52 ± 0.04	1.26 ±0.04	**82.8**
Sb	0.063 ± 0.006	0.03 ±0.076	**53.7**
Hg	0.034 ± 0.004	0.02 ±0.031	**68.9**

	Not certified mass fraction (w_B) (mg/kg)		
Ag	0.017	0.01 ±0.01	**85.8**
Sr	85	70.6 ±3.14	**83.0**
Mo	0.46	0.33 ±0.05	**71.1**
Cs	0.053	0.04 ±0.002	**79.0**
Ba	63	51.0 ±2.75	**80.9**
Gd	0.17	0.18 ±0.007	**107**
Mg	12000	10300 ±525	**86.1**
U	0.035	0.03 ±0.003	**72.8**
S	9600	10200 ±980	**106**

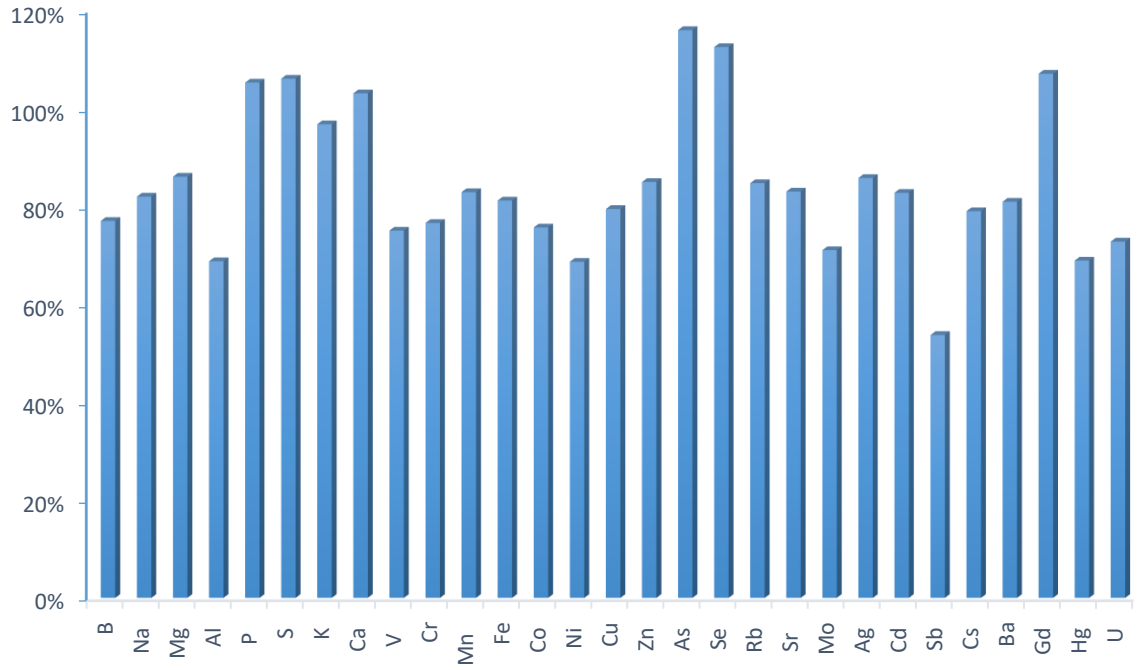


Figure 15. Trueness for each element expressed in %

Quality control of measurement for total element analysis in Sarcosphaera coronaria extract was checked using the certified water standard reference material M144A and M144B. The certified values of the constituent element were compared with the obtained values in *Table 11*.

Table 11. Certified concentrations for M144A (a) and M144B (b) in comparison with the concentrations obtained from the measurements (total element analysis from extract)

a) M144A

Element	**M144A certified concentration (µg/kg)**	**Determined concentration (µg/kg)**	**%**
Al	47.9	51.7 ± 2.4	**108**
Cr	1.9	2.0 ± 0.5	**105**
Mn	18	17 ± 2.2	**96.7**
Fe	141.6	134 ± 15	**94.4**
Ni	1.91	1.42 ± 0.8	**74.3**
Cu	2.39	2.12 ± 1.1	**88.7**
Zn	9.44	5.11 ± 3	**54.1**
As	1.9	1.8 ± 0.9	**94.7**
Se	4.1	3.4 ± 1.5	**82.9**
Cd	0.076	0.062 ± 0.05	**81.6**
Hg	1.4	0.5 ± 0.9	**38.6**
U	1.39	1.26 ± 0.5	**90.6**

b) M144B

Element	M144B certified concentrations (µg/kg)	Measured concentrations (µg/kg)	%
Al	25.2	27.5 ± 5.2	**109**
Cr	3.6	3.2 ± 2.2	**88.0**
Mn	30.0	28.5 ± 7.21	**95.0**
Fe	42.0	41.3 ± 4.4	**98.3**
Ni	3.2	3.0 ± 1.5	**93.8**
Cu	6.0	5.0 ± 3	**82.8**
Zn	30.3	23.0 ± 9	**75.9**
As	0.7	0.6 ± 0.2	**84.5**
Se	1.4	1.0 ± 0.8	**72.5**
Cd	1.1	1.0 ± 0.5	**93.6**
Hg	0.5	0.1 ± 0.9	**20.0**
U	3.2	2.9 ± 2.1	**90.6**

Quality control of measurement for total arsenic species analysis was checked with the certified standard reference material (SRM) IPE 120 Mushroom/Agaricus bisporus and NIST 1640a. The certified concentrations of the elements are compared with the measured concentrations in *Table 12.* a) anion-exchange chromatography and b) cation-exchange chromatography.

Table 12. Certified concentrations for NIST 1640a for a) anion-exchange chromatography and b) cation-exchange chromatography.

a) anion-exchange chromatography

NIST1640a	Unretained	MA(III)	DMA	MA(V)	As(V)
	0.0293	0.1867	0.1892	0.0657	7.8067
Obtained conc. (µg/g)	8.277				
Certified conc. (µg/g)	8.075				
	102.51%				

IPE 120	Unretained	MA (III)	DMA	MA(V)	As(V)
Obtained conc. (µg/g)	0.0768	-	0.0449	0.0015	0.0099
Certified conc. (µg/g)	0.0760	-	0.0470	0.0017	0.0330

IPE 120 ox.	Unretained	MA (III)	DMA	MA(V)	As(V)
Obtained conc. (µg/g)	0.0775	-	0.0484	0.0031	0.0151
Certified conc. (µg/g)	0.0760	-	0.0470	0.0017	0.0330

b) cation-exchange chromatography

NIST1640a	Unretained	AB	TMAO	AC	TETRA
	8.10	-	-	-	-
Obtained conc. (µg/g)			8.10		
Certified conc. (µg/g)			8.075		
			101%		

IPE 120	Unretained	AB	TMAO	AC	TETRA
Obtained conc. (µg/g)	0.0898	0.0616	0.0034	-	0.0028
Certified conc. (µg/g)	0.0970	0.0670	<0.0093		

IPE 120 ox.	Unretained	AB	TMAO	AC	TETRA
Obtained conc. (µg/g)	0.0859	0.0624	0.0034	-	0.0029
Certified conc. (µg/g)	0.0970	0.0670	<0.0093		

One more quality control parameter during ICPMS analysis was the measurement of internal standards (ISTD). In this work beryllium, germanium, indium and lutetium were added online via a t-piece to the sample to all sample solutions. Which internal standard was used for which elements is shown in *Table 3.* The stability of signals for the internal standards during the measurements is important because on this way we can check the stability of measurement system, correct for possible matrix effects and evaluate the quality of the results obtained. One example of ISTD stability graph during the analysis is shown on *Figure 16.*

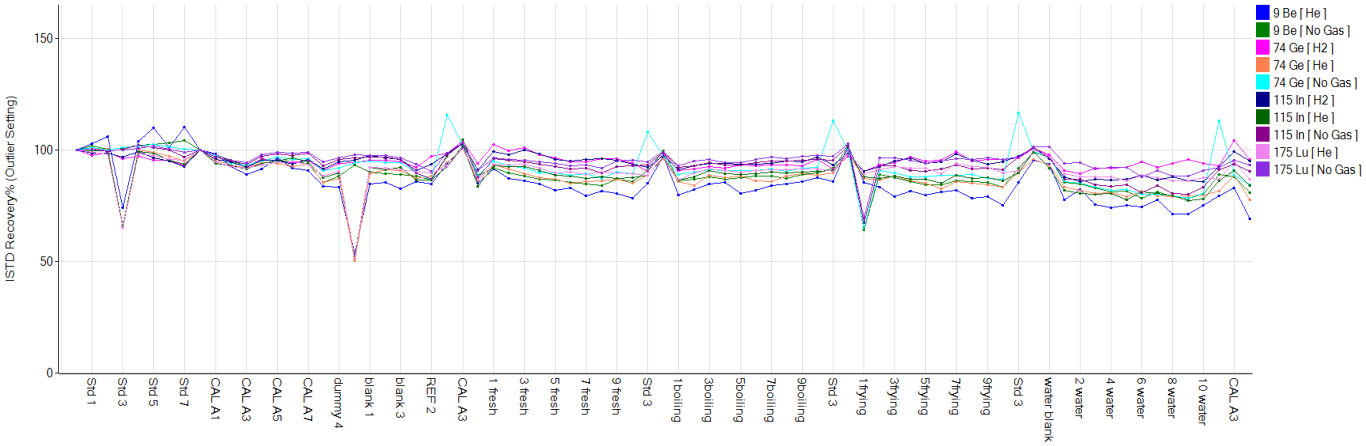


Figure 16. The example of ISTD stability graph

We also measured „drift standards“ in every batch (one calibration solution) after every 10 samples to check the stability of the measurements.

All of the mentioned quality control checks were done before every measurement series. According that, the quality of the measurement was monitored during this work.

3.3. Sarcosphaera Coronaria results

3.3.1. Total element analysis with digestion process

Digested samples are subjected to total elemental analysis with ICPMS that included analysis of 36 elements. Total concentration of elements given in µg/l by instrument, is expressed in µg/g, according the equation:

$$conc\left(\frac{\mu g}{g}\right) = [sample\ conc\left(\frac{\mu g}{l}\right) - blank\ conc.\left(\frac{\mu g}{l}\right)] * dilution\ coefficient \qquad (8)$$

where dilution coefficient (co. dil) is:

$$co.dil = \frac{volumen\ of\ dilution\ (ml)}{mass\ for\ digestion\ (g)} \qquad (9)$$

Further, concentration of each element per gram of fresh piece is calculated according the equation:

$$conc.\left(\frac{\mu g}{g}per\ fresh\ piece\right) = \frac{[mass\ sample\ after\ cooking\ process\ (g) \cdot sample\ conc\left(\frac{\mu g}{g}\right)]}{mass\ sample\ before\ cooking\ process\ (g)} \qquad (10)$$

For arsenic, concentrations in µg/g and concentrations in µg/g per fresh piece in Sarcosphaera coronaria samples are shown in *Table 13.*

Table 13. Arsenic concentrations in µg/g and in µg/g of fresh piece in fresh and fried samples, samples boiled in water and samples that are boiled in water with vinegar for Sarcosphaera coronaria samples, after digestion process.

Sample	conc. As (µg/g)	conc. As (µg/g fresh piece)
1 fresh	79.3	79.3
2	145	145
3	94.7	94.7
4	52.0	52.0
5	57.4	57.4
1 fried	214	71.1
2	238	61.1
3	325	72.8
4	147	56.3
5	155	66.2
1 boiled in water	8.50	6.20
2	14.5	10.1
3	10.5	6.51
4	14.9	9.19
5	16.7	10.2
1 boiled in water with vinegar	19.7	14.6
2	15.2	10.1
3	22.3	14.8
4	19.5	12.2
5	19.4	11.7

According to obtained results and the way how we took the samples shown previously on *Figure 13*, we can conclude that we have a inhomogeneous distribution of arsenic and other elements in the fruit body. Especially the results obtained from the different parts of the same mushroom (blue colour on *Figure 13*) differ significantly from adjacent parts (purple, sampling for extraction). This is a very interesting results and needs some further investigations in the future.

3.3.2. Arsenic species analysis

3.3.2.1. Extraction efficiency and column recovery

To check the accuracy of the results a comparison of the results obtained from anion- and cation-exchange chromatography were compared. Therefore, the total arsenic concentrations from the extracts were compared to the results from the different cooking procedures (see *Table 14.*).

The obtained results are compared with total element concentrations and the extraction efficiency was calculated according to equation:

$$Eff\ \% = \frac{conc\left(\frac{\mu g}{g}\right)_{extract}}{conc\left(\frac{\mu g}{g}\right)_{digestion}} \cdot 100 \qquad (11)$$

where concentration (µg/g) in extracts is calculated according to the equation (8) but dilution coefficient (co. dil) is:

$$co.dil = \frac{volumen\ of\ dilution\ (ml)}{mass\ for\ extraction\ ()} \qquad (12)$$

The extraction efficiencies are shown in *Table 15.*

Table 14. Arsenic concentrations in μg/g and in μg/g of fresh piece in fresh and cooked samples from Sarcosphaera coronaria

Sample	conc. As (μg/g)	conc. As (μg/g fresh piece)
1 fresh	86.7	86.7
2	90.3	90.3
3	91.7	91.7
4	92.6	92.6
5	91.1.	91.1
1 fried	218	72.7
2	236	60.4
3	349	78.4
4	151	57.9
5	154	66
1 boiled in water	6.86	5.01
2	12.9	8.93
3	9.19	5.69
4	13.3	8.18
5	14.6	8.91
1 boiled in water with vinegar	19.0	14.1
2	13.0	8.61
3	25.7	17.0
4	16.9	10.6
5	21.9	13.9

It should be noted that for calculating the extraction efficiency for fresh samples we took the median of all concentration values given from digestion process and divided concentrations obtained from extractions with this median value. The inhomogeneity of the elemental distribution and the fact that the fresh samples were taken from different areas is the reason for this strategy. In

other words, the five samples which we analysed from the extract are not from the same area of mushroom as the five samples which we used for total element determination (*Figure 16.).* For the others samples, fried, boiled in water and boiled in water with vinegar analysis from extract and total analysis after digestion are made from the same area of the mushroom. Results are similar and extraction efficiency is acceptable. You can see correlation between sample 1 of digested samples and sample 1 from extract, because these are from same area of the fruit body.

Also, for quality control the measurement of arsenic species by HPLC-ICPMS we calculated column recovery for cation and anion chromatography according to equation:

$$column\ recovery(\%) = \frac{sum\ conc.\left(\frac{\mu g}{g}\right)\ of\ anions/cations}{conc\left(\frac{\mu g}{g}\right) from\ extraction} \cdot 100 \qquad (13)$$

The obtained results are depicted in *Table 15.*

Table 15. Extraction efficiency and column recovery (%)

Sample	Eff %	Column recovery (%), anions	Column recovery (%), cations
1 fresh	109	104	90.5
2	114	104	89.8
3	116	102	87.0
4	117	106	88.5
5	115	104	88.9
1 fried	102	95.1	93.1
2	98.8	97.7	92.6
3	108	92.7	88.4
4	103	93.3	90.7
5	99.5	96.7	91.4
1 boiled in water	80.8	133	114
2	88.6	121	108
3	87.5	130	110
4	89.1	122	105
5	87.2	118	104
1 boiled in water with vinegar	96.1	105	96.4
2	85.5	124	108
3	115	92.8	81.9
4	86.8	116	102
5	119	98.6	85.4

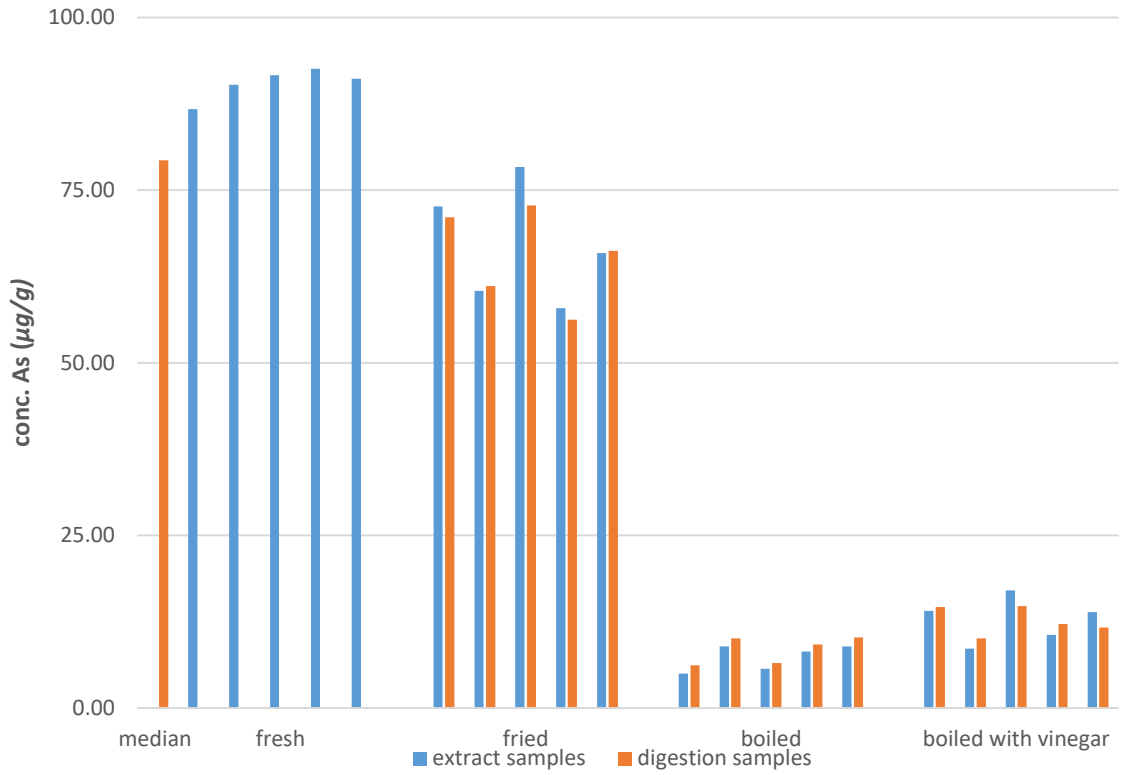


Figure 17. Comparison of As concentration in µg/g fresh piece given by total analysis from digested samples and total analysis from extract for fresh, fried, boiled in water and samples boiled in water with vinegar

We can see that after cooking process we have less arsenic in samples than in fresh samples. Through boiling we lost more arsenic than with frying process. This can be explained with the fact that the arsenic compounds in the fruitbody are small polar molecules and therefore much better extracted into the water than into the corn oil.

3.3.2.2. Anion-exchange chromatography

With anion-exchange chromatography we analysed three arsenic species: DMA, MA(V), As(V) and the unretained compounds indicated as AB. Calibration solutions for these compounds were in the range from 0.05 µg/l to 100 µg/l. A typical chromatogram at a concentration of 10 µg/l is shown on *Figure 18.*

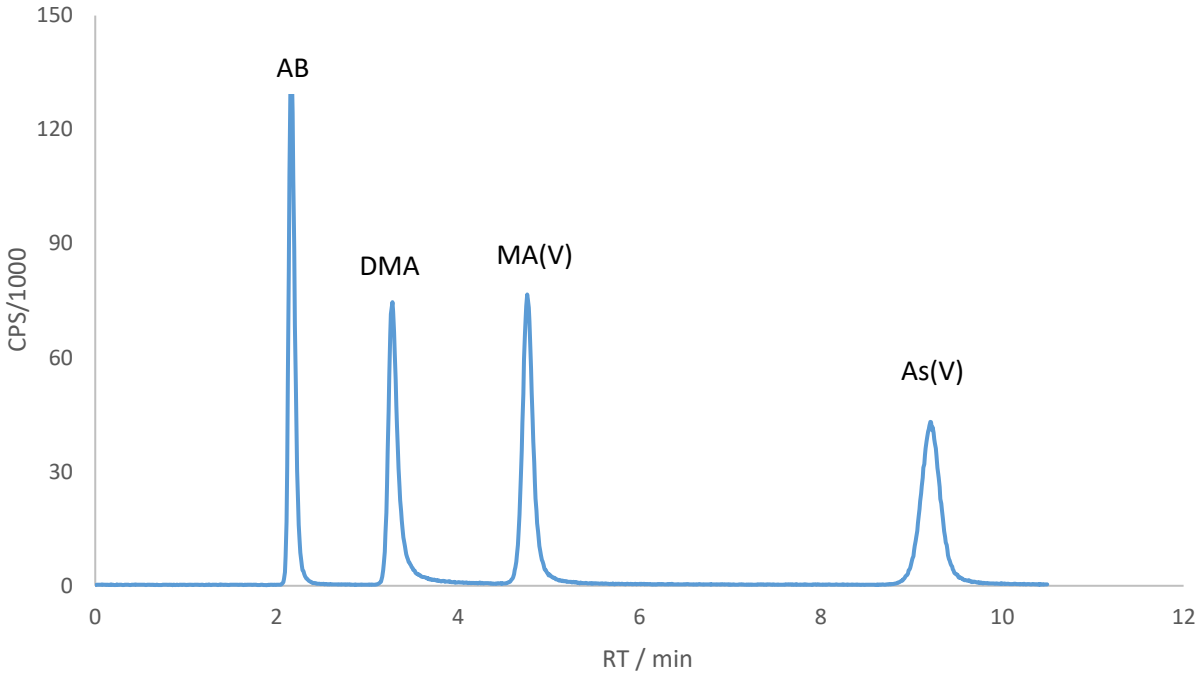
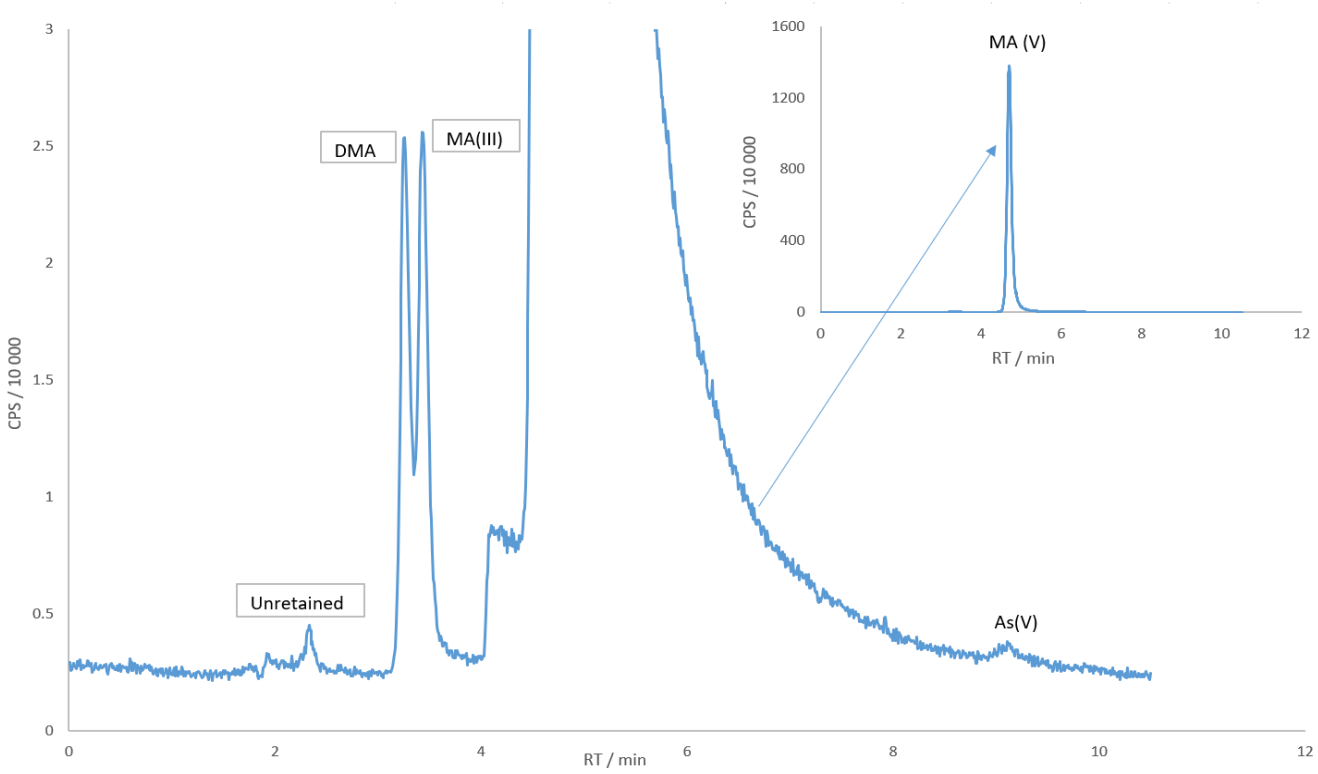
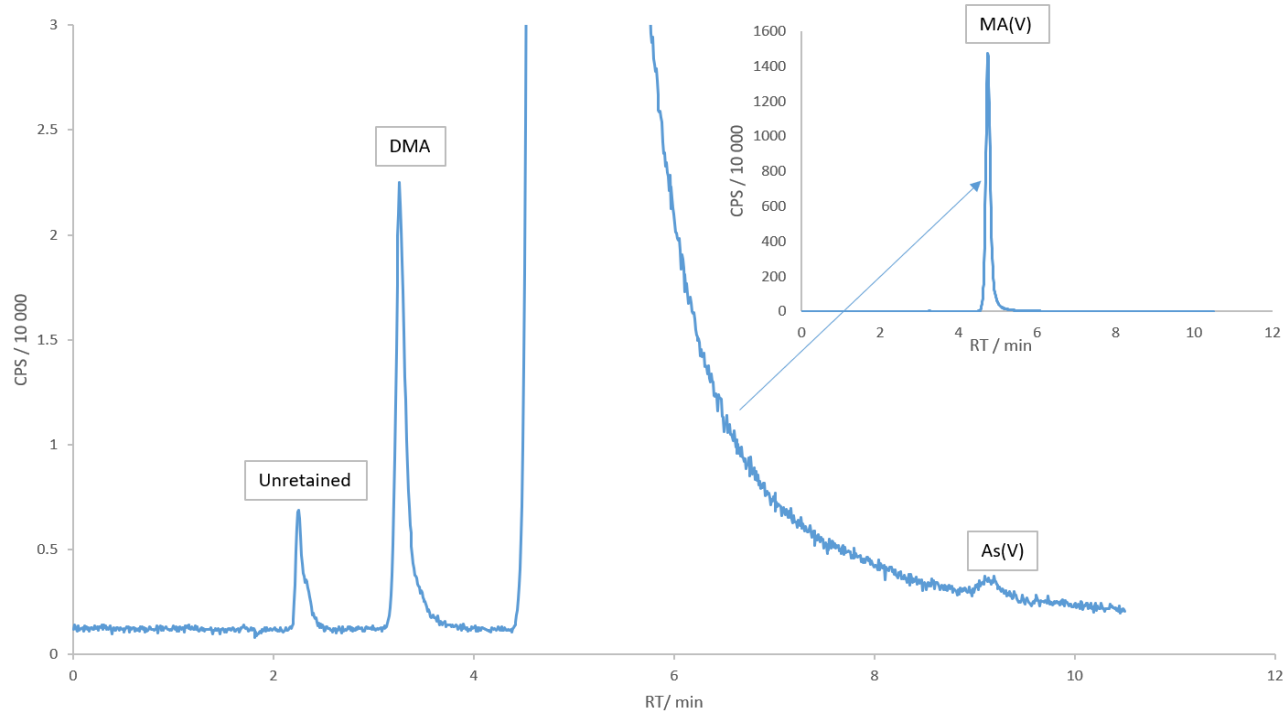


Figure 18. Chromatogram of compounds AB, DMA, MA (V) and As (V), calibration solution of 10 µg/l.

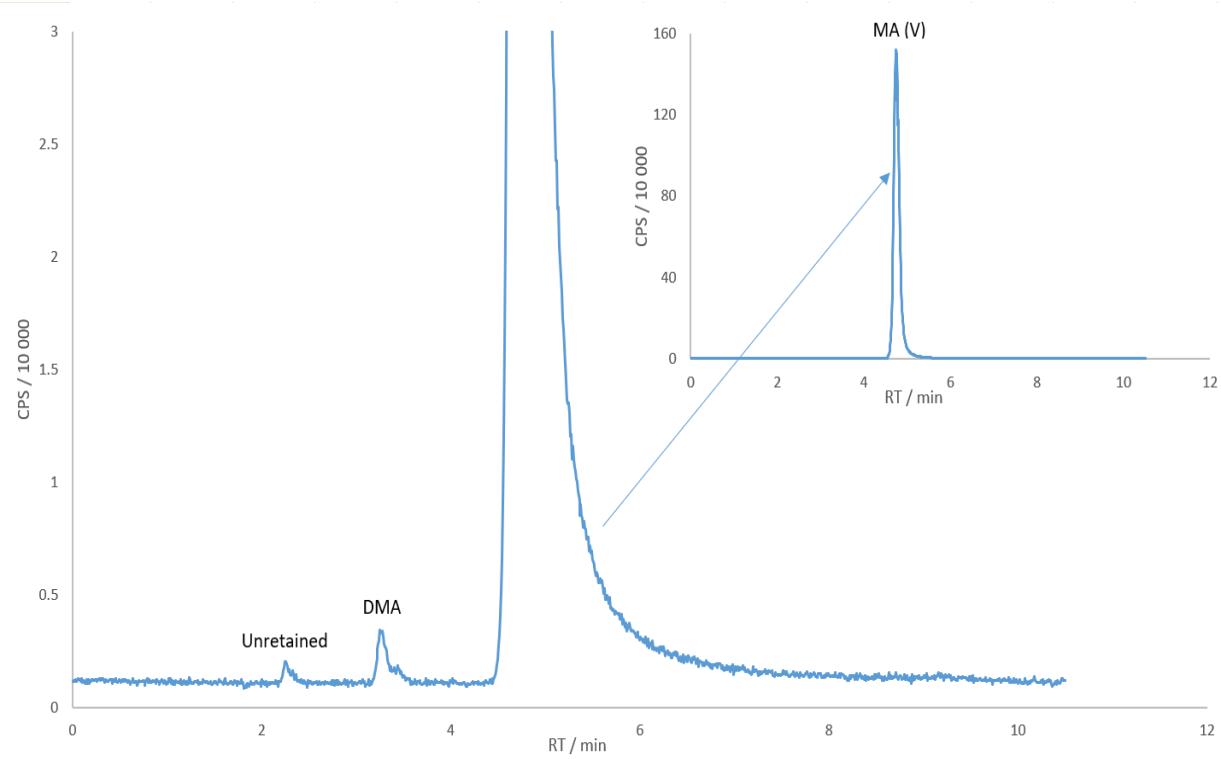
In the extracts of Sarcosphaera coronaria we detected DMA, MA(III), MA(V) and As(V), shown on *Figure 19.* To quantify MA(III) we used the calibration of DMA. Additionally, one unretained compound was detected but could not be confirmed as AB with cation-exchange chromatography. MA(III) was detected in fresh samples at a concentration between 0.13-0.14 µg/g. In fried samples and samples boiled in water MA(III) is not detected at all, but in samples which were boiled in water with 10 % vinegar we detected MA(III) in concentration between 0.018-0.031 µg/g. From these results we can conclude that the highly toxic MA(III) is very likely oxidized to the much less toxic pentavalent compound MA(V). In the presence of vinegar the oxidation was not as pronounced. Preparing this mushroom in vinegar without cooking might have some impact on its toxicity.


DMA
MA(III)
Unretained
As(V)
MA (V)
CPS / 10 000
RT / min

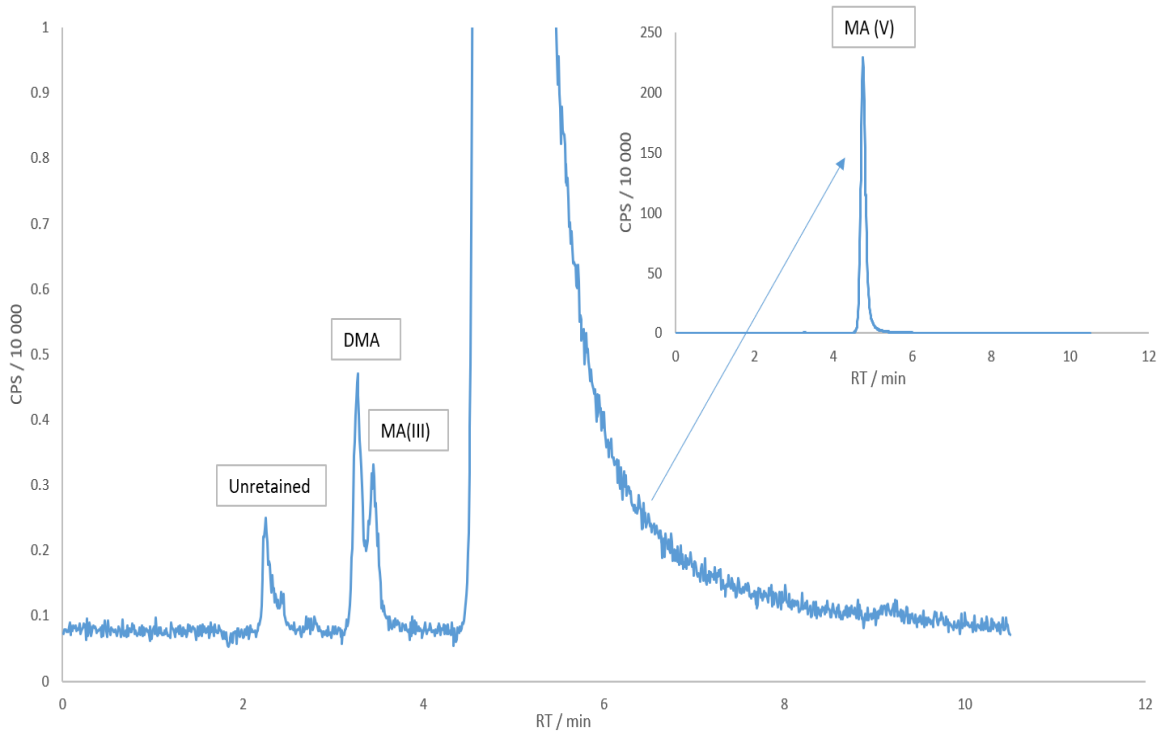
a)


DMA
Unretained
MA(V)
As(V)
CPS / 10 000
RT/ min

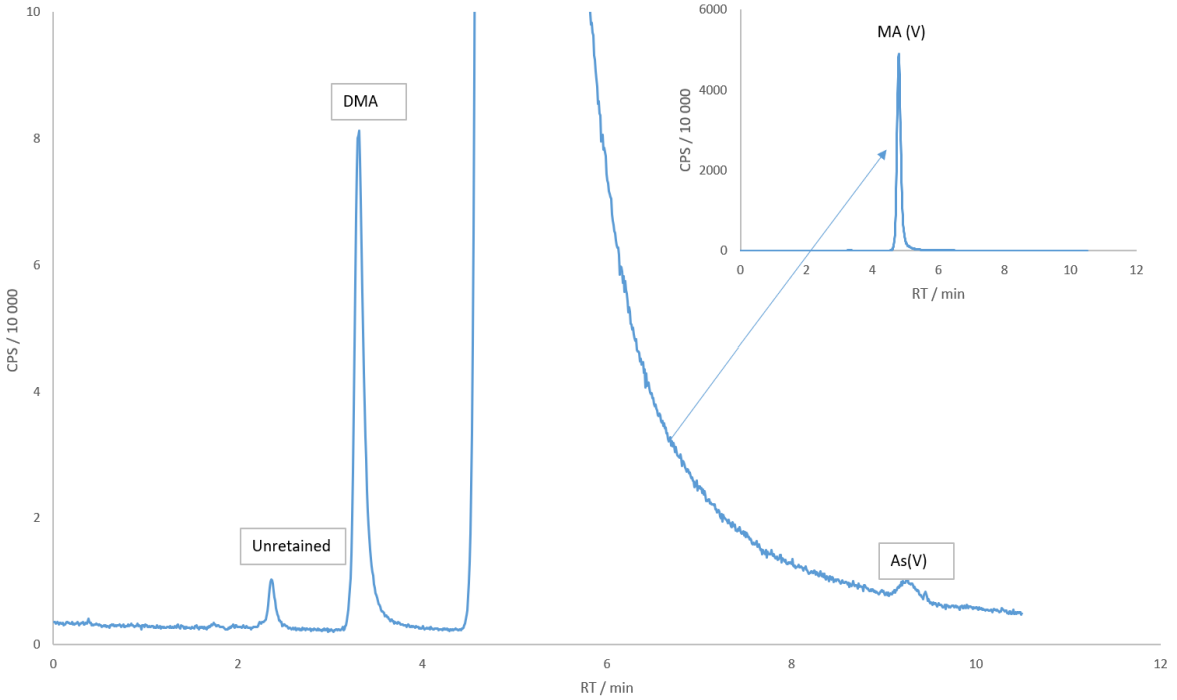
b)


CPS / 10 000
RT / min
Unretained
DMA
MA (V)

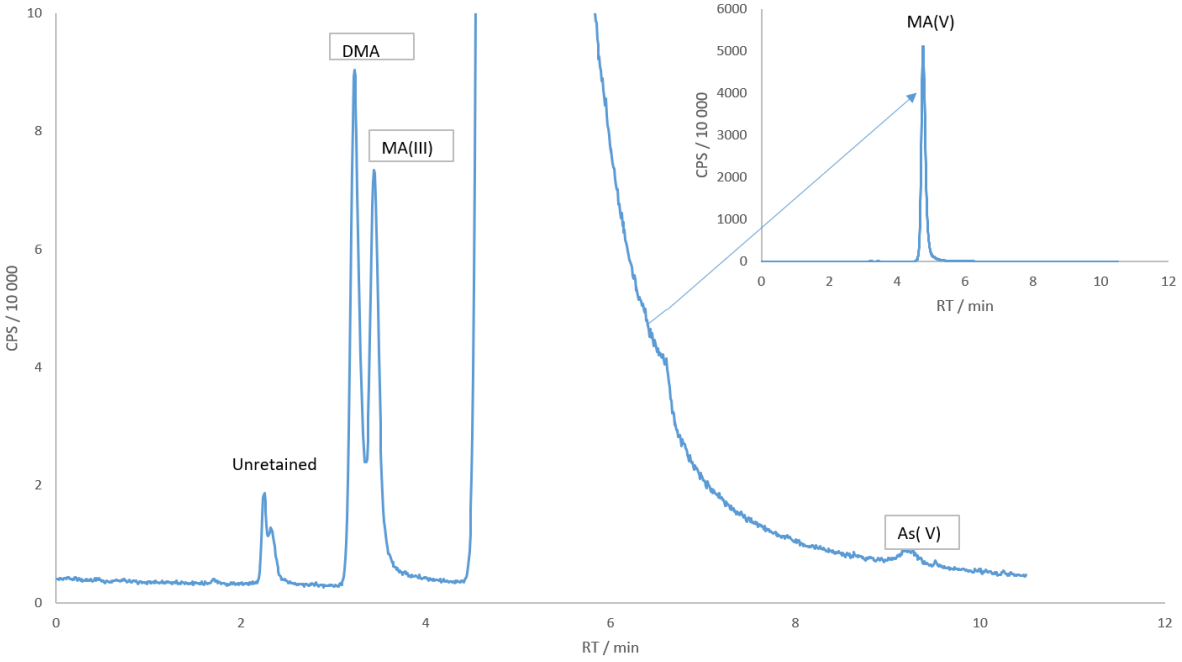
c)


CPS / 10 000
RT / min
Unretained
DMA
MA(III)
MA (V)

d)


DMA
Unretained
As(V)
MA (V)
CPS / 10 000
RT / min

e)


DMA
MA(III)
Unretained
As(V)
MA(V)
CPS / 10 000
RT / min

f)

Figure 19. Chromatograms of a) fresh samples, b) fried samples, c) sample boiled in water, d) sample boiled in water with 10 % vinegar, e) boiled water, f) boiled water with 10 % vinegar, anion-exchange chromatography

The concentrations of MA(III) in µg/g fresh piece for fresh, fried, boiled in water and samples boiled in water with vinegar are shown on *Figure 20.* The relative proportion of MA(III) is shown on *Figure 21*.

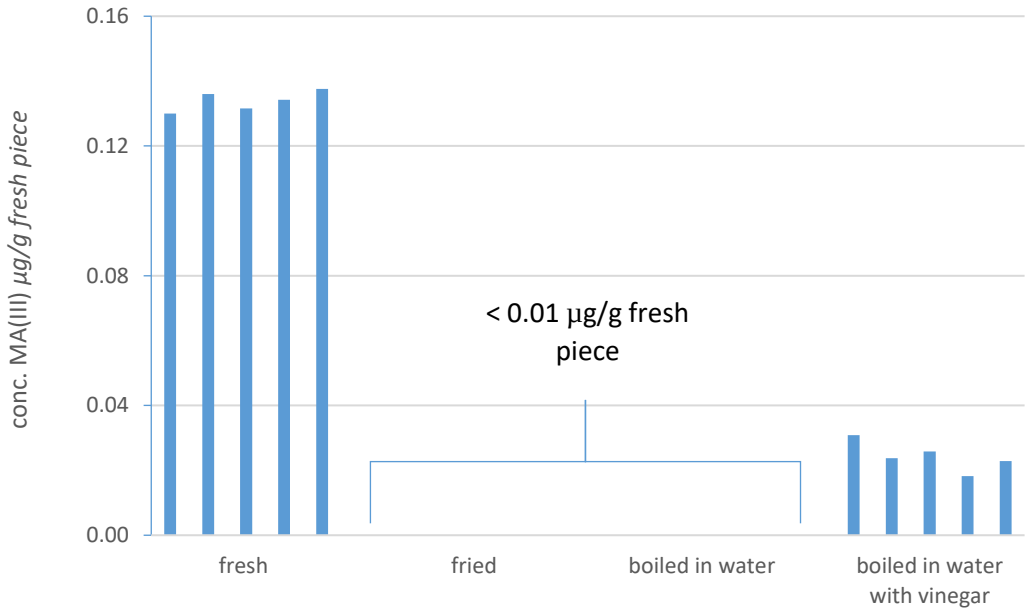


Figure 20. Concentration of MA(III) arsenic species in µg/g fresh piece

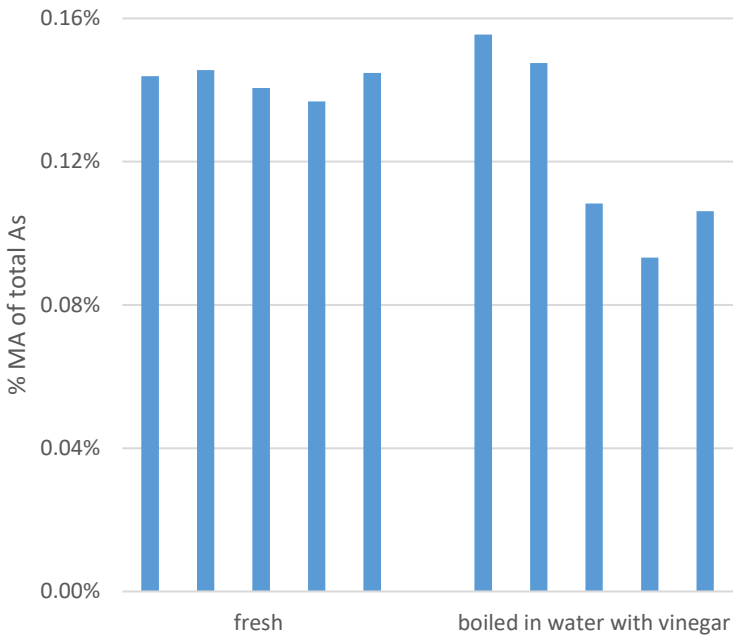


Figure 21. Relative proportion (%) of MA(III) of total As

DMA was detected in all samples, fresh, fried, boiled, boiled with vinegar albeit at low concentrations as shown on *Figure 22.* In fresh samples concentration is between 0.106-0.112 µg/g. Cooking reduced the concentration of DMA, especially boiling the samples in water.

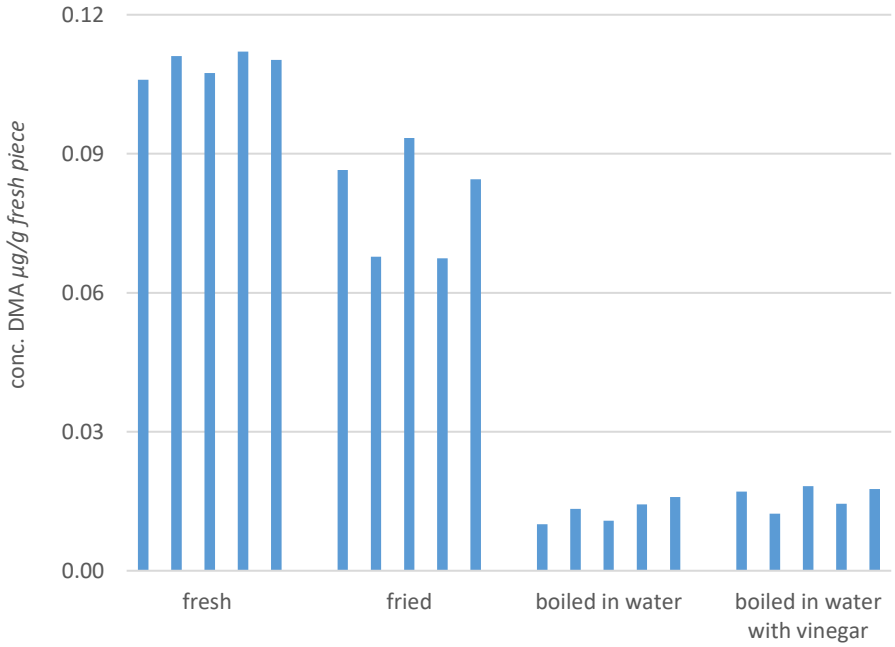


Figure 22. Concentration of DMA arsenic species in *µg/g* fresh piece

From anion-exchange chromatography we found MA(V) as major species. This specie is detected in all samples, fresh and cooked in every way. Concentrations of MA(V) in µg/g fresh piece in samples are shown on *Figure 23*. Through frying around 30 % of MA are lost, but with boiling the loss is around 80-90 %.

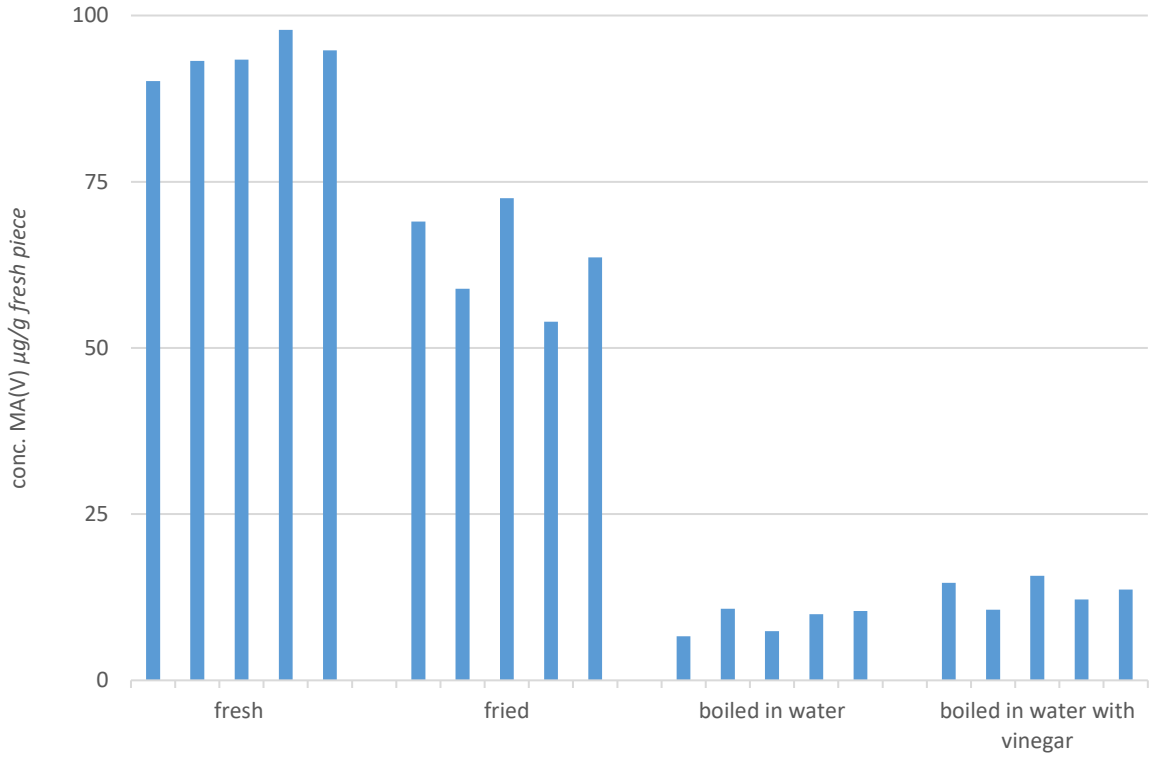


Figure 23. Concentrations of MA(V)

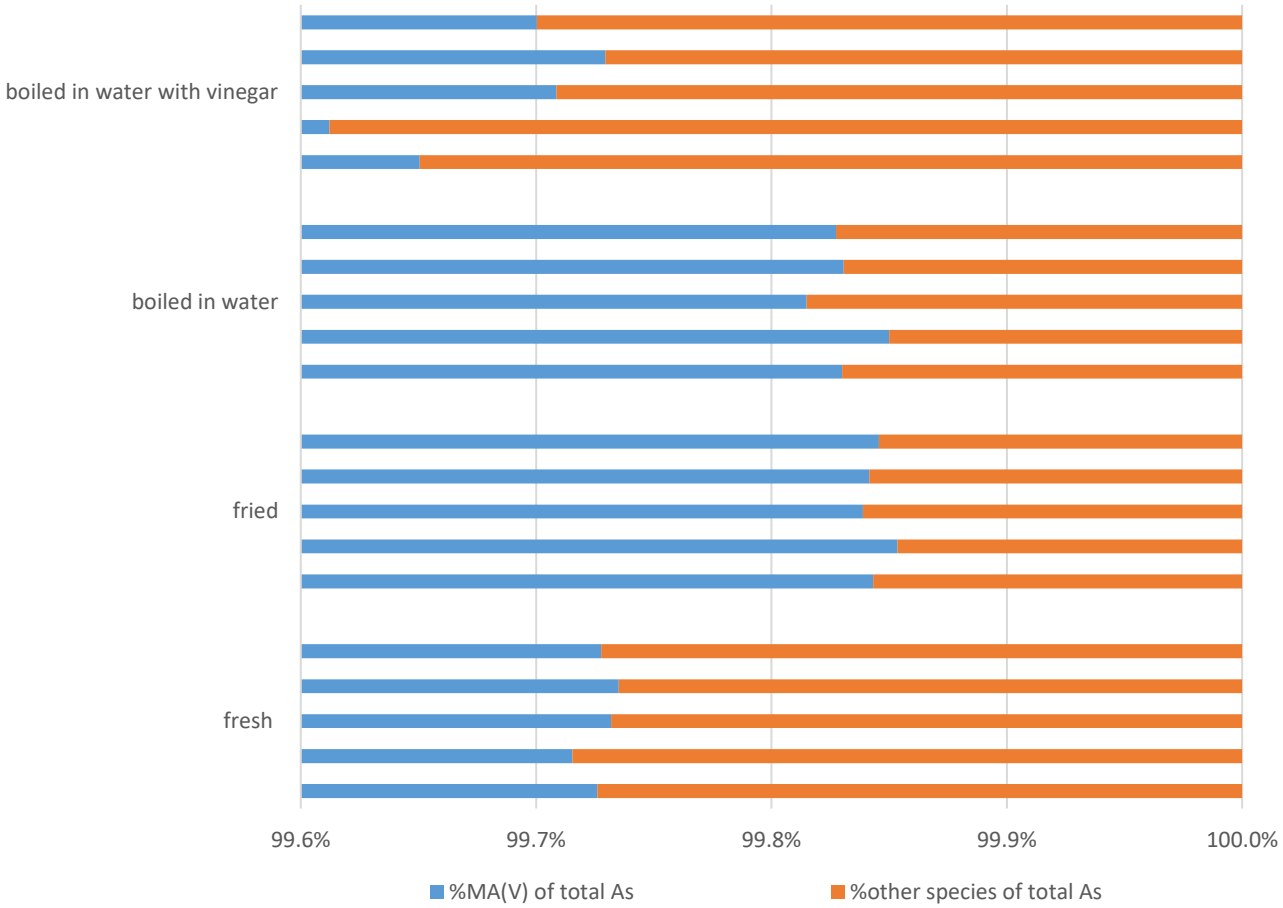


Figure 24. Relative proportion (%) of MA(V) and other arsenic species of total As

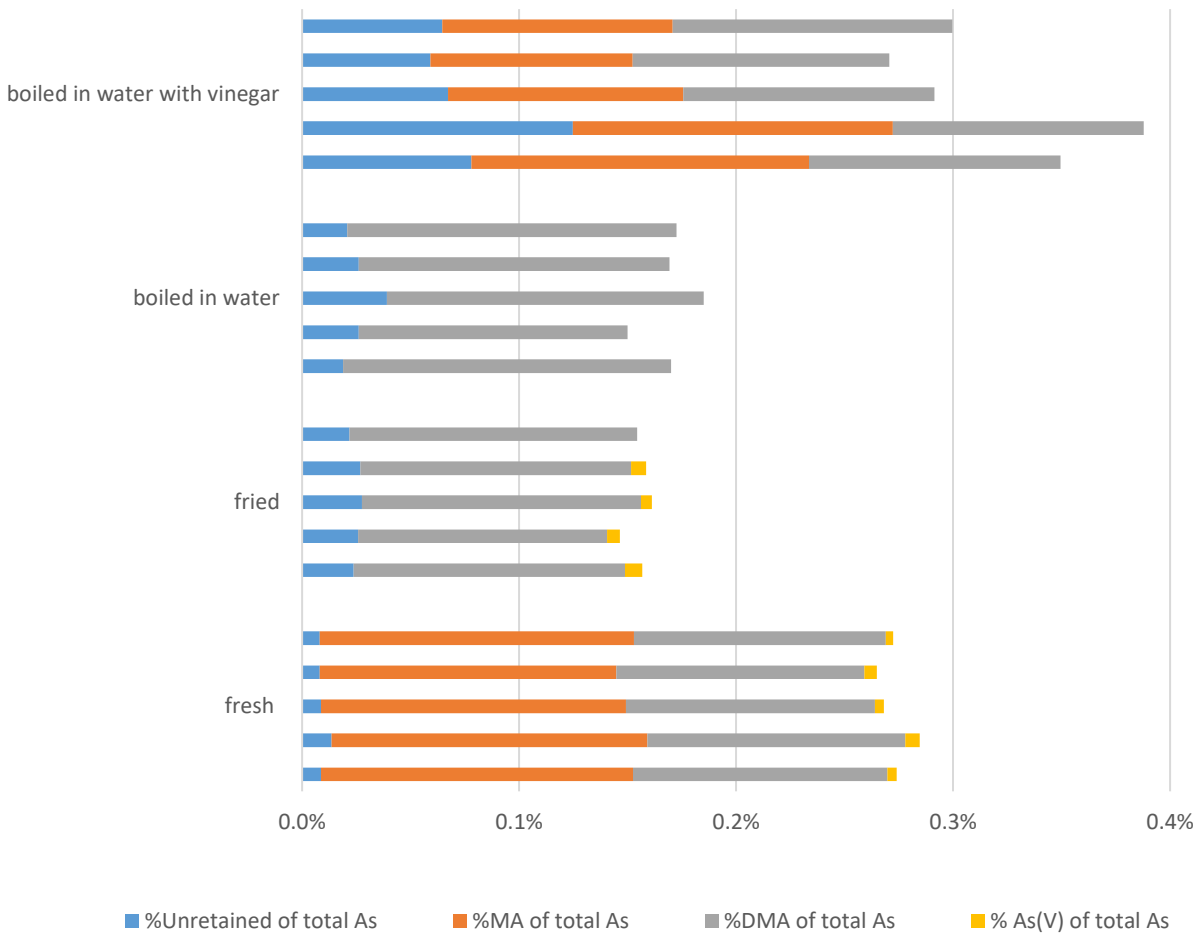


Figure 25. Relative proportion (%) of other arsenic species of total As

As(V) is detected only in fresh and fried samples, but concentration in µg/g fresh piece is quite low.

Generally, we have less anion arsenic species in cooked sample then in fresh samples which is shown in the following figure (*Figure 26*).

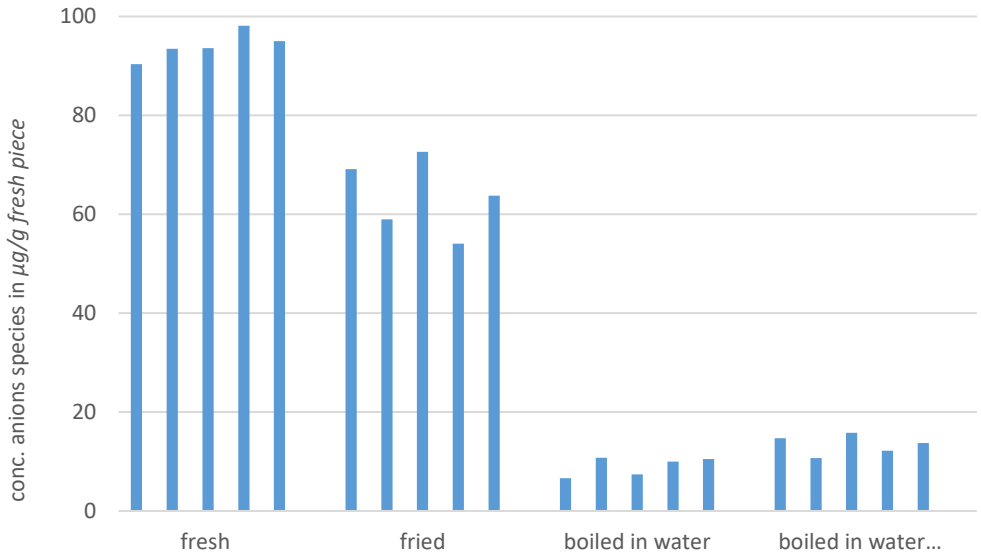


Figure 26. Sum of concentrations of anionic species obtained from anion-exchange chromatography

3.3.2.3. Cation-exchange chromatography

Cation-exchange chromatography was used for the determination of four arsenic species: AB, TETRA, TMAO and AC. Calibration solutions were prepared in the concentration range from 0.05 µg/ l to 100 µg/l. A typical chromatogram at a concentration of 10 µg/l (each compound) is shown on *Figure 27.*

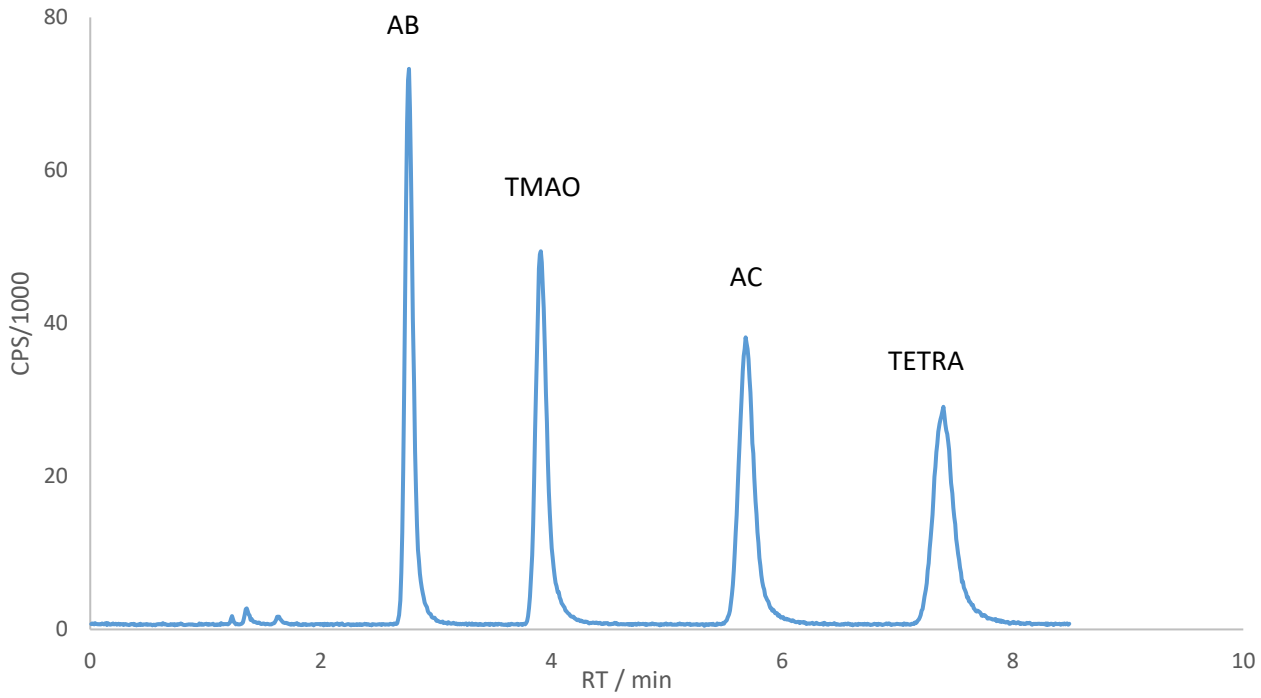


Figure 27. Cation-exchange chromatogram of standard solution (concentration 10 µg As/l AB, TMAO, AC and TETRA) with 10 mM pyridine, pH 2.3.

AC and TETRA were not detected in any Sarcosphaera coronaria samples (fresh, fried, boiled, oxidised). AB species is detected only in non-oxidised samples which are boiled in water with 10% vinegar, chromatogram shown on *Figure 30.d).* The determined concentrations are between 0.002-0.010 µg/g in these samples. In samples with H_2O_2 we did not find AB at all. TMAO was detected in fresh and fried samples of Sarcosphaera coronaria *Figure 30.a)* and *Figure 30.b),* oxidised and non-oxidised. In samples boiled in water we did not detected TMAO, shown on *Figure 30.c).* In fresh, non-oxidised samples concentrations were between 0.0065 and 0.0068 µg/g, and in oxidised samples concentrations are between 0.0061 and 0.0078 µg/g what is actually pretty similar. In fried, non-oxidised samples concentrations are between 0.0153 and 0.0250 µg/g. In oxidised samples concentrations are between 0.0123 and 0.0275 µg/g. Also, we found TMAO in oxidised samples which were boiled with vinegar. Concentration of TMAO in those samples ranged from 0.0014 to 0.0022 µg/g. The concentration of TMAO is shown on *Figure 28.*

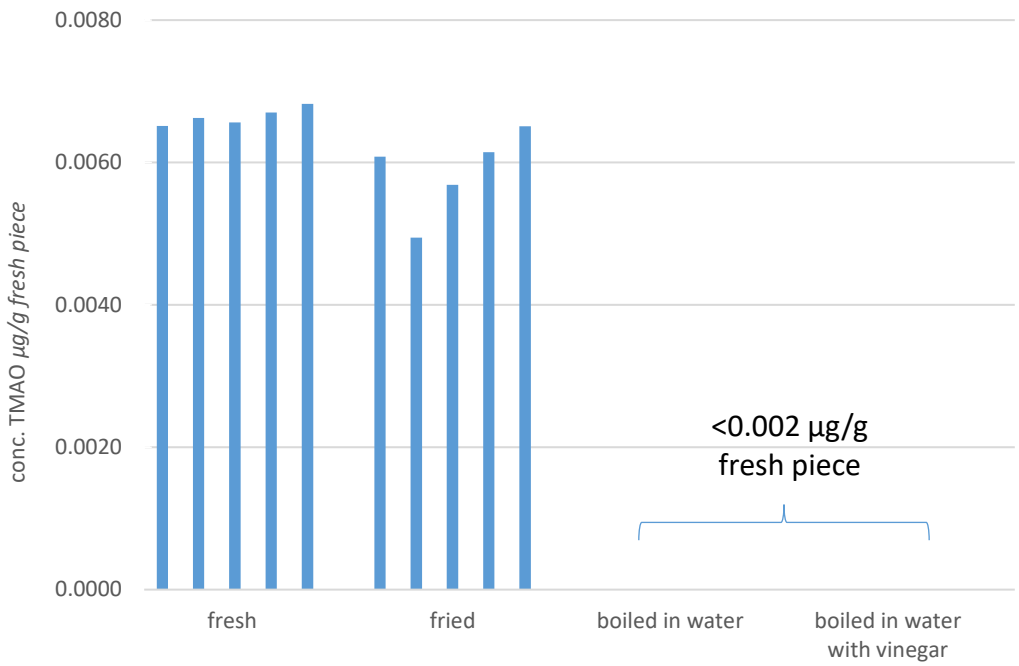


Figure 28. Concentration of TMAO arsenic specie in *µg/g* fresh piece

Generally, less arsenic was determined in cooked sample then in fresh sample (*Figure 29*).

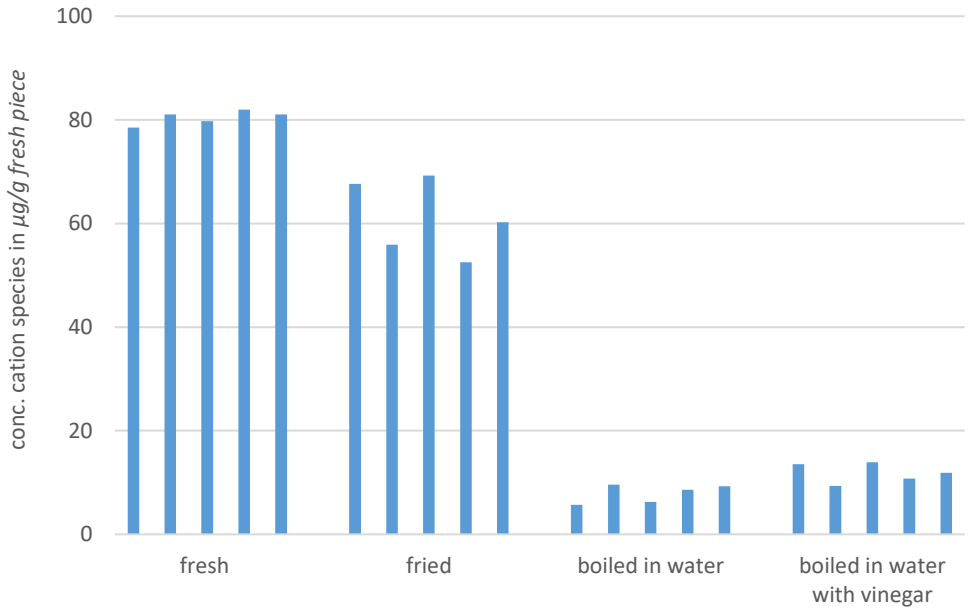
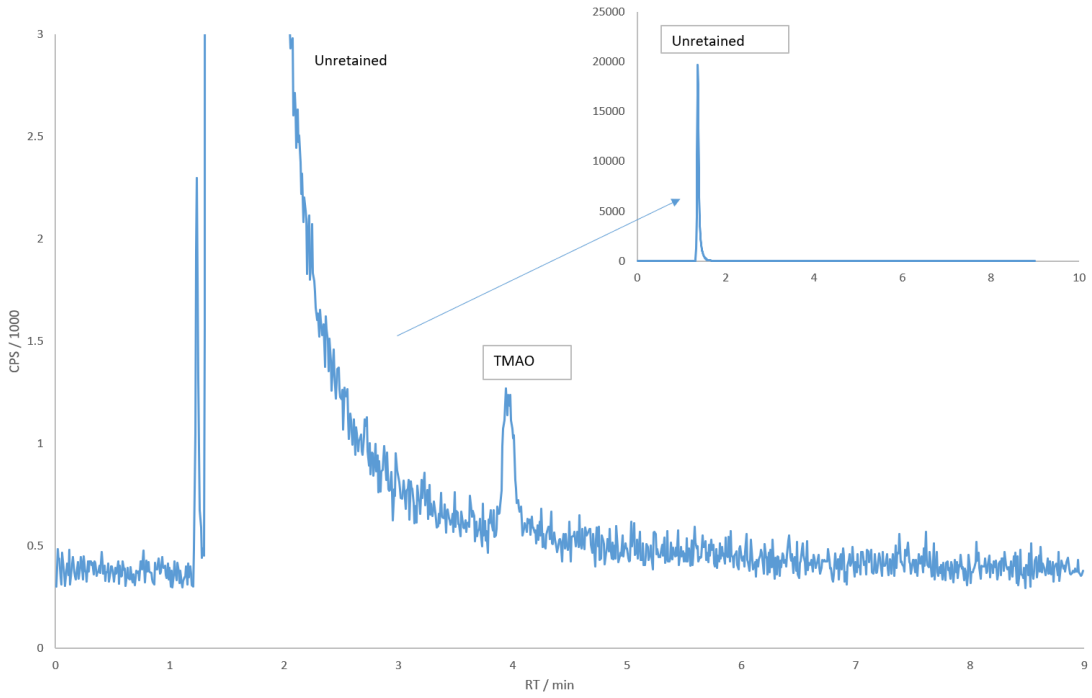
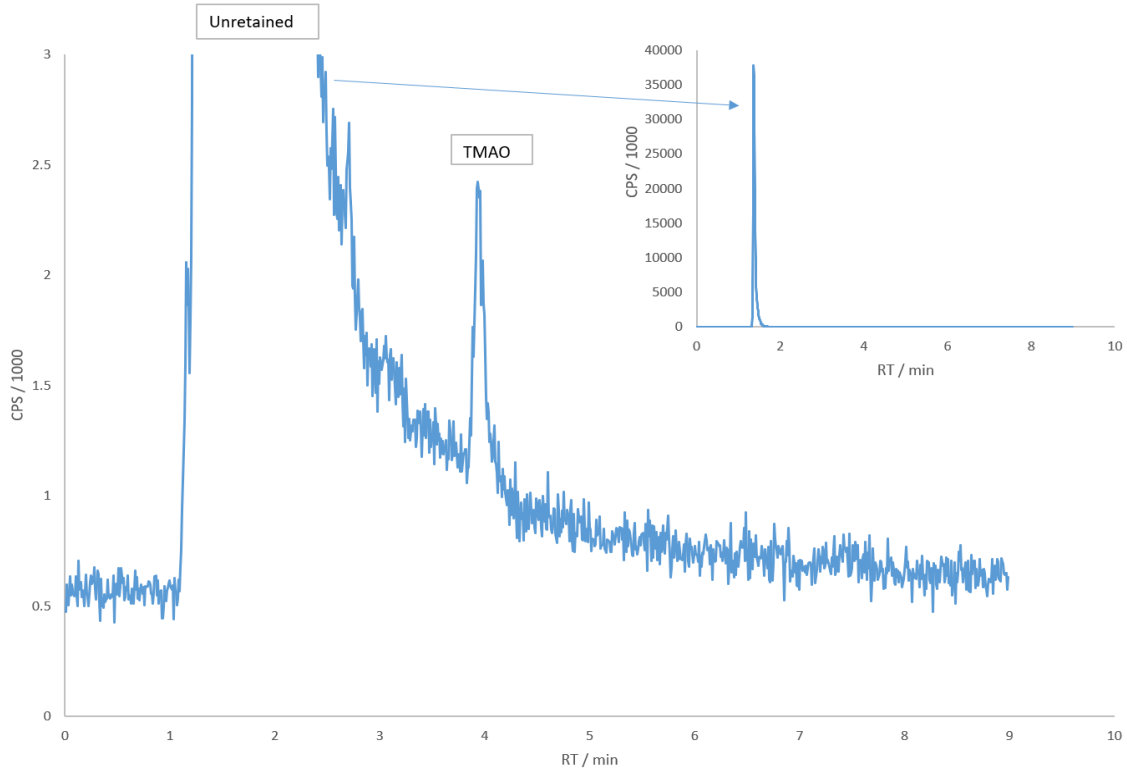


Figure 29. Total concentrations of cationic species obtained from cation-exchange chromatography

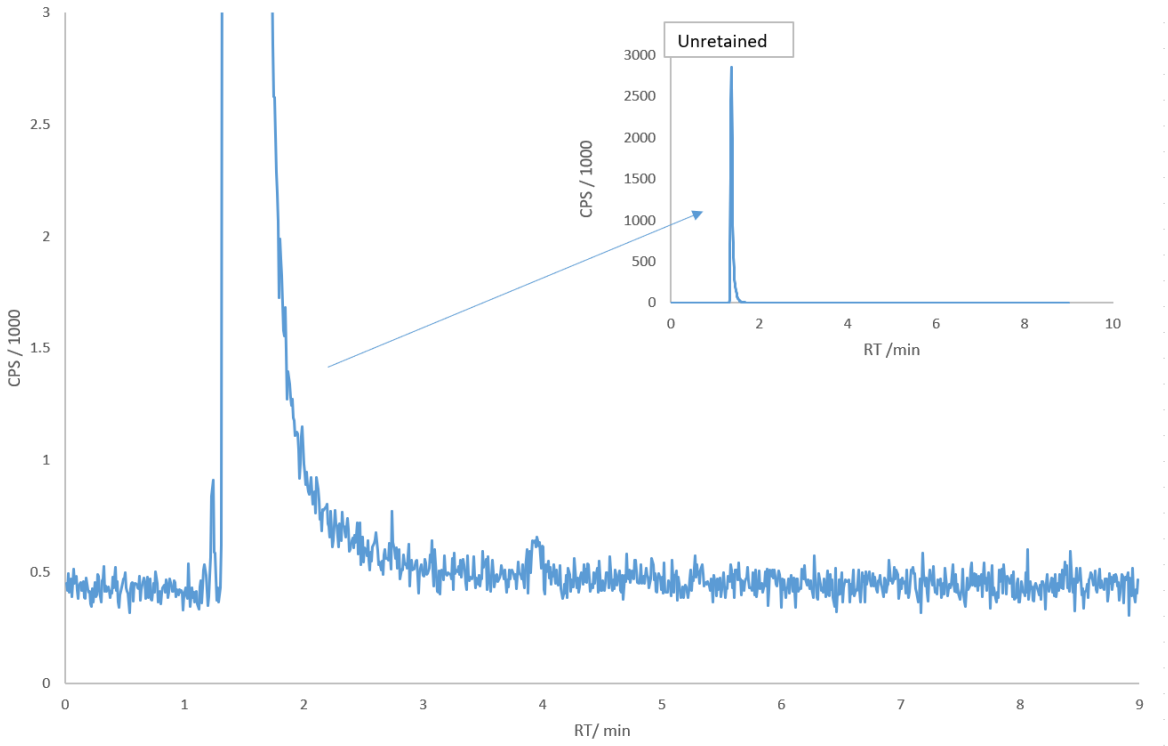
In water where samples were boiled, from cation arsenic species, we found only TMAO at concentrations between 0.35 – 0.78 µg/l shown on *Figure 30.e).* The similar is in water where samples were boiled with 10 % vinegar shown on *Figure 30 .f).*



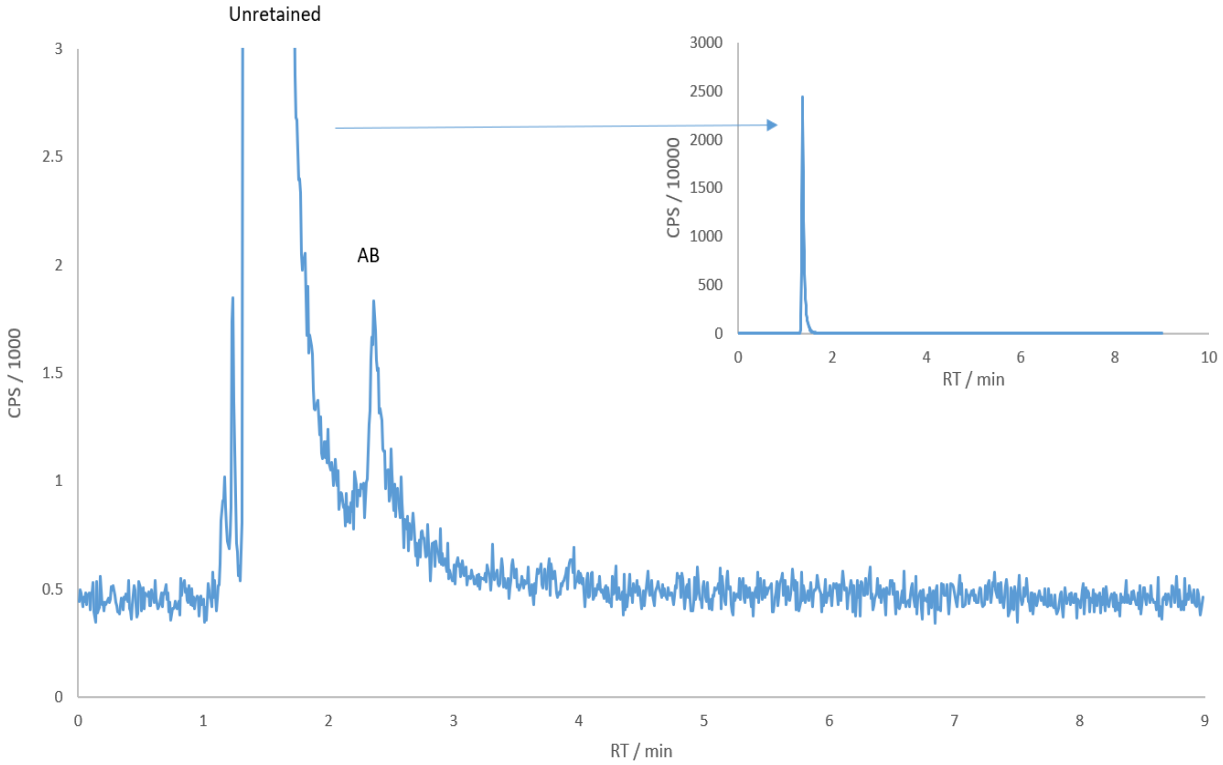
a)


Unretained
TMAO
CPS / 1000
RT / min
CPS / 1000
RT / min

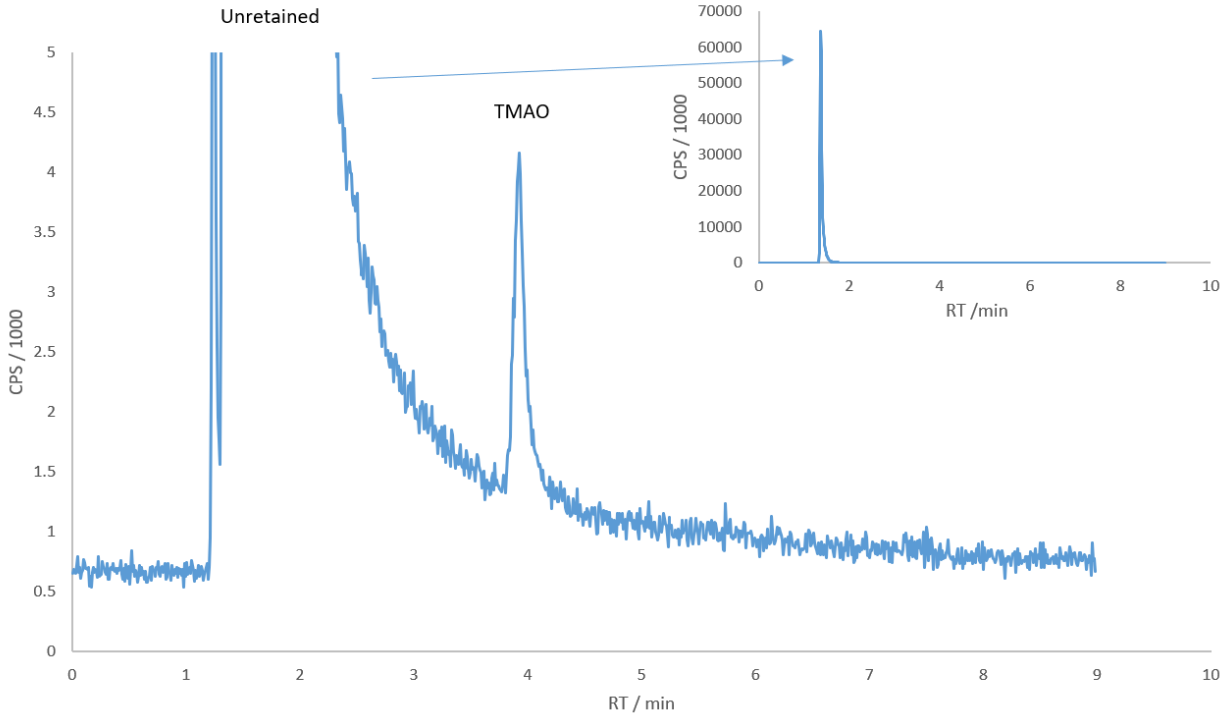
b)


Unretained
CPS / 1000
RT/ min
CPS / 1000
RT /min

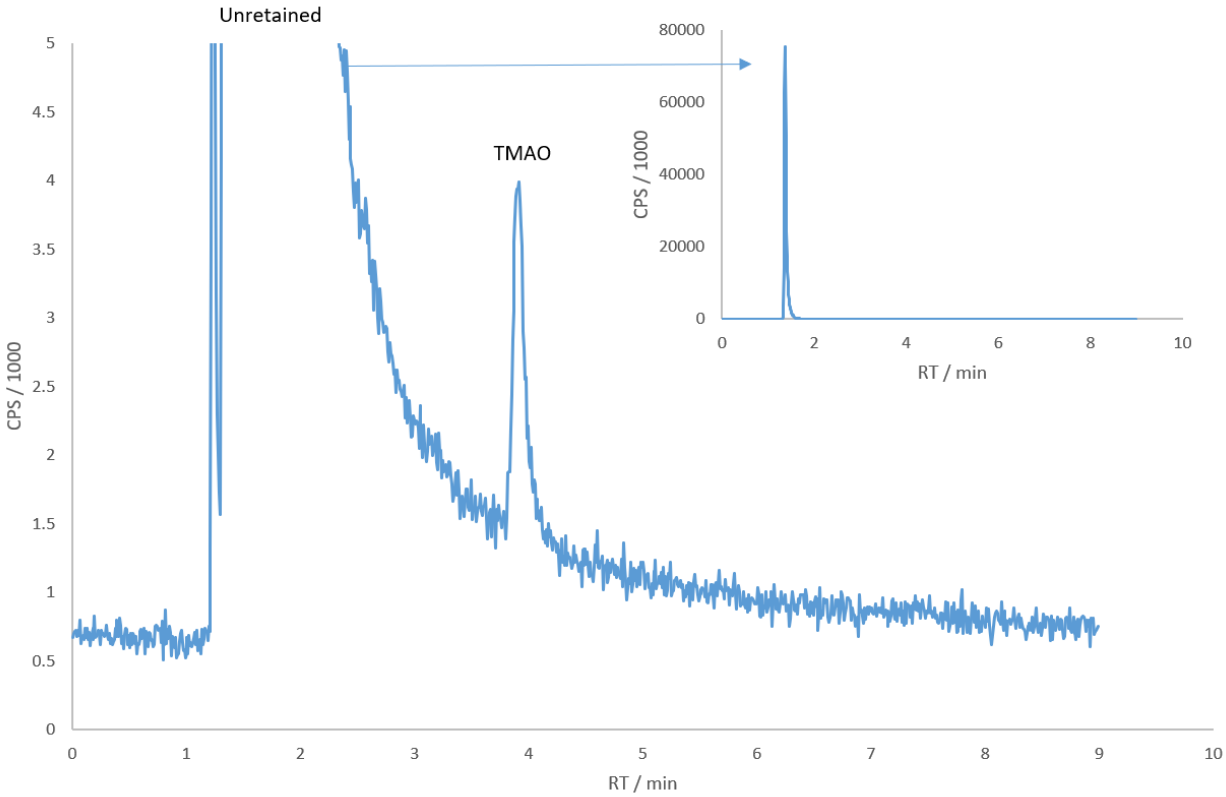
c)


Unretained
AB
CPS / 1000
RT / min
CPS / 10000
RT / min

d)


Unretained
TMAO
CPS / 1000
RT / min
CPS / 1000
RT /min

e)



f)

Figure 30. Chromatograms of a) fresh samples, b) fried samples, c) sample boiled in water, d) sample boiled water with 10 % vinegar, e) boiled water, f) boiled water with 10 % vinegar, cation-exchange chromatography

3.3.2.4. Removal of arsenic with boiling

After the boiling process in water and boiling in water with vinegar, we calculated how much arsenic was extracted in water during the cooking process. Arsenic in cooking water was calculated according to formula:

$$As\ in\ water\ after\ cooking\ = \frac{\left(As\ conc.\left(\frac{\mu g}{L}\right)_{water\ sample} - As\ conc.\left(\frac{\mu g}{L}\right)_{water\ blank}\right) \cdot mass\ of\ water\ after\ cooking(g)}{1000} \qquad (14)$$

The percentage of arsenic in water and in mushrooms after cooking was calculated according to equations:

$$\% \, As \; in \; water \; after \; cooking = \frac{As \; (\mu g) in \; water \; after \; cooking}{(As \; (\mu g) in \; water \; after \; cooking + As \; (\mu g) in \; cooked \; mushroom)} \quad (15)$$

$$\% \, As \; in \; mushrooms \; after \; cooking = \frac{As \; (\mu g) in \; cooked \; mushroom}{As \; (\mu g) in \; cooked \; mushroom + As \; (\mu g) in \; water \; after \; cooking} \quad (16)$$

Results are shown on *Figure 31*. We can see that more than 80 % of arsenic are extracted with boiling process, independently is it cooked in water or in water with vinegar.

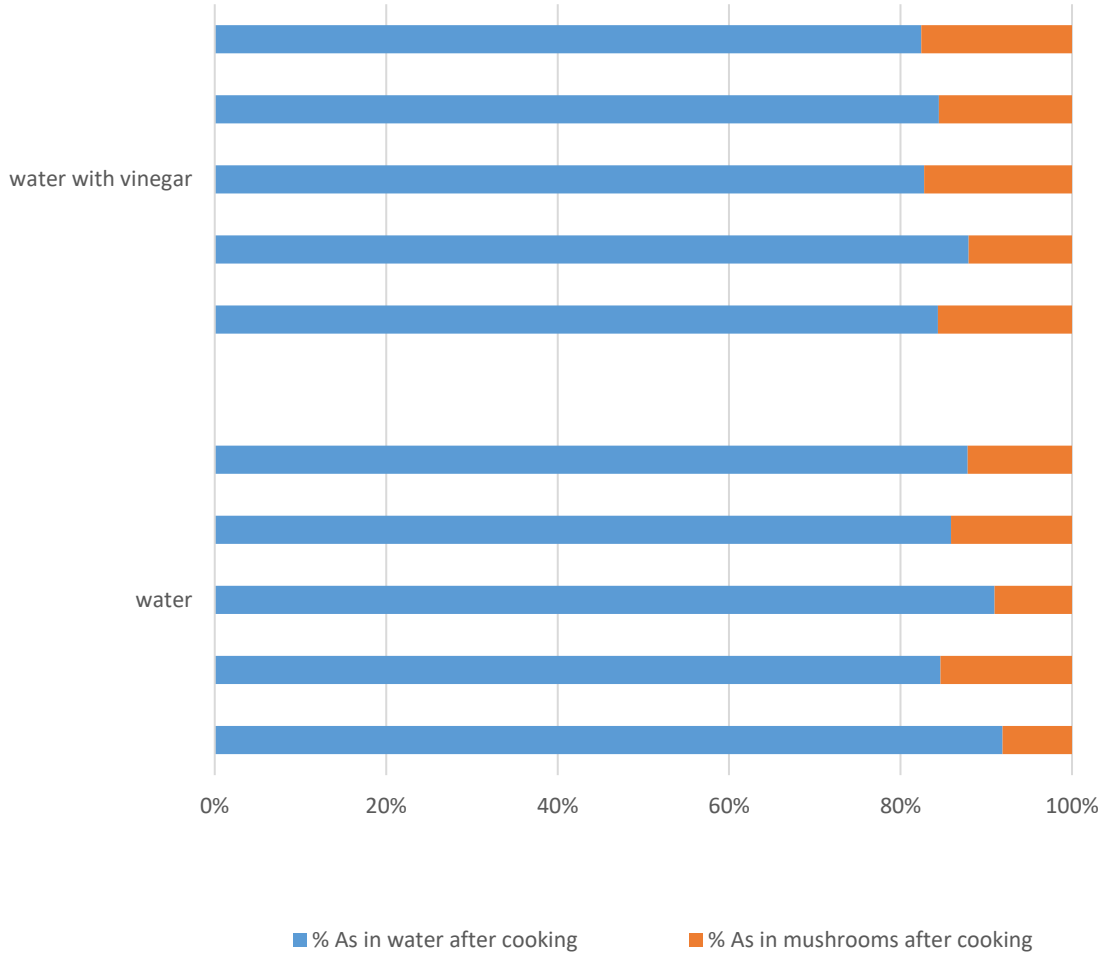


Figure 31. Proportion (%) of total As in water and in mushrooms after cooking

3.4. Chantarelle results

3.4.1. Extraction efficiency and column recovery

The extraction efficiency and column recovery for cation- and anion-exchange chromatography were calculated according to equations (11) and (13) and results are shown in *Table 16.*

Table 16. Extraction efficiency and column recovery for measurements for Chantarelle samples

Sample	Eff %	Column recovery (%), anions	Column recovery (%), cations
1 fresh	35.3	85.3	120
2	49.2	86.7	113
3	48.3	86.7	126
4	43.5	76.1	105
5	54.5	81.5	109
6	53.1	82.2	108
7	64.0	86.7	111
8	51.0	82.3	113
9	53.7	79.8	105
10	52.4	78.8	97.1
1 fried	29.9	83.4	105
2	38.4	79.9	103
3	50.0	76.8	103
4	27.9	81.4	108
5	39.9	76.4	101
6	62.5	75.7	101
7	48.3	79.6	106
8	59.2	78.4	103
9	62.5	77.9	99.4
10	51.5	67.9	86.5
1 boiled	39.5	60.1	74.7
2	75.1	57.0	72.9
3	42.8	53.6	72.7
4	64.2	71.2	95.0
5	43.3	73.2	97.7
6	42.1	77.2	99.0
7	27.2	80.6	105
8	31.2	82.7	98.9
9	29.0	77.2	96.7
10	74.6	35.9	43.8

To check the accuracy of ion-exchange chromatography, quality control are observed. Total arsenic concentrations in the extracts were compared with total elemental concentrations (*Figure 32*).

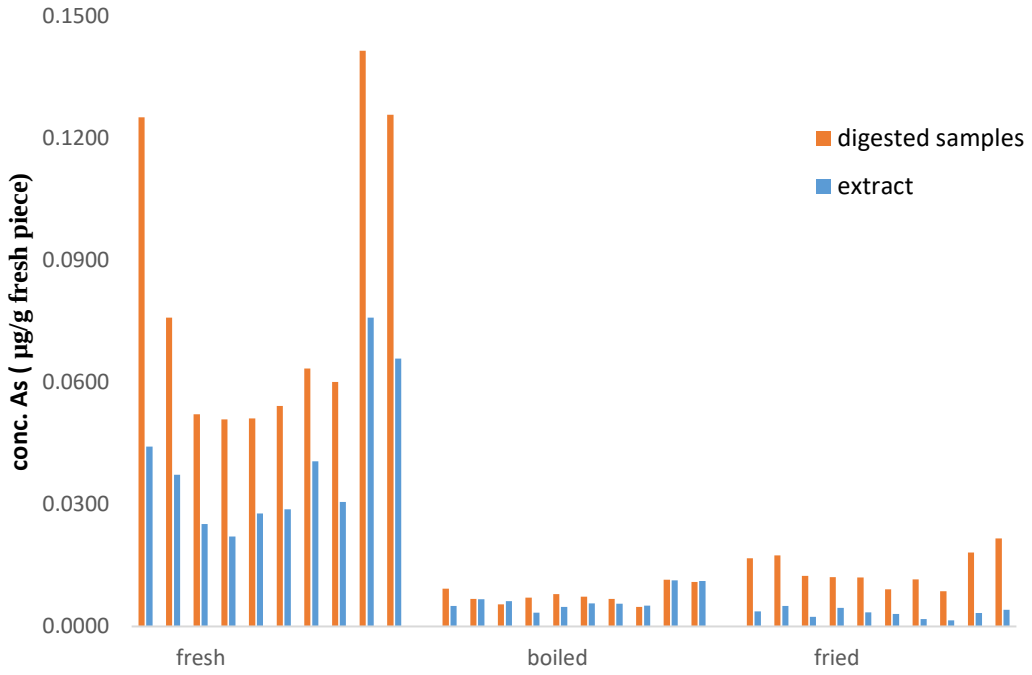


Figure 32. Comparison of As concentrations in µg/g fresh piece given by total analysis from digested samples and total analysis from extract for fresh, boiled in water and fried samples.

3.4.2. Anions exchange-chromatography

Anion-exchange chromatography was used for determination three arsenic species: DMA, MA(V), As(V) and the unretained arsenicals expressed as AB. Calibration solutions for these compounds were in the range from 0.05 µg/l to 100 µg/l. A typical chromatogram is shown on *Figure 18.*

In fresh, boiled and fried samples of Chantarelle we detected DMA, MA(V) and As(V) species, shown on *Figure 33.*

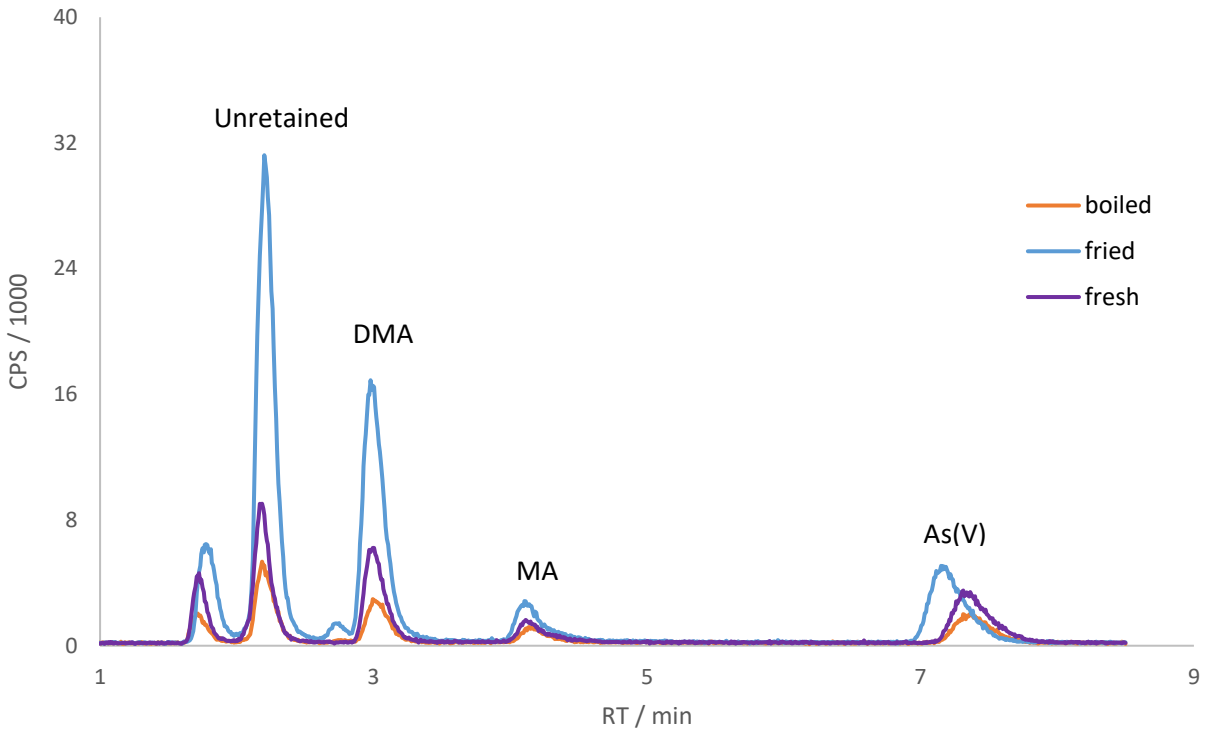


Figure 33. Overlayed chromatograms of fresh, fried and boiled Chantarelle samples

We can see in the chromatograms of fresh, fried and boiled Chanterelle samples that DMA, MA and As(V) were detected in every samples. In chromatogram of fried sample one more peak appeared, just before DMA peak. This is very interesting and might indicate that through the frying process another arsenic species, with a retention time of 2.7 min under the chromatographic conditions employed is formed. Further studies are needed to confirm this. Frying also increases the relative proportion of As(V) in the fruit body.

3.4.3. Cation exchange-chromatography

Cation-exchange chromatography was used for the determination of four arsenic species: AB, TETRA, TMAO and AC. Calibration solutions for these compounds were in the range from 0.05 to 100 µg/l. A typical example of chromatogram is shown on *Figure 27.*

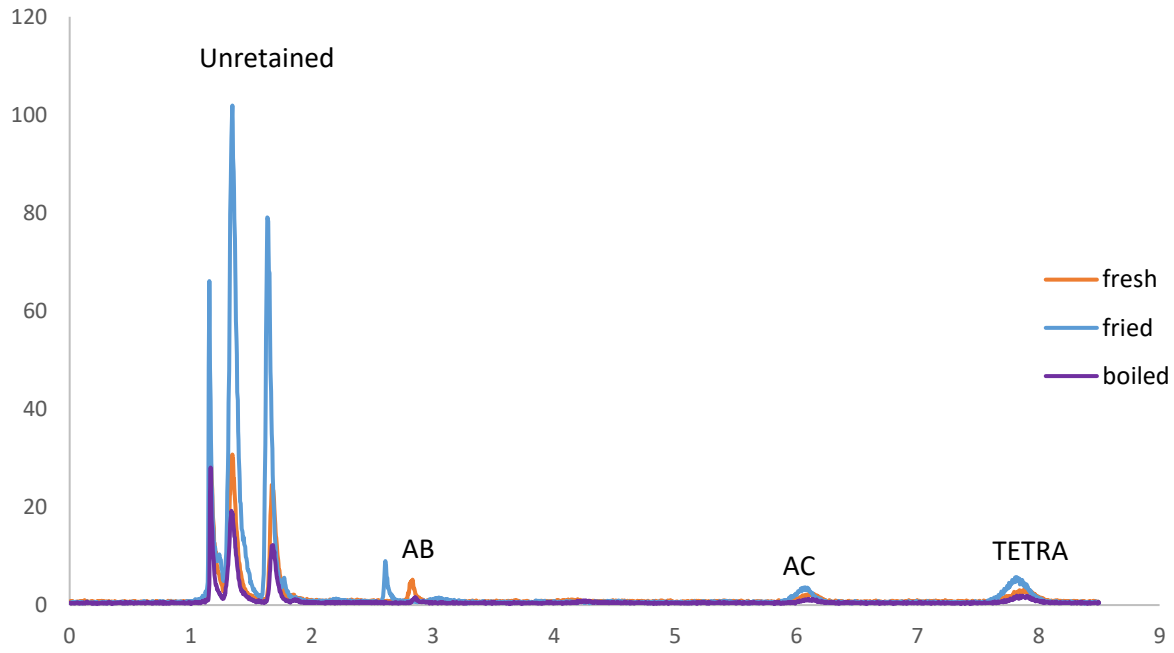


Figure 34. Chromatograms of detected cationic arsenic compounds in fresh, fried and boiled Chantarelle samples

From the chromatograms shown on *Figure 34.* it is obvious that AC and TETRA are present in this mushroom irrespective of the cooking process. Also, we can see that we have two different peaks in the area between 2 and 3 min retention time, and just for fresh and fried sample. In boiled sample this peak is not exist. Anyway, we can say that both peaks are from AB species, because AB is a very stable arsenic compound and not present in the boiled sample. So, probably the matrix just shifted AB and then we see this like two different peaks.

3.4.4. Other elements

Besides arsenic, we also analysed 36 other elements in Chantarelle samples and compared concentration of elements in mushrooms samples before and after cooking process. Some of them were interesting to process and shown graphically, especially some toxic elements, essential trace elements and the most important minerals for human body. The extraction efficiency (%) for the essential trace element selenium is shown on *Figure 35.* It is clearly visible that the highest extraction efficiency is obtained in fresh, non-cooked samples, between 30 – 90 %. After boiling lower extraction efficiencies were observed, which is even worse 10 – 50 % and with after frying the Chantarelle. Also, it is clearly visible that with typical cooking process we reduces concentration of selenium in mushrooms.

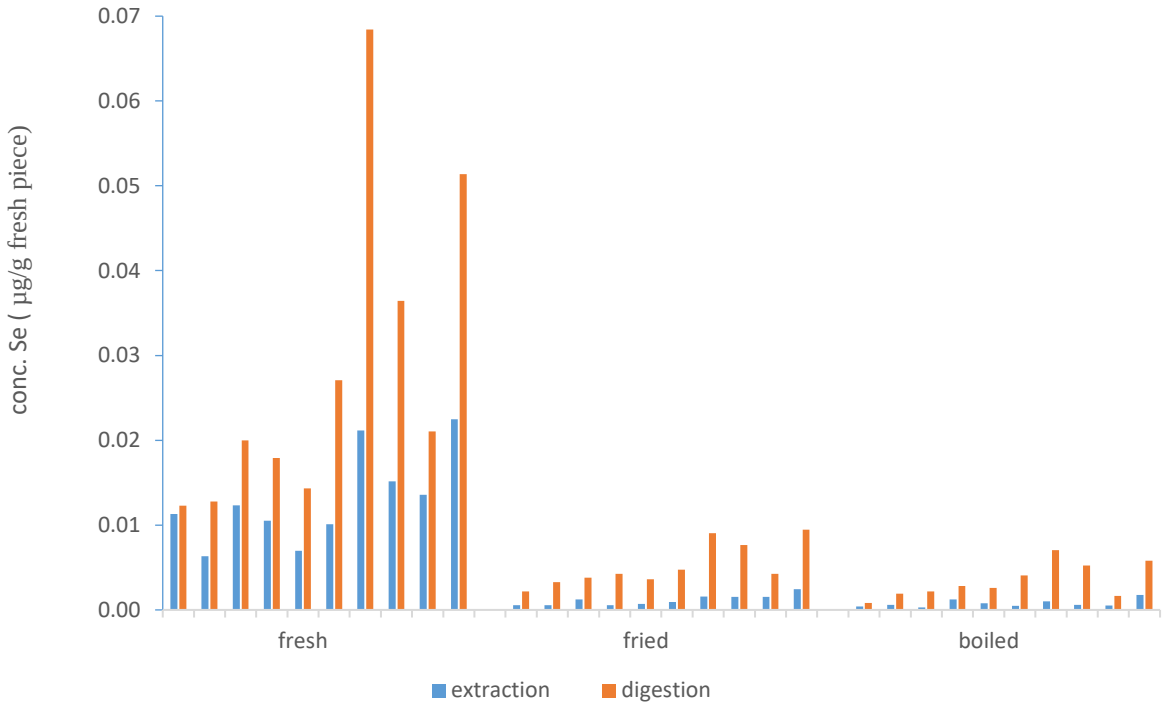


Figure 35. Extractability of selenium from the differently treated mushrooms

Very interesting results were obtained for strontium, which are shown on *Figure 36.* The extraction efficiencies for the fresh samples were really low and not higher than 10 %, but after frying the extraction is almost quantitative. Anew we can see a decrease in concentration of strontium by cooking, especially with frying.

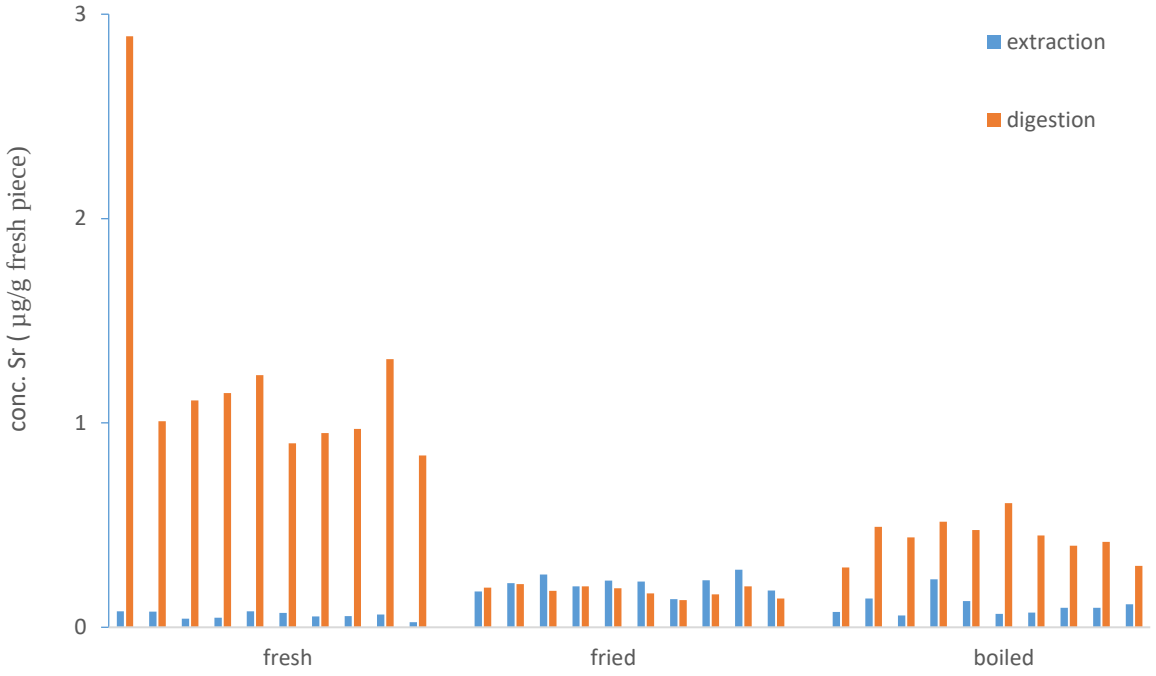


Figure 36. Extractability of strontium from Chantarelle mushrooms

Results were obtained for toxic element lead, which are shown on *Figure 37.* Lead is a metal that preferentially replaces other metals in human body like the iron, calcium and zinc, which we need for normal function. The extraction efficiency for the fresh samples were really low and not higher than 10 %, after frying the extraction is also around 10 %, but after boiling in water it is lower than 5 %. This is satisfying, because we confirmed that with typically cooking process, especially with boiling, reduces its concentration in mushrooms.

The extraction efficiency for iron is extremally low, for fresh and fried samples it is around only 3 %, and for boiled samples around 2 %. Results are shown on *Figure 38.*

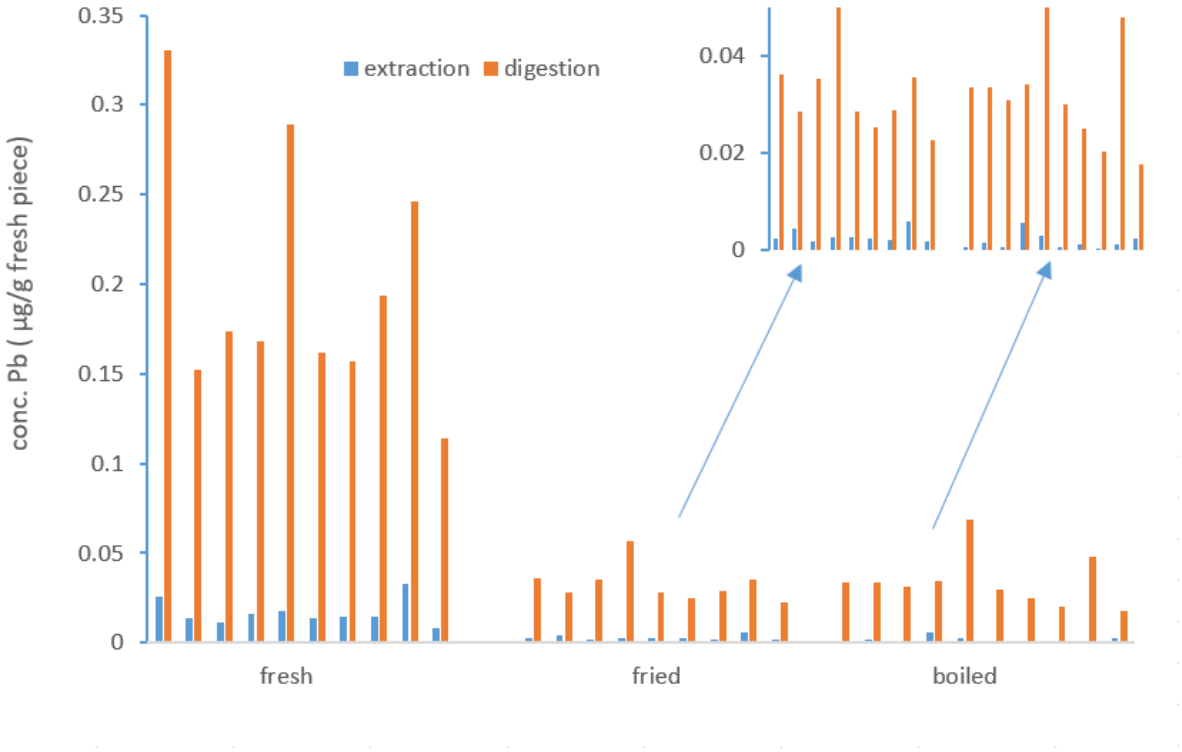


Figure 37. Extractability of lead from Chantarelle mushrooms

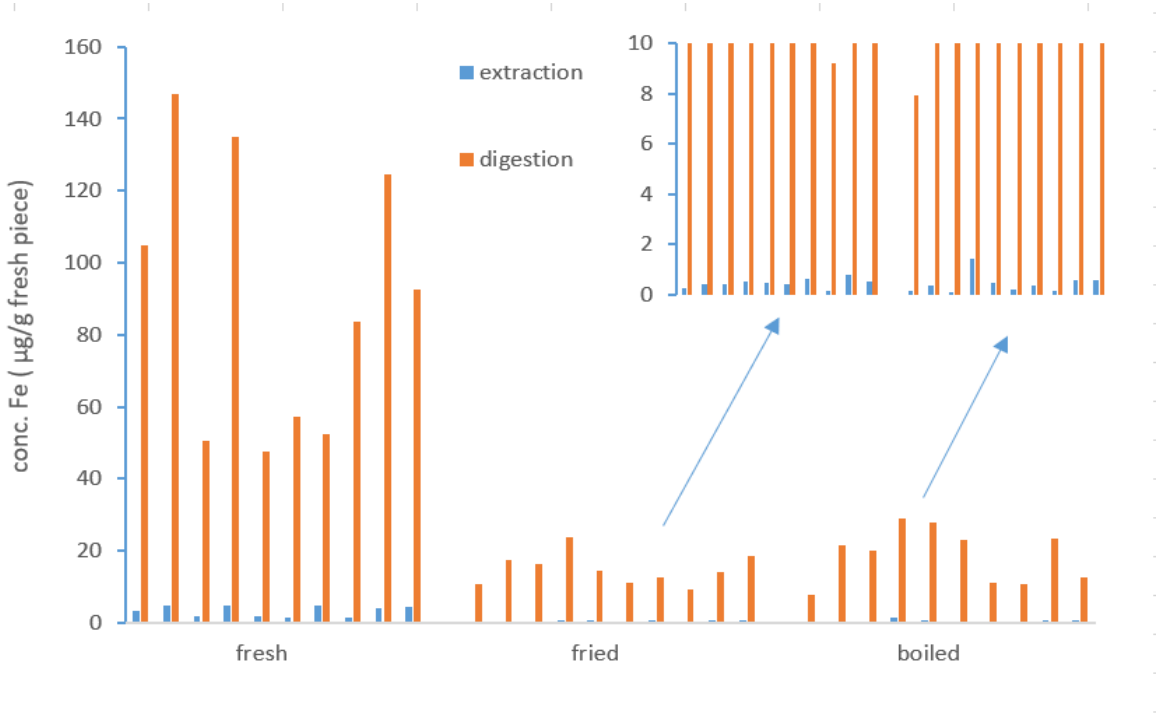


Figure 38. Extractability of iron from Chantarelle mushrooms

Calcium, magnesium, sodium and zinc are some of the most important minerals for human body and because of that it is interesting to see what is happened with their concentration during typical cooking process, like frying and boiling.

Calcium concentration in fresh samples is around 220 µg/g fresh piece. With boiling we lose half of the amount and concentration is around 100 µg/g fresh piece and with frying we lose around 80 % from the initial amount and concentration is around 35 µg/g fresh piece. The extraction efficiency is the highest after frying process, around 65 % and very low after boiling process, around 25 %. On fresh samples, it amounts around 45 % (*Figure 39*).

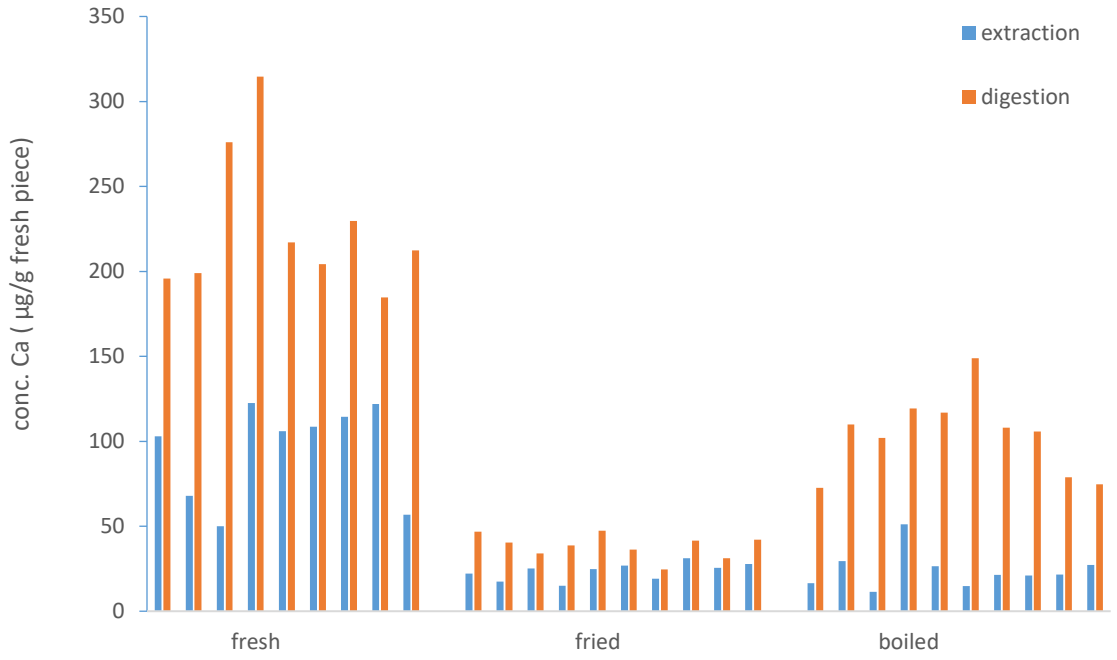


Figure 39. Extractability of calcium from Chantarelle mushrooms

Magnesium concentration in fresh samples is around 500 µg/g fresh piece. With frying we lose 75 % from the initial amount but with boiling the loss is greater than 90 %. The extraction efficiency is the highest after frying process, around 55 % and very low after boiling process, around 25 %. On fresh samples, it amounts around 45 % (*Figure 40*). The extraction efficiency is very similar to calcium.

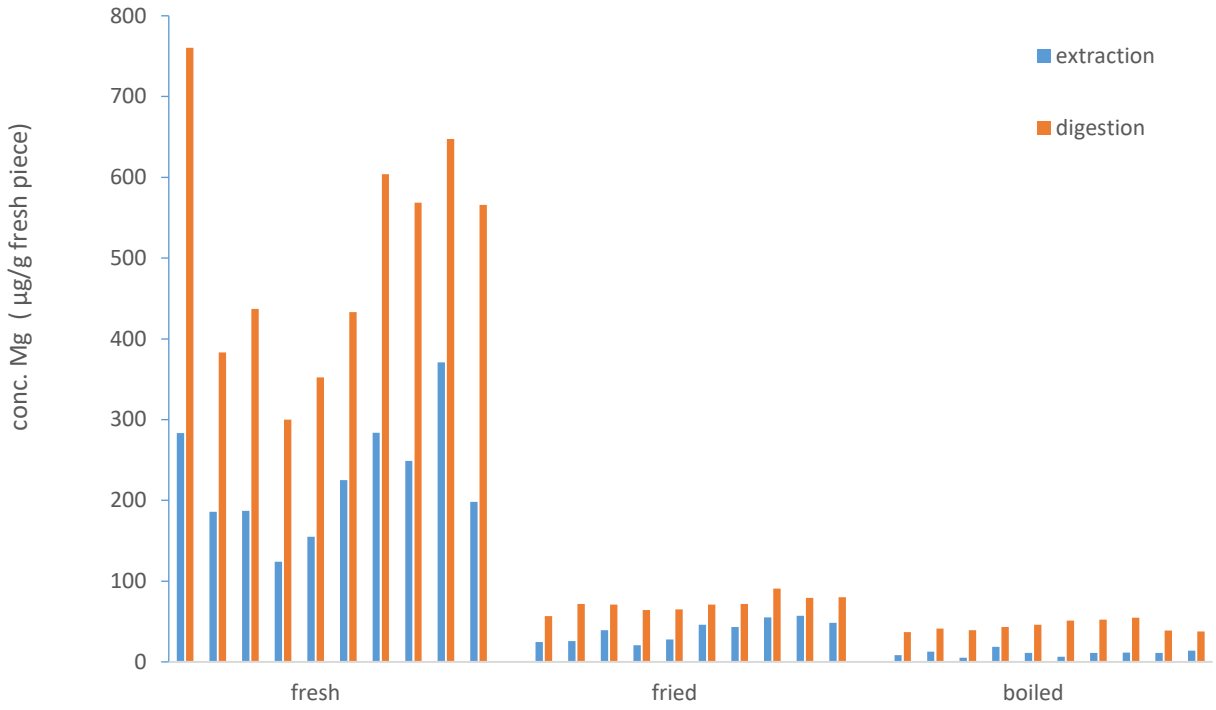


Figure 40. Extractability of magnesium from Chantarelle mushrooms

For mineral sodium the extraction efficiency for fresh and boiled samples is similar, around 90%, but after frying it is lower, around 65 %. Concentration is reduced by cooking, especially with frying. In fresh samples concentration is around 160 µg/g fresh piece, in fried around 30 µg/g fresh piece and in boiled samples around 80 µg/g fresh piece. Result for sodium are shown on *Figure 41*.

For zinc results are shown on *Figure 42.* The extraction efficiency for fresh samples is around 25 %, for fried samples around 35 % and for boiled samples less than 10 %. Concentration in fresh samples is 24 µg/g fresh piece, but after both cooking process it is reduced to around 4 µg/g fresh piece.

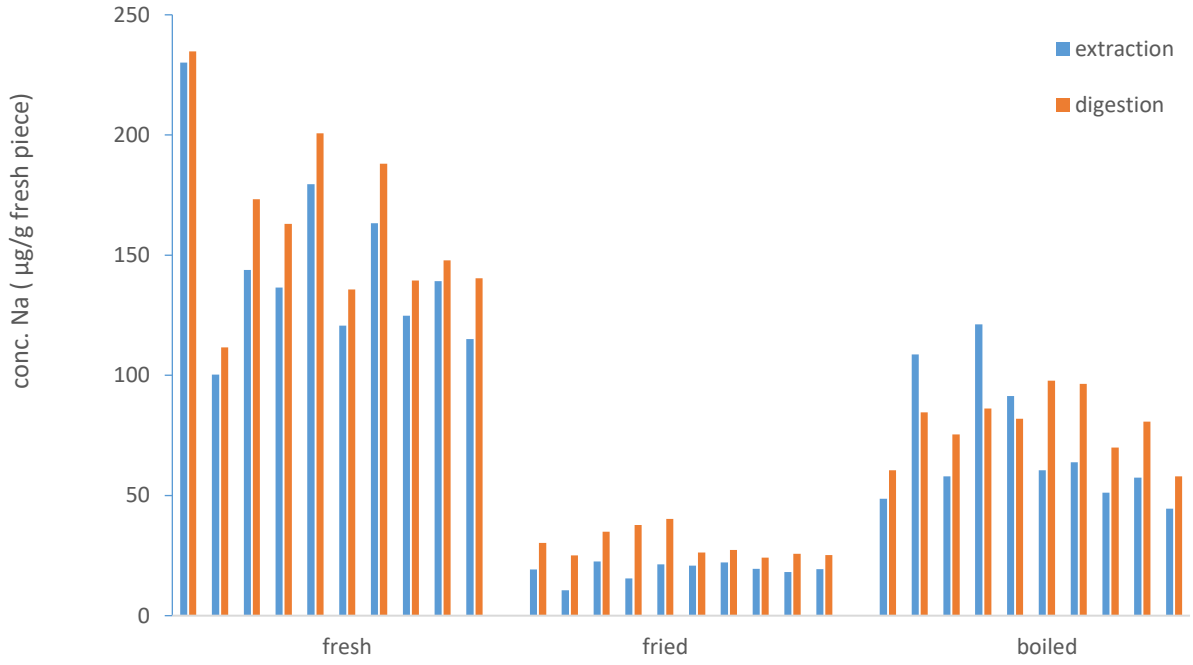


Figure 41. Extractability of sodium from Chantarelle mushrooms

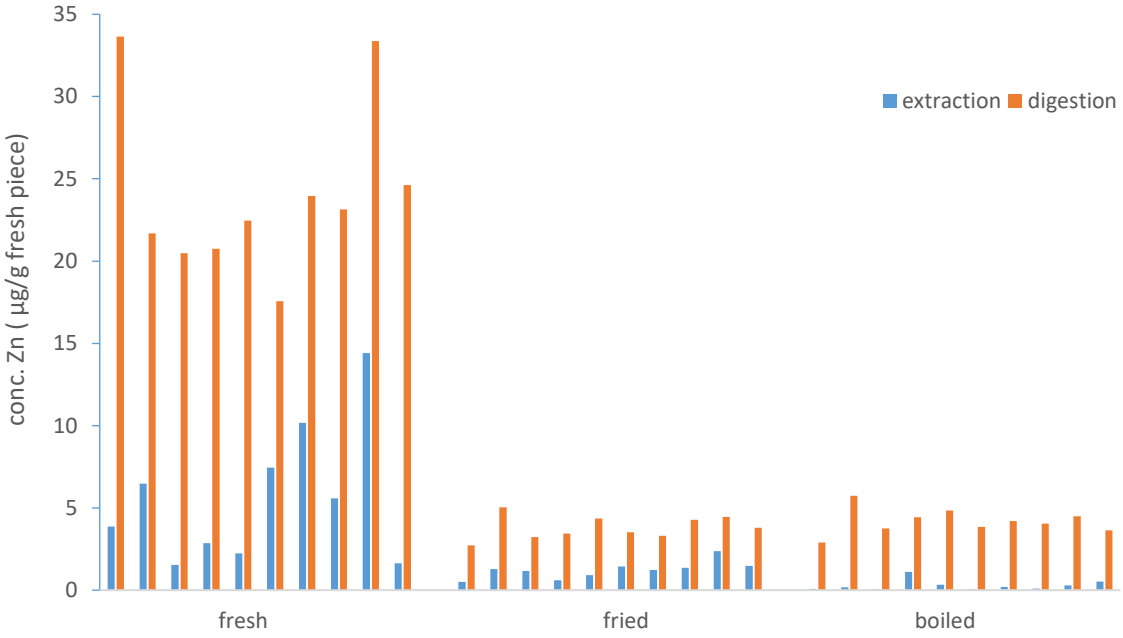


Figure 42. Extractability of zinc from Chantarelle mushrooms

All amounts in µg/g fresh piece, for every elements, were calculated as the average of 10 tested samples.

4.CONCLUSION

After all the measurements made during this scientific research on fungi Sarcosphaera Coronaria and Chantarelle we can conclude the following:

- very polar arsenicals are efficiently removed with boiling but to a lesser extent with frying. As a drawback we also lose minerals (Na, Ca, Mg and Zn), other essential trace elements (Se and Fe) and also toxic elements (As, Pb and Sr).
- boiling with vinegar (at least the one we used) does not significantly increase the formation of the MA(III) but keeps it steady as opposed to boiling in pure water, causing MA(III) to disappear completely.
- the formation of a new compound during frying needs some further investigations, also it can be interesting to examine the behaviour of arsenic, arsenic species and other elements during the thermic process of other edible mushrooms
- ICPMS method is a satisfactory method for this kind of research

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6. CURRICULUM VITAE

Elvira Pek, [redacted] After finishing elementary school, enrolled in 2009 at the Technical High School and Natural Science Gymnasium Ruđer Bošković in Osijek, Croatia. After graduating high school, enrolled in 2013 at the Faculty of Chemical Engineering and Technology, the University of Zagreb, undergraduate study in Chemistry and Material Engineering. In third university year, she completed a one-month internship in Pliva, pharmaceutical company, at pilot department, TAPI R&D, under the mentorship of dr.sc. Martina Hrkovac. The undergraduate study completed with topic *Development and validation of a chromatographic method (HPLC) for the determination of hydroxychloroquine* under the mentorship prof. dr. sc. Sandra Babić. During her college education, she was an active member of the largest student volunteer association of the University of Zagreb, the *eSTUDENT* association, for 4 years. As part of that association, she was the member for one year and the team leader for two years of the *Popularization of Science and Technology* team, whose goal was to transfer and share knowledge and love for science to younger generations. As part of this, she has gained extensive experience in designing, organizing, conducting science workshops, writing projects, working in a team and working with children. She also served as a *Community Coordinator* for one year and was a part of the *Associations Presidency.* As a member of Student Section of *Croatian Society of Chemical Enigneers - CSCE* she started project *"Science through a Test Tube".* She did the project *Hydrolytic and photolytic degradation of cefdinir in the environment* under the mentorship dr. sc. Martina Biošić and *Erasmus*$^{+}$ *Internship* at Karl-Franzens University of Graz, Austria for 5 months under the mentorship ao. univ. prof. mag. dr. Walter Goessler in his research group "Analytical Chemistry for Health and Environment".