RISK ASSESSMENT OF ARSENIC IN ARABIC AREA RICE USING ON-LINE LEACHING AND SPECIATION ANALYSIS BY ION EXCHANGE CHROMATOGRAPHY COUPLED TO INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

By

Randa Althobiti

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Abstract

For the first time, a simple on-line continuous leaching method, where artificial gastrointestinal fluids are sequentially pumped through a mini-column of food while As is continuously monitored by inductively coupled plasma mass spectrometry (ICP-MS), is used to assess the bio-accessibility of As in Arabic area rice. This on-line leaching method offers several advantages over batch methods; most notably, it provides real-time leaching data and involves a shorter and simpler sample preparation, which reduces the risk of contamination. There was no significant difference between the bio-accessible As concentrations measured by on-line and batch methods, although, with the on-line method, the concentration sum of As leached and As left in the residue was closer to the total As concentration according to a Student's t-test at the 95 % confidence level, which is commensurate with the batch method being more subject to contamination. Leaching with artificial saliva released As the most, followed by artificial gastric juice and intestinal fluid. Both the on-line and batch methods showed that 20%-95% of As in rice samples is bio-accessible. The results of As bio-accessibility in unwashed raw rice and in washed cooked rice after simply washing the rice with water prior to cooking were similar, indicating that cooking had no significant effect on As bio-accessibility.

A method based on anion-exchange chromatography with on-line detection by ICP-MS was also used for the speciation analysis of As. For better risk assessment, speciation analysis was performed on washed and cooked samples. Because saliva leached the most

As, the determination of As species was only conducted in saliva leachates. The results revealed that the concentration of inorganic As species in rice varies to some extent according to the samples' location. In general, As(V) predominated over As(III), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA). Although As(V) is considered to be more toxic than MMA and DMA, this investigation indicates that the concentration of As(V) can be reduced significantly by washing rice several times, thereby decreasing the risk of arsenic poisoning.

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List of Abbreviations

AAS: Atomic absorption spectrometry

AFS: Atomic Fluorescence Spectroscopy

a.m.u: Atomic Mass Unit

As(III): Arsenite

As(V): Arsenate

AsB: Arsenobetaine

AsC: Arsenocholine

CRI: Collision/reaction interface

CRC: Collision reaction cell

CRM: Certified reference material

DDW: Doubly deionized water

DMA: Dimethylarsinic acid

EPA: Environmental Protection Agency (based in the United States)

GF: Graphite furnace

HG: Hydride Generation

HPLC: High performance liquid chromatography

IARC: International Agency for Research on Cancer

ICP-MS: Inductively coupled plasma mass spectrometry

IEC: Ion exchange chromatography

MMA: Monomethylarsonic acid

MS: Mass spectrometry

OES: Optical emission spectrometry

PEEK: Polyether ether ketone

PTFE: Polytetrafluoroethylene

SHIME: Simulator of the Human Intestinal Microbial Ecosystem

WHO: World Health Organization

Chapter 1

General Introduction

1.1 Food safety risk analysis

Food analysis is essential to ensure its safety for human health, and to assess food quality. Potentially toxic elements must be measured for risk analysis. Different organizations play a major role in enforcing food safety standards through different ways, such as laws, regulations, inspections and the establishment of threshold values. Both regulating and renewing food safety guidelines help people have more confidence in the safety of their food or agricultural products. Moreover, reducing food additives and the incorporation of food contaminants is also important, as they have been shown to significantly contribute to toxicity.[1,2] The World Health Organization (WHO) and the Environmental Protection Agency (EPA) are two examples of organizations that have been regulating the contamination of food with toxic elements.[1,2]

Different studies have applied quantitative sensitive analytical methods to evaluate the hazard level with different food matrices, such as seafood,[5,6] corn bran,[7] edible seaweed,[8] water samples,[9] rice,[3,4,10] and various fruits and vegetables.[10] The main purposes of these studies was to assess the levels of potentially toxic elements to identify issues with food and to provide realistic and applicable steps that consumers can take to further ensure the safety of their food, such as washing rice before cooking it to remove a large portion of arsenic (As).[3] A significant fraction of As in rice is in inorganic form,[10] i.e. most toxic, a large portion of which is bio-accessible.[3,4]

Inorganic As compounds are soluble, which can lead to acute toxicity if contaminated drinking water, food cooked in that water or crops irrigated with that water are consumed.[15] The consumption of such compounds over time can indeed result in dangerous diseases, such as cancer [3-6]. In 1980, the International Agency for Research on Cancer proved that there was a relationship between skin cancer and exposure to inorganic As compounds.[11] Therefore, WHO and EPA have each assessed the level of inorganic As in water that is safe to human health and arrived at 10 µg/L and 66 µg/L respectively.[11] The USA and Canada have reduced their level of safe intake of As in food from 50 µg/L to 25 and 10 µg/L respectively.[11] The maximum permissible level of inorganic As in rice is 2 µg/kg of body weight per day.[4] In contrast, organoarsenic compounds that are found in high concentration in seafood are less harmful to the human body because mostly arsenobetaine is present, which is non-toxic.[5,6]

Apart from ensuring food safety, organizations (WHO, EPA, Health Canada, etc.) also inform consumers through the internet, publications and conferences. The European Food Safety Authority even gathers people's perspectives when establishing scientific policies and threshold values. Such a process significantly increases people's awareness and knowledge about protecting themselves from contaminated food, water and environmental materials.[13,14] However, in order to make and justify food safety decisions, powerful tools are needed to enable risk analysis.[1]

1.2 Arsenic in nature

Historically, As has been used as a poison, as it was difficult to detect because it lacks color, odor and taste.[15] Substantial evidence exists documenting that exposure to As is

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linked with a significant risk of developing tumors in lungs, skin and liver [3]. For example, in some regions, farmers and their families are exposed to chemical contamination from extensive past use of pesticides;[16] consequently, a majority of these people suffer from cancer and other health problems.[15] Many studies have concluded that ingestion or inhalation of As interferes with the metabolism process.[22] Because As in nature poses a significant health concern around the world, environmental geochemical analysis is important.[3] Arsenic can indeed be found in the environment, including in the atmosphere, soil, rocks, water and various food sources. Furthermore, numerous As compounds are man-made such as fertilizers, drugs, feed additives, insecticides, wood preservatives and herbicides. In fact, approximately 80% of the As in agricultural areas used to come from such supplements. Nowadays, this percentage has dropped for many reasons, such as people's awareness along with many regulations and policies.[15]

A high level of As has been measured in the ground water of some countries, such as India and West Bengal, and people living in these regions are experiencing the toxicity of As from that source.[17] Ground water in Western Turkey is contaminated with some toxic elements, including As at concentrations in the range of 33-911 µg/L, which exceed the threshold value.[18] Because arsenic-contaminated ground water is a global issue, its concentration is monitored in many regions around the world, including Spain, Greece, Germany, Romania, Hungary, Iran, Turkey and Finland.[18]

Arsenic in soil can come from both natural and industrial sources, but is normally present at trace level, except in geological regions that are naturally rich in As-containing minerals.[19] The geology must therefore be taken into consideration when determining As concentration.[19] For instance, the As level in rice was 149 mg/kg in a region where the As content of soil was 312 mg/kg, confirming that As in soil can be transferred to crop.[19] In fact, rice is a crop that requires an enormous volume of water, which can leach As from agricultural soil, allowing its eventual accumulation in crops such as rice. For instance, the concentration of As in irrigation water was 0.11-0.76 mg/L versus 1.38-12.7 mg/kg in soil, resulting in rice with an As content of 7.2-18.3 mg/kg.[20]

Arsenic can exist as inorganic and organic compounds in the environment. For instance, drinking water may be a major source of inorganic As.[26] Inorganic compounds are arsenite (As(III)) and arsenate (As(V)). Organo-arsenic compounds include monomethylarsonic acid (MMA) or dimethylarsinic acid (DMA). Their toxicity depends on their oxidation state, which dominates their physiochemical and biological activities. While these four compounds are toxic, As(III) and As(V) are more toxic than MMA and DMA to the human body as shown in Table 1.[3-6]

Table 1. Toxicity of selected inorganic and organic arsenic species.[2]

Species	Name	Chemical formula	LD50*
As(III)	Arsenite	AsO_3^{-3}	25-45 mg/kg
As(V)	Arsenate	$AsO(O)_3^{-3}$	55 mg/kg
MMA	Monomethylarsonic acid	$AsOCH_3(O)_2^{-2}$	970 mg/kg
DMA	Dimethylarsinic acid	$AsO(CH_3)_2O^-$	650-2000 mg/kg
AsB	Arsenobetaine	$As(CH_3)_3CH_2COO^-$	Non toxic

* LD50 = lethal dose required to kill 50% of a population of animals

1.3. Arsenic in rice

It is widely recognized that the presence of As in high concentration can cause a variety of adverse health effects after acute and chronic exposures as mentioned in the previous section.[24] Rice is of particular interest because it can contain a high level of As and is a fundamental food in many cultures, with the average person consuming between 100-650 g/person/day, as it is a good source of energy and is rich in vitamins.[22] There are 40,000 types of rice around the world, which each have the potential to accumulate hazardous levels of toxic As compounds from soil naturally rich in As and from three major pollution sources:[22]

> ➢ using irrigation water containing a high As level, as occurs in Bangladesh;
>
> ➢ growing rice in soil contaminated by industrial activity; and
>
> ➢ growing rice in soil previously treated with arsenical pesticides.

As a result, the As concentration in rice grains may vary greatly.[21,28] The total As concentration in rice was found to be lower in the USA than in Bangladesh,[29] and lower in India and Egypt than in USA and France[21] (Table 2). Many researchers have proven that, regardless of the rice variety, As accumulation was 28-75 fold higher in the root than in rice grain. According to 2009 research, As accumulation follows the order of root > straw > husk > grain.[20,25] Furthermore, some researchers have reported that brown rice (not polished, so with bran) contained more inorganic As than white rice (polished, without bran).[26]

In general, As(III) and DMA are the prevalent As species in rice, DMA concentration usually increasing with the total As concentration while that of As(III) remains

constant.[22] Rice and rice products contain significant amounts of inorganic As,[26,27] the global percentage of As present as inorganic As in rice being 11-90%.[26-28] In some regions, this inorganic As comes from past or continuous extensive use of pesticides on agricultural soil, which ultimately accumulated in rice.[12] However, the risk associated with exposure to inorganic As depends on the concentration of inorganic As and on the quantity of rice that a person consumes daily.[21] For adequate risk assessment, the chemical forms of As must also be identified. For instance, fish and shellfish contain As at 10-100 fold the levels found in rice. However, the majority of this As is in the form of arsenobetaine and arsenocholine, which are considered innocuous to the human body.[30]

Table 2. As concentration in rice samples collected from different countries.

Sample location	Sample preparation	Detection method[a]	Concentration (mg/kg)	Reference
Egypt	Raw rice	ICP-MS	0.05	21
France	Raw rice	ICP-MS	0.25	21
Italy	Raw rice	ICP-MS	0.15	21
Bangladesh	Raw rice	ICP-MS	0.31	21
	Rice washed with deionized water, then dried for 3 h before analysis	HG-AAS	0.70	25
Japan	Raw rice	ICP-MS	0.19	21
	Prewashed and boiled	ICP-MS	0.542	23
Spain	Raw rice	ICP-MS	0.20	21
Thailand	Raw rice	ICP-MS	0.14	21
	Rice was grinded and dried in an oven at 85ºC; kept in glass bottles at room temperature until analysis	AAS	0.125	28
USA	Raw rice	ICP-MS	0.25	21
	Raw rice	ICP-MS	0.560	3
West Bangal, India	Raw rice	ICP-MS	0.451	21
	Washed rice and dried at 60ºC for 72 h	FI-HG-AAS	0.334	17
China	Raw rice	ICP-MS	0.41	21
	Rice was grinded and then oven dried at 80ºC for 24 h before analysis	ICP-MS	0.46	24

[a] ICP-MS: inductively coupled plasma mass spectrometry; HG: hydride generation; AAS: atomic absorption spectrometry; FI: flow injection

The cooking procedure is another variable that must be considered because rice cooking can increase, decrease or have no effect on As concentration. For example, the concentration of As increased from 0.17 to 0.21 mg/kg after cooking,[31] because the cooking water, which was contaminated with As, was absorbed by rice grains. Cooking with a small volume of water is thus advisable to not only reduce the risk of As (or other toxins) absorption, especially in regions known for having drinking water contaminated with As, such as Bangladesh and West Bengal,[24] but also preserve nutrients in rice.[21] If water is not contaminated, then the cooking process has no impact on As concentration in rice.[3,4] In fact, washing rice before cooking it can eliminate 90% of bio-accessible As.[3,4].

Given the number of variables involved, which include the rice variety (brown or not, organic or not, region where it is grown, etc.), purity of the water used for cooking, cooking conditions, chemical forms of As present, extent to which they are absorbed by the human body, etc. (Figure 1),[35] much research is still needed to update the guidelines and the threshold As values for human health and safety.

1.4 Bio-accessibility and bio-availability of As

In order for toxic As species present in food to exert their detrimental effect on the human body, they must first be solubilized in the gastro-intestinal tract and then be absorbed into the blood system.[32] What ends up in the bloodstream is the bio-available portion, which is measured by in-vivo methods, whereas the concentration of analyte leached by gastro-intestinal fluids from food is the bio-accessible portion, which is

usually estimated by in-vitro methods.[3] Ideally, for realistic risk assessment, bio-availability should thus be taken into account.

Most approaches used to measure bio-accessibility are static gastrointestinal assays, which mimic transit through the human digestive tract by sequential exposure of the samples to artificial saliva, gastric juice and small intestine juice.[5,6] Because static models are easier to implement than dynamic models,[33] only a few researchers have implemented dynamic gastrointestinal procedures, which simulate the gradual transit of extracted food mixtures through the simulated physiological conditions in the digestive tract of the human body. An example of in-vitro method is the "Simulator of the Human Intestinal Microbial Eco-system" (SHIME), which uses human gastro-intestinal microbiota to determine the bio-accessible fraction of a compound.
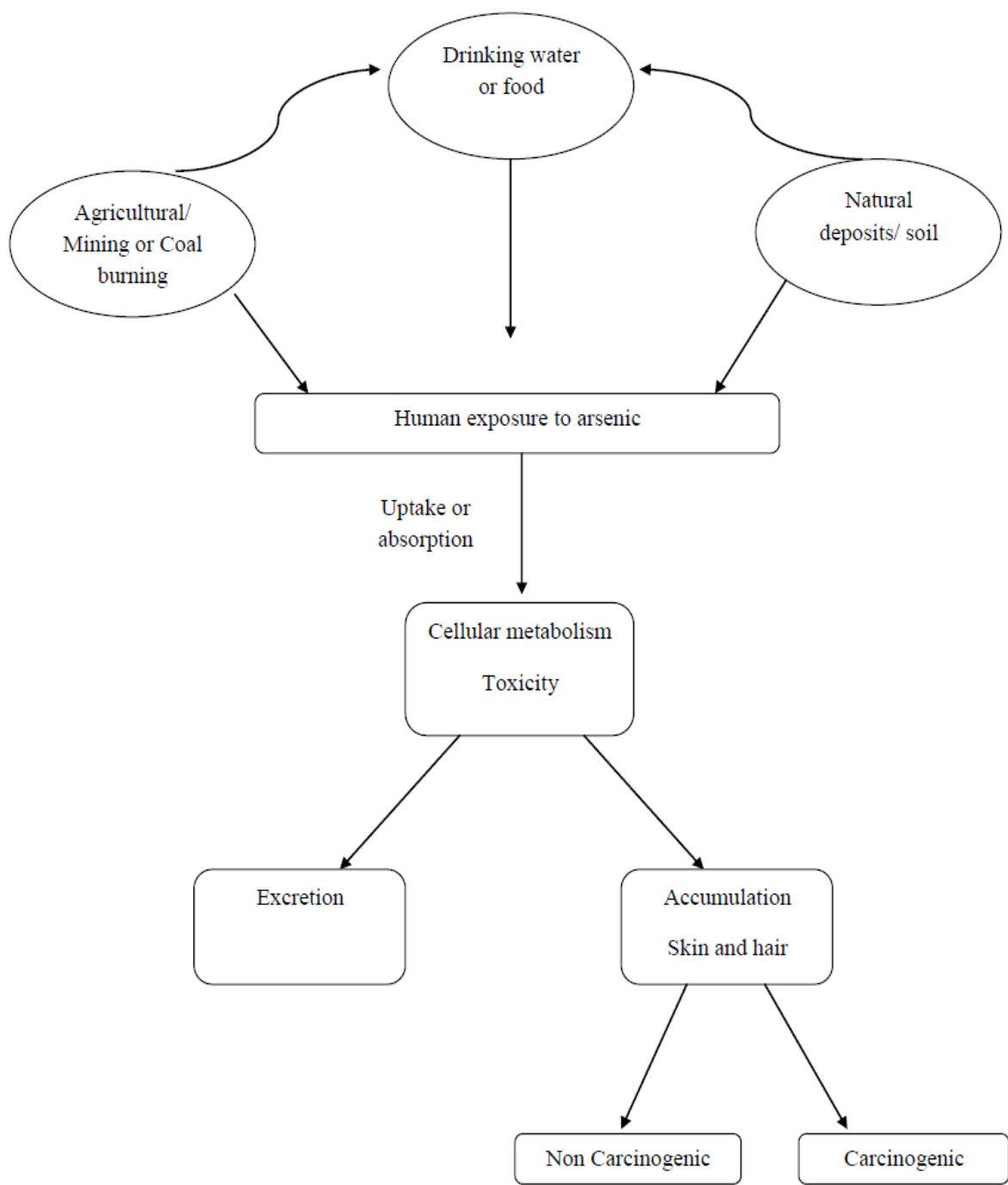
Figure 1. Flow chart of As uptake by the human body (adopted from reference 23).

To best evaluate the health and safety claims for functional food, medication and supplements, the conditions in the human body should be closely mimicked.[34] Minekus *et al.* described a novel system simulating the conditions in the large intestine,[34] to complement the simulated stomach and small intestine systems developed by the same group. Instead of looking at digested food, this novel approach deals only with undigested food in the large intestine, focusing on metabolic activities in the colon.[34] It can be used to measure the effect of genetically modifying food or of antibiotic residues in the colon to ensure their safety for the human body.[34] Bradham and his group combined in-vivo, in-vitro and physiochemical approaches to enable a responsible comparison between them and provided more compelling evidence of food safety through better risk assessment of As.[36] However, further investigation of these models is required in order to improve the experimental conditions and facilitate data analysis.

In any case, the bio-accessibility of As depends on its chemical form and on the food matrix, as well as on extraction conditions, such as temperature, enzyme composition and pH. The main concern with the different approaches that are available is the wide range of bio-accessibility values that result because of different laboratory conditions. Moreover, many studies did not include saliva in their model, as they assumed that anything that would be leached out by saliva will be released by gastric juice.[33] However, in the case of rice, the majority of bio-accessible As was reported to be leached out by saliva.[3,4] Therefore, this step should be taken into consideration for more informative measurement of bio-accessibility, as it provides information on the fraction of As that is not tightly bound to rice.[3-5]

1.5 Techniques for the determination of total As concentration

The determination of trace levels of As in environmental and biological samples requires high-sensitivity detection. Several spectrometric techniques are able to measure As concentration at those levels. Five element-specific detectors in particular have been used most often: inductively coupled plasma (ICP) mass spectrometry (MS); ICP **o**ptical emission spectrometry (OES) (often called atomic emission spectrometry)**;** hydride generation (HG) coupled to atomic fluorescence spectrometry (AFS) and graphite furnace atomic absorption spectrometry (GFAAS), as shown in Table 1. Table 3 summarizes their advantages and disadvantages.[22]

Of all the atomic spectrometry detection techniques that can be used to quantify As, ICP-MS is commonly regarded as the most sophisticated tool for ultra-sensitive analysis of food samples.[3] It indeed provides the lowest detection limit, with the widest linear dynamic range.[3] Its multi-element and isotope ratio measurement capabilities are useful if other elements also have to be determined simultaneously. Therefore, despite the fact that is has the highest operating cost, and is less robust than ICP-OES, ICP-MS has been increasingly used to measure As in environmental and biological samples, including rice.[22,40]

Table 3. Pros and cons for different analytical approaches for the determination of total element concentration.[22]

Technique	Pros	Cons
GFAAS	Sensitive Moderate operation cost 20-µL sample size Sub-ppb detection limit	Determination of a single or few elements per analysis Susceptible to matrix effects 10^2 linear dynamic range
ICP-OES	Multi-element detection Moderate operation cost ppb detection limit 10^6 linear dynamic range Relative freedom from matrix effects	Complex spectrum 300 µL/min-1 mL/min sample uptake Watch out for spectroscopic interferences
HG-AFS	Low operation cost Easy to maintain detection limit below ppb	Large sample size Determination of a single or few elements per analysis
ICP-MS	Multi-element detection ppt detection limit 10^9 linear dynamic range Isotopic analysis	High operation cost Limited to <0.2% total dissolved solids Prone to matrix effects Watch out for spectroscopic interferences 300 µL/min-1 mL/min sample uptake

1.6. Inductively coupled plasma mass spectrometry

Thirty years ago, ICP-MS was introduced to analytical laboratories for trace element analysis.[41] The usage of this technique for routine analysis has increased ever since because of its many features, in particular its very low detection limit, isotope measurement capability, wide linear dynamic range and multi-element capability, which allow the fast determination of numerous elements, thereby ensuring a high sample throughput.[3,4,44,43] As a result, it has become the analytical technique of choice in a wide variety of application areas such as nuclear industry, environmental analysis, biomedical applications, food analysis, geochemical analysis, petrochemical industry, and metals analysis.[43-46]

An ICP-MS instrument involves sample introduction, aerosol generation, and ion production in an argon plasma source, followed by separation of ions according to mass-to-charge ratio (m/z) and their detection, as schematically shown in Figure 2.
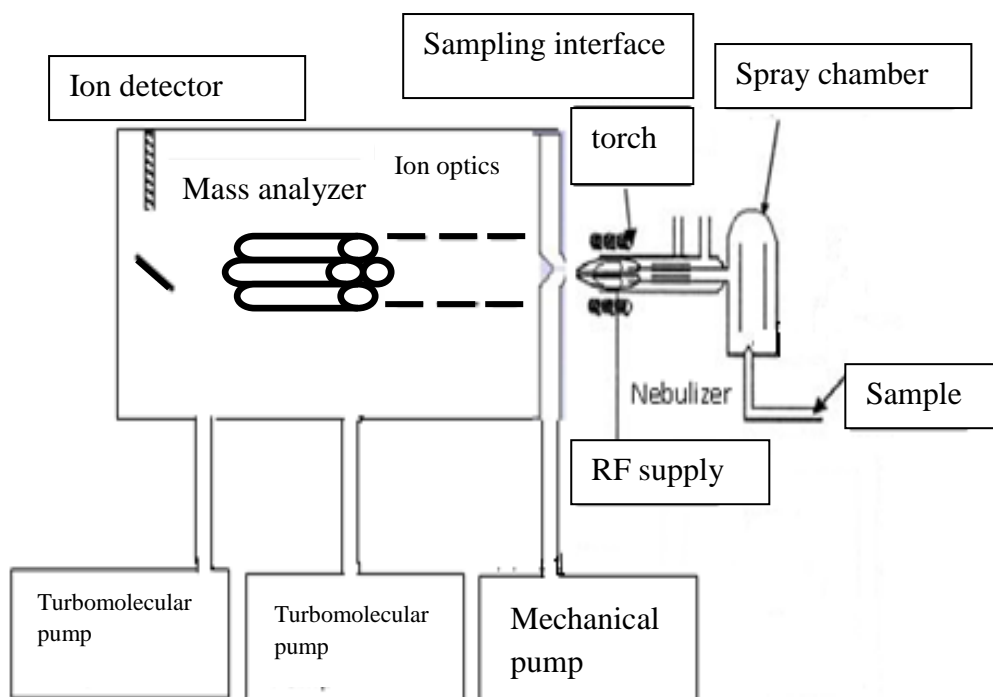
Figure 2. Basic components of an ICP-MS instrument (adopted from reference 41).

1.6.1. Sample introduction

The standard sample introduction system in ICP-MS is a nebulization system consisting of a nebulizer and a spray chamber. As a result, the most common sample in ICP-MS is an aqueous solution. The sample solution is pumped using a three-channel peristaltic pump at a flow rate normally in the range of 0.2-1 mL/min. The first channel is used to pump the sample to the nebulizer. The second one is for pumping the waste away from

the spray chamber. This ensures that pressure inside the spray chamber is uniform, maintaining the nebulizer gas flow rate into the plasma constant, and it compensates for physical interferences, such as differences in viscosities between solutions. The last channel is sometimes used to add an internal standard solution on-line, by continuously merging it with the flow of sample or standard solution through a tee before the nebulizer.[43-46]

The sample solution is transformed into a fine mist by the nebulizer upon interaction with an argon nebulizer gas. The resulting aerosol then passes through a spray chamber where large droplets are removed to the drain. These processes are necessary to produce droplets that are small enough to result in analyte ions during the few mist that they reside in the ICP.[43-46]

1.6.2 Inductively coupled plasma

Argon gas is commonly used for the three gas flows that are used to generate an ICP in a torch consisting of three concentric quartz tubes. The tangential plasma gas flow in the outer tube is used to both maintain the plasma and prevent the latter from melting the outer tube. The tangential auxiliary gas positions the plasma within the torch and also prevents the plasma from melting the inner tube. Finally, the nebulizer gas flow is used to generate and carry the sample aerosol from the spray chamber through the torch into the heart of the plasma.[47-51] As this cools the plasma in its centre, it results in the so-called central channel of the plasma, where the sample aerosol is confined.

The fine droplets exiting the spray chamber and penetrating the plasma rapidly undergo four processes sequentially due to the very high temperature (6000-10000 K) in the

ICP: desolvation, vaporization, atomization, and ionization. All elements, with the exception of He, Ne and F (which have a higher ionization potential than Ar), are ionized in an Ar ICP.[43-46] The majority are singly ionized. Only elements with a second ionization potential lower than that Ar may be partially doubly-ionized, depending on operating conditions.

1.6.3 Interface between the ICP and mass analyzer

The interface allows a sample from the ICP portion of the instrument to be introduced into the mass analyzer where ions are separated and analyzed according to their mass-to-charge ratios (m/z). The interface portion enables coupling of the ICP and MS.

The first component of the interface is the sampler cone. This is a water-cooled cone with a small orifice, allowing for the hot plasma gas to enter a depressurized chamber. In this chamber, rapid cooling, and thus rapid expansion, of the gas occurs. A fraction of this gas then passes through a skimmer cone, and into a chamber that is maintained at a vacuum that is suitable for the MS. This two-step pressure reduction allows the ions to enter the MS.

Positive ions emerging from the skimmer cone are isolated by the ion optics prior to entering the mass analyzer. The electrical potentials are set to negative values so as to guide the positively charged ions and repel the negatively charged electrons. Most photons and neutral species are also filtered out by appropriately designed ion optics.[42]

1.6.4 Mass analyzer

The quadrupole is the most widely used mass analyzer in industrial, government and academic research laboratories because of its simplicity and low cost compared to other mass analyzers. For instance, it does not require a magnetic field for the separation of ions, which reduces the cost, makes the instrument more compact and lighter, and allows a faster m/z scan speed.[42-51] A quadrupole indeed simply consists of four parallel cylindrical rods, which are normally 9-mm in diameter and 220-mm long. Through suitable application of direct current and alternating current potentials, only ions of one particular m/z are able to take a stable path between the four rods, the others being forced out of this path and evacuated. As a result, a quadrupole mass analyzer is called a mass filter, which sequentially lets ions of different m/z pass through in order to generate a mass spectrum. An important pumping system is needed to continuously create a vacuum inside the spectrometer. Several pumps are typically used to gradually reduce pressure before the ion stream reaches the quadrupole.[52]

1.6.5 Detector

A discrete dynode electron multiplier is typically used to detect ions exiting the quadrupole mass analyzer. It is installed off axis, with ions deflected into it, to minimize the background from photons. Under a high negative potential, the positively charged ions hit the cathode, causing it to produce electrons. Upon these electrons striking a dynode, many more electrons are released. This amplification process is repeated many times, ultimately resulting in a pulse of current.[42-49] The number of pulses gives an indication of the number of ions. However, not every ion striking the

detector will result in a count, especially when many ions are arriving at the detector at the same time, i.e. at high concentration.

1.6.6 Spectroscopic interferences

Even though ICP-MS has great advantages over other techniques, it possesses some limitations. One of the major problems is that of mass spectroscopic interferences, which arise when ions originating from the plasma, the sample, or a combination of the two have a m/z that is identical to that of the analyte ion.[42-51] They include isobaric interferences, such as $^{82}Kr^+$ interfering with the quantification of $^{82}Se^+$, and polyatomic interferences, such as $^{40}Ar^{35}Cl^+$ interfering with the quantification of monoisotopic $^{75}As^+$. Some common interferences and the analyte ions impacted are shown in Table 4.

These interferences are particularly problematic in the case of monoisotopic elements, such as As, as there is then no alternative isotope available for the measurement. It also leads to a significant decrease in sensitivity when the alternative isotope that is interference-free is much less abundant than the most abundant isotope (such as for V, K, etc.).

Table 4. Common interferences in quadrupole-based ICP-MS

Polyatomic ion	Interfered analyte
$^{12}C^{15}N^+$, $^{12}C^{14}N^1H^+$	$^{27}Al^+$
$^{38}Ar^1H^+$	$^{39}K^+$
$^{40}Ar^+$	$^{40}Ca^+$
$^{35}Cl^{16}O^+$	$^{51}V^+$
$^{35}Cl^{16}O^1H^+$, $^{36}Ar^{16}O^+$, $^{40}Ar^{12}C^+$	$^{52}Cr^+$
$^{38}Ar^{16}O^+$	$^{56}Fe^+$
$^{40}Ar^{35}Cl^+$	$^{75}As^+$
$^{40}Ar^{40}Ar^+$	$^{80}Se^+$

Several approaches can been used to overcome spectroscopic interferences, from physically removing the source of the interference through appropriate sample pretreatment to using a high-resolution mass analyzer, which involves double focusing of the ion beam with both an electrostatic analyzer, which acts as energy filter, and a magnetic sector for m/z selection. However, analyte loss or contamination can occur during sample pretreatment, and higher mass resolution is achieved by decreasing the width of the entrance and exit slits, which substantially decreases sensitivity. A high resolution mass analyzer can resolve several polyatomic interferences but not isobaric ones, which may be possible with sample pretreatment if the analyte and isobaric interferent have different chemical properties. A high resolution mass analyzer is also substantially more expensive than a quadrupole mass spectrometer and requires more extensive maintenance. Sample pretreatment is widely used, not only for solving the spectroscopic interference problem but also for simultaneously improving sensitivity and detection limit if the analyte can be concurrently pre-concentrated as part of the separation process.

A collision/reaction cell (CRC) is another approach that can be used to minimize spectroscopic interference. It is usually located between the skimmer cone and the

mass analyzer. The cell is pressurized with a small amount of collision or reaction gas, which can reduce a polyatomic interference by breaking it up or by reacting with either the interferent or the analyte to form an ion with a different m/z. A CRC can be used to resolve an isobaric interference if only the interferent or the analyte reacts with the reaction gas to form an ion with m/z free of interference.

A simpler approach, which does not require a more expensive instrument (i.e. equipped with CRC or double-focusing sector field) or sample pretreatment, involves using a so-called cool plasma, where the operating conditions of the ICP are changed. Under low plasma power and high aerosol carrier gas flow rate, Ar-containing polyatomic ions are drastically suppressed. However, this is achieved with a substantial sacrifice in sensitivity. Nonetheless, this approach suppresses the formation of interfering ions before they reach the mass analyzer.[42-50]

A different approach, used in this work, is a collision reaction interface (CRI), which became commercially available in 2005. As shown in Figure 3, the collision or reaction gas is introduced through a hollow skimmer cone or sampler cone, where it can act on the polyatomic interference before its entrance in the mass analyzer. Usually, He or H_2 are used CRI gases, for collision and reaction, respectively. Introduction of H_2 through the skimmer cone allows more efficient collision/reaction than through the sampler cone. For example, in the case of the $^{40}Ar^{35}Cl^+$ interference on As, the reaction proceeds as follows, resulting in ions that do not have the same mass as the analyte:

$$^{40}Ar^{35}Cl^+ + H_2 \longrightarrow {}^{40}Ar^1H^+ + {}^{35}Cl^1H$$
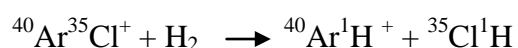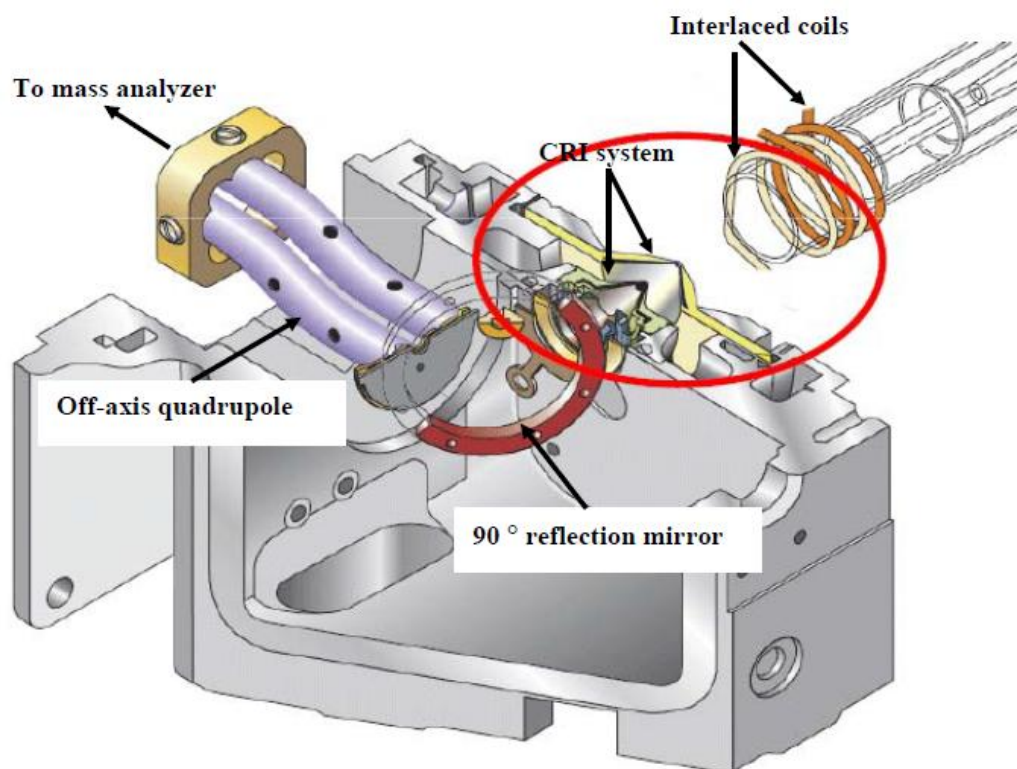
Figure 3. The CRI location in ICP-MS (picture taken from reference 45) .

1.7 Speciation analysis

In environmental chemistry, nutrition, and medicine, it is well known that the chemical form of an element present in a sample determines its biological and toxicological properties. Knowing the chemical forms of As is important for accurate risk assessment because of its species-dependent toxicity, bio-availability and physiology[3,4]. As already mentioned, the most toxic forms are inorganic forms, As(III) and As(V), followed by their methylated metabolites, monomethyl arsenic acid (MMA) and dimethylarsinic acid (DMA), whereas most other organic forms are essentially non-toxic[3,4,5]. Because As exists in both inorganic toxic forms and organic non-toxic forms in environmental and biological samples, often at trace levels, a robust, sensitive and

efficient analytical procedure should be used to selectivity and quantitatively determine the toxic forms. The determination of the chemical forms of an element is called speciation analysis.[37] A powerful analytical method is thus important for the determination of As speciation and, consequently, its potential effect on human health.[3-6]

Epidemiological studies have indicated that analyzing urine is better than blood, as it is a pathway for elimination of As from the human body.[37] Furthermore, blood has a complex matrix, which complicates analysis. To understand how As compounds are metabolized from water or food matrices, it is crucial to develop techniques that can measure metabolites and intermediate compounds formed in the process to further understand As toxicity.[37-38]

Speciation analysis is most often carried out through hyphenation of a separation technique (such as chromatography or electrophoresis) with a powerful detector. For the speciation analysis of As, the most widely used approach involves high performance liquid chromatography (HPLC) coupled to an atomic spectrometry detection, in particular ICP-OES or especially ICP-MS. Liquid chromatography techniques are the most useful separation methods because As species have soluble forms and HPLC can separate them without altering them. Moreover, HPLC is easy to couple with various detectors, as it needs a simple interface.[39] For instance, it is readily coupled to an ICP using a narrow-bore tubing.[40] However, an extraction step is required to bring the As species from solid matrices into solution, which may not be efficient or may alter some As species in the process.[38] Nonetheless, HPLC-ICP-MS is

widely used for As speciation analysis, as exemplified by selected examples in Table 5, with the majority involving ion-exchange chromatography (IEC).

Table 5. Selected examples of application of HPLC-ICP-MS to the speciation analysis of As (taken from reference 37).

Analytes	Matrix	Analytical column	Mobile phase
As(III), As(V), MMA, DMA	Waters (surface, mining, and underground)	Hamilton PRP-X100 Dionex AS7	75 mM Na_3PO_4, 2.5–50 mM HNO_3
As(III), As(V), AB MMA, DMA, AC	Water	Waters IC-Pak CM/D	$NaHCO_3/Na_2CO_3$, HNO_3
As(III), As(V), AB MMA, DMA, AC	Fish tissue	Hamilton PRP-X100	0.3% HNO_3, 10% CH_3OH
As(III), As(V)	Soils	Dionex AS9	NaOH, Na_2CO_3, $NaHCO_3$
As(III), As(V), MMA, DMA, AB	Rice, soil, straw, hair, nails	Hamilton PRP-X100	$NH_4H_2PO_4$, NH_4HPO_4, CH_3OH

1.8 Thesis objectives

The goal of this thesis is to investigate the levels of As in Arabic area rice, which has not been studied extensively before, for risk assessment of its safety. A part of this thesis also aims to educate the consumers on how to minimize the risk associated with toxic As species. To this end, the total concentration of As in rice from the Arabic area was determined, the bio-accessibility of As from that rice was measured, speciation analysis of the bio-accessible As fraction was performed to determine which portion of the bio-accessible As is toxic, and the effect of washing rice prior to cooking on the toxicity level was studied, as this would constitute a simple way for the consumer of protecting him/herself. The following approach was used:

➢   use a simple, rapid, and inexpensive on-line leaching method with artificial gastro-intestinal fluids to measure in real-time the bio-accessibility of As from rice samples, with and without first washing it with water;

➢   compare the results obtained by on-line leaching to those with a typical batch method to ensure that the on-line method provides similar results as the more time-consuming batch method; and

➢   assess the potential toxicity of As in the samples by speciation analysis with HPLC-ICP-MS.

Chapter 2

Experimental

2.1 Instrumentation

2.1.1 Inductively coupled plasma mass spectrometry

A Varian 820MS quadrupole-based ICP-MS instrument (Mulgrave, Victoria, Australia) was used. This instrument is equipped with a MicroMist concentric nebulizer (Glass Expansion) fitted into a Scott double-pass spray chamber (Figure 4) maintained at 0 °C via a computer-controlled Peltier cooling system. A tuning solution containing 5 µg/L As, Ba and In was used to optimize the torch position, gas flow rates and ions lens settings daily so as to maximize and stabilize the As signal while minimizing the level of oxide and doubly-charged ions. Typical operating conditions are listed in Table 6.
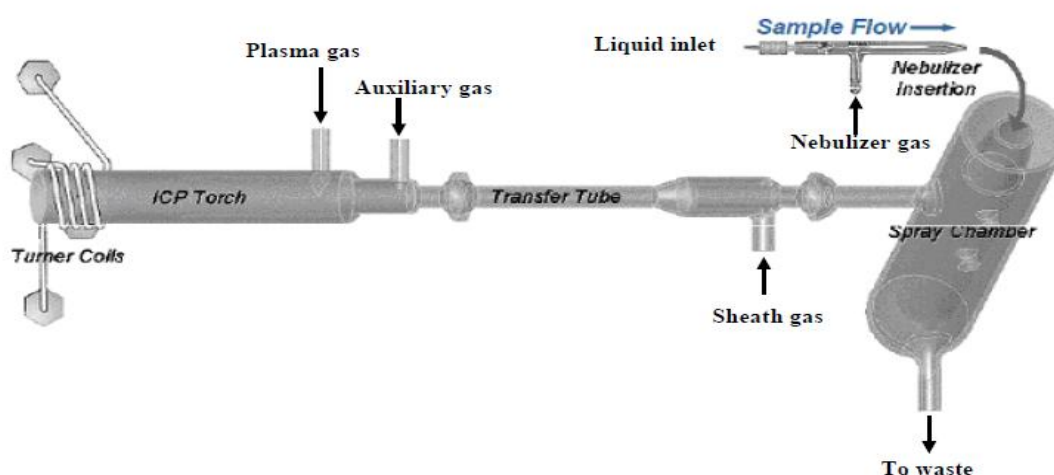
Figure 4. Sample introduction for Varian 820MS (picture taken from reference 42).

Table 6. Optimal ICP-MS instrumental parameters for the determination of As

Parameters	Selected setting
Ar plasma gas flow rate (L/min)	18
Ar auxiliary gas flow rate (L/min)	1.8
Ar nebulizer gas flow rate (L/min)	0.99
Ar sheathing gas flow rate (L/min)	0.15
Sample uptake rate (mL/min)	0.8
H_2 CRI gas (mL/min)	80

In this research, the gastric reagent had a high chloride concentration, resulting in an important spectroscopic interference ($^{40}Ar^{35}Cl^+$) on the only As isotope. Optimization of the CRI, with H_2 being introduced through the skimmer cone, was necessary to reduce this interference, the extent of which also depended on the sample matrix.[5] As indicated in Table 5, a compromise flow rate of 80 mL/min was selected to minimize the polyatomic interference without sacrificing analyte sensitivity too much. [44]

Data acquisition was done in time-resolved mode with three points per peak, one scan per replicate and 0.025 a.m.u. spacing for the proposed on-line leaching approach and for speciation analysis. In the case of the batch approach and for the analysis of digests, data acquisition was done in steady-state mode with 10-s integration while the solutions were continuously nebulized.

2.1.2 Ion-exchange chromatography

A DX600/BioLC liquid chromatography system was used for speciation analysis. This instrument consists of a GS50 gradient pump (Dionex, Oakville, Canada), an injection valve with a 25-µL injection loop, an Ion Pac AG7 guard column, and an Ion Pac AS7

(25-cm long, 4-mm diameter) anion exchange column (all Dionex, Oakville, Canada). The connection between the IEC analytical column and the nebulizer for of ICP-MS was done with PEEK tubing (0.17-mm internal diameter, 40-cm long). Gradient elution was used, starting with 0.5 mM HNO_3 in 1% of methanol in order to separate As(III), MMA and DMA, and ending with 50 mM HNO_3 in 1% of methanol for the elution of As(V). The sample solution was manually loaded into the sample loop using a syringe and automatically injected when the elution program was executed. Before injecting the sample, the syringe was rinsed with the sample solution. The column was rinsed for two hours using 0.5 mM HNO_3 to avoid peak broadening. Furthermore, stabilization with 0.5 mM HNO_3 was done for 20 min prior to each series of chromatographic separations on a given day. The experimental conditions, which were as previously optimized,[3,4] are summarized in Table 7.

Table 7. Separation conditions for IEC.

Parameter	Setting (anion exchange)
Column	IonPac AG7 (guard)
	IonPac AS7 (analytical)
Column temperature	20ºC
Mobile phase	A: 0.5 mM HNO_3, 1% MeOH
	B: 50 mM HNO_3, 1% MeOH
Elution program	100% A, 3 min; 100% B, 2.5 min
Flow rate (mL/min)	1.5
Sample injection volume (µL)	25

2.2 Reagents and standards preparation

For the determination of total As concentration, standard solutions and an internal standard solution were prepared from 1000 mg/L As and In solutions (SCP Science, Baie d'Urfé, Quebec, Canada), respectively. A 5 µg/L tuning solution in 5% (v/v) nitric acid was prepared by dilution of a 10 mg/L multi-element (Ba, Be, Ce, Co, In,

Mg, Pb, Th and Tl) standard solution. For speciation analysis, 1000 mg/L solutions of each chemical form were prepared from arsenic (III) oxide (99.999%), arsenic (V) oxide (99.9%) (all Alfa Aesar, Ward Hill, USA), disodium methyl arsenate (MMA) (97.5%) (ChemService, West Chester, USA), and cacodylic acid (DMA) (≥98%) (Sigma–Aldrich, St. Louis, USA). Furthermore, all these standard solutions were diluted to 10 mg/L and stored at 4°C in the dark to prevent decomposition or oxidation. For external calibration, standard solutions of 5, 15, 20, 25, 30, 50 and 100 µg/L were made daily by appropriate dilution. For the IEC mobile phase, doubly deionized water (DDW) (18.2 MΩ) purified using an Arium Pro UV|DI water purification system (Sartorius Stedim Biotech, Göttingen, Germany), sub-boiled HNO_3 and methanol were utilized (ACS grade; Fisher Scientific, Ottawa, Canada).

Artificial saliva, gastric juice and intestinal fluid were prepared as follows. For saliva, 6.8 g of KH_2PO_4 (ACS grade; Fisher Scientific, NJ, USA), 77 mL of 0.2 mol/L NaOH (ACS grade; BioShop, Burlington, Canada), diluting to 1 L using DDW and finally the pH was adjusted to 6.5 using 0.2 mol/L NaOH. Artificial gastric juice was prepared by diluting 2.0 g of NaCl (ACS grade; BioShop, Burlington Canada), 3.2 g of pepsin (Sigma-Aldrich, Oakville, Canada), 7.0 mL of sub-boiled HCl (ACS grade; Fisher Scientific, Ottawa, Canada) to 1 L using DDW. For intestinal fluid, 6.8 g of KH_2PO_4, 10 g of pancreatin (Sigma-Aldrich, St.Louis, USA), 77 mL of 0.2 mol/L NaOH, were diluted to 1 L using DDW and finally adjusted to pH 6.8. All HNO_3 and HCl were purified prior to use by a DST-1000 sub-boiling distillation system (Savillex, Minnetonka, USA). For the digestion of rice and residues from the bio-accessibility studies, sub-boiled HNO_3 and H_2O_2 (J.T. Baker, Phillipsburg, USA) were utilized.

2.3 Rice samples

A total of 10 rice samples were collected from local markets in Saudi Arabia representing the Middle East countries (cities): Iraq (Mosul), Iraq Tamn (Alnajaf), Egypt (Giza), Lebanon (Brucke), Saudi Arabia (Al Hasa), Morocco (Tangier), Sudan (Aweil), Yemen (Qishn), Turkey (Samsun) and Iran (Rasht), as shown in Figure 5. These samples were analyzed for total As, As bio-accessibility and speciation of the bio-accessible As fraction. The rice samples were sub-divided into two groups:

> ➢ raw rice, as purchased, was ground with ceramic mortar and pestle.

> ➢ washed and cooked rice, as consumed, was prepared by washing the rice for 5 min with DDW, adding 20 g of washed rice to 50 mL of boiling DDW and cooking it until all water was absorbed. Finally, the cooked rice sample was grinded.

Generally, five replicates were analyzed in each case and the mean was used in the calculations.

Figure 5. Sources of rice samples (picture taken from Google)

2.4 Analytical procedures

2.4.1 Continuous on-line leaching

The continuous leaching procedure was performed using mini-columns prepared as described in previous papers.[3,4] Each mini-column was prepared by packing about 0.2 g of rice wrapped in quartz wool into a PTFE tube (10-cm long, 3/15-in outer diameter, 1/8-in inner diameter). A quartz wool plug was then inserted at each end of the column. A blank mini-column was prepared by inserting two quartz wool plugs in an empty tube.

As shown in Figure 6, on-line continuous leaching simply involved continuously pumping reagents through the mini-column which was connected to the nebulizer of the ICP-MS instrument. Saliva, followed by gastric juice, followed by intestinal fluid, were then sequentially pumped through the mini-column. Both the leaching reagents and the mini-column were maintained at 37 °C using a thermostatically controlled water bath.
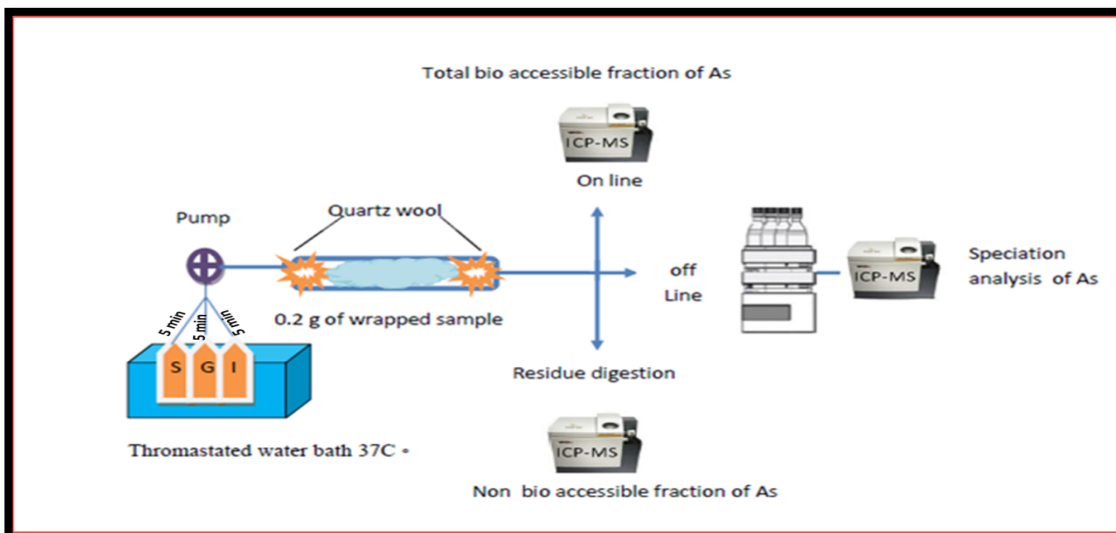
Figure 6. Summary of experimental procedure (adopted from reference 3).

Because As is weakly bound to rice,[3,4] the leaching time was set to 5 min. It was no more As was released after 5 min of leaching with each of the three reagents. Continuous ICP-MS measurements in the time-resolved mode were made during leaching to provide real-time leaching data.

A simple single-line flow injection manifold was used to carry out external calibration. Standard solutions and a blank were prepared in each reagent matrix and then injected in order of increasing concentration using a 100-µL injection loop attached to a universal automatic actuator (Anachem Ltd., Luton, England). In this procedure, because of peak area was observed to be more reproducible, it was used instead of peak height.

2.4.2 Batch method

This procedure was used to validate the on-line leaching results. For each replicate, 1 g of rice was placed in a 50-mL falcon tube with, sequentially, saliva, gastric juice and intestinal fluid. Between each addition, the test tube was shaken for 10 min for saliva and 2 h for other juices at 37°C, followed by centrifuging the samples and collecting the supernatant. The shaking time was set to simulate the human digestive tract. These supernatants were quantitatively analyzed by external calibration with internal standardization. Standard solutions were prepared in the corresponding matrix while the internal standard solution (5 ppb In) was prepared in 4% HNO_3. These solutions were mixed on-line through a Y-connector.

2.4.3 Verification of mass balance

To verify mass balance, 2.5 mL of concentrated nitric acid and 0.5 mL of hydrogen peroxide were used to digest the residue from the mini-column and batch approaches. Digestion was done at 50°C for one hour. Each digest was then diluted to 25 mL with DDW. A blank was prepared following the same procedure but without sample. Standard solutions used for external calibration were prepared in the same matrix. Internal standardization was done through the on-line addition of 5 ppb In through a Y connector. A mass balance was performed on all on-line leaching residues and batch residues. This step is important to ensure that the sum of bio-accessible fraction and that remaining in the residue was the same as the total concentration of As in the rice samples.

2.4.4 Quality assurance

To monitor for contamination during experiment procedures, a blank was used. The series of standard solutions used to establish calibration curves also allowed an assessment of linearity. A standard solution was also aspirated after every two samples and at the end to monitor for instrumental drift. External calibration was used for quantitation of total As concentration as well as for that of As species. The retention times of the different peaks during LC separation were used to identify As species in the sample through comparison with those measured using standard solutions of the four As species.

Chapter 3

Results and discussion

3.1   Analytical characteristics of ICP-MS for As determination

The limit of detection (LOD) was calculated as three times the standard deviation of 10 replicate measurements of the blank signal in each matrix, divided by the corresponding sensitivity, which is defined as the slope of the calibration curve.   All the calibration curves showed good linearity, with the square of the correlation coefficient being 0.997 or higher in the different matrices. Table 8 compares the LOD and sensitivity obtained in the different matrices, including using on-line and batch leaching procedures. They are fairly constant, considering that these values were not all obtained on the same day and thus also reflects changes in instrumental operating conditions.

Table 8. Analytical characteristics (limit of detection and sensitivity) for arsenic in different matrices using batch and on-line leaching methods

Matrix	Leaching method	LOD (µg/L)	Sensitivity ± standard deviation (counts/s per µg/L)
Saliva	On-line	0.3	500±10
	Batch	0.2	750±10
Gastric	On-line	0.4	450±13
	Batch	0.4	450±10
Intestinal	On-line	0.4	950±50
	Batch	0.2	900±10
10 % HNO_3	Full digestion	0.2	760±5

In addition, the reproducibility of the on-line leaching and batch methods for the determination of As in artificial saliva and gastric juice leachates are compared in Table 9.

Table 9. Reproducibility (expressed as relative standard deviation (RSD), n=5) of the on-line leaching and batch methods for As in saliva and gastric juice leachates

Sample	Method	Reagent	% RSD
Iraq	On-line	Saliva	6
		Gastric juice	2
	Batch	Saliva	8
		Gastric juice	6
Egypt	On-line	Saliva	10
		Gastric juice	5
	Batch	Saliva	1.4
		Gastric juice	2
Lebanon	On-line	Saliva	5
		Gastric juice	4
	Batch	Saliva	17
		Gastric juice	3
Saudi	On-line	Saliva	8
		Gastric juice	17
	Batch	Saliva	5
		Gastric juice	30
Morocco	On-line	Saliva	4
		Gastric juice	13
	Batch	Saliva	15
		Gastric juice	3
Sudan	On-line	Saliva	10
		Gastric juice	4
	Batch	Saliva	5
		Gastric juice	4
Turkey	On-line	Saliva	10
		Gastric juice	17
	Batch	Saliva	5
		Gastric juice	8
Yemen	On-line	Saliva	3
		Gastric juice	4
	Batch	Saliva	5
		Gastric juice	2
Iran	On-line	Saliva	6
		Gastric juice	7
	Batch	Saliva	15
		Gastric juice	17
Iraq Tamn	On-line	Saliva	3
		Gastric juice	2
	Batch	Saliva	2
		Gastric juice	4

3.2   Total concentration of As in rice

One main objective of this study was to evaluate the variability and trend in total As concentration in Arabic area rice. Table 10 summarize the concentrations found in rice from different Middle East countries while Figure 7 shows the percentage of As left in the samples after washing step. Rice from nine different countries was analyzed to provide a larger scope than previous studies, in an attempt to derive a more global "normal" range of As concentration. The rice from Iraq Tamn had the lowest concentration at 93 ng g $^{-1}$, followed by rice from Morocco, Saudi Arabia, and Iran. The highest concentration, larger by about an order of magnitude than in Iraq Tamn was also found in rice from Iraq, but from a different region than Tamn. This is a result of the fact that many factors contribute to As uptake by rice plants and,

Table 10. Mean As concentration (ng/g) in unwashed raw rice and washed cooked rice samples (n=5) with Student's test at 95% confidence level.

Country	Rice type	Unwashed raw rice	Washed cooked rice	Wash water	Cooked rice + wash water	Student's t-test	
						Table	Found
Iraq	Long, white	989±29	780±100	200±90	980±140	2.57	0.14
Egypt	Short, white	254±50	205±80	5±1	210±80	2.36	1.04
Lebanon	Long, white	454±60	330±29	20±12	350±31	2.26	2.07
Saudi	Long, brown	153±29	116±17	51±19	167±26	2.57	1.83
Morocco	Short, white	113±20	77±16	10±12	87±20	2.57	0.8
Sudan	Long, brown	312±60	215±38	40±15	255±41	2.26	1.75
Turkey	Long, white	298±35	216±27	17±8	233±28	2.36	2.05
Yemen	Long, white	220±27	139±5	24±1	163±5	2.26	1. 57
Iran	Short, white	193±35	108±8	27±2	135±8	2.57	2.27
Iraq Tamn	Long, white	93±11	66±11	25±3	91±11	2.26	1.14
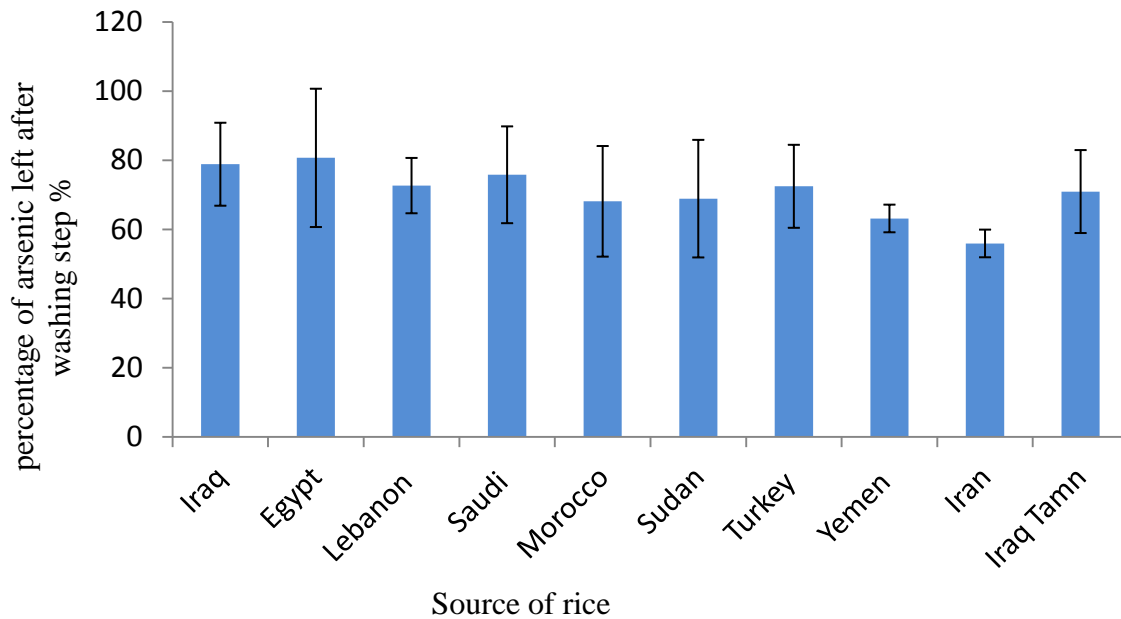
Figure 7. The percentage of As left in rice after the washing step

ultimately, determine the As content of rice grains, including the levels of As and As-containing pesticides in soil and irrigation water.[3] Indeed, the As content may correlate with environmental contamination,[55] although it is not possible to distinguish whether the higher As concentrations in these rice samples are due to genetic or other factors.[56]

Another objective of this study was to compare the data with those from the literature as well as with the recommended permissible limit in rice. Studies by Meharg *et al*., 2003[56] and Islam *et al*., 2004[57] reported that rice grain could accumulate As up to 2000 µg/kg, which is much above the WHO recommended safety limit in rice of 2.1 µg / kg of body weight per day.[22] Indeed, assuming a body weight of 60 kg, a person would only have to consume 63 g of rice per day to achieve the safety limit, whereas the average rice consumption is 100-650 g/person/day.[22] Therefore, it is curial to evaluate Arabic area rice for better risk assessment. Shraim *et al*. found a mean total As

concentration of 47 ng/g in Egypt rice (*n=2*),[58] which is comparable to 50 ng/g (*n=110*) reported by another group.[21] Both of these results are much lower than the 254 ng/g found in this work. These variations could be due to differences in rice in different districts of Egypt, just as was observed for the two different sources in Iraq.

Most of the studies conducted so far have focused on unwashed raw rice even though people consume cooked rice. These studies considered the total concentration as well as that of inorganic As. Recently, a few investigations have reported that cooked rice can be different from raw rice in terms of total As concentration as well as in content of different species of As, as As concentration in cooking water and the rice cooking process may affect the final As content of rice. Furthermore, washing rice before cooking it can make a significant difference. For example, Nolan and Beauchemin have determined that 90% of the inorganic As could be removed from one type of rice by washing with DDW.[4] However, Sengupta *et al.* reported that only up to 28% of the As washed out during the washing process,[60] while, according to Raab *et al.*, washing rice removed only 1–4% of the As from general types of rice, with the exception of Basmati rice, from which about 13–15% of the As could be removed by washing.[61] This indicates that the effect of washing varies according to the type of rice or the washing time.

Table 10 shows that, regardless of the region from which the rice came from, washing rice with DDW decreased its As content. Nonetheless, after washing, rice from Iraq and Egypt contained a significantly higher level of As than rice from Morocco, Iraq Tamn and Saudi Arabia. Figure 7 clearly shows that washing rice with DDW could

extract up to 41 % of the As. Such data is important, as a washing step can easily be added prior to cooking. The significant loss of As upon washing indicates that it is loosely bound or adsorbed on the surface of rice grains. The low level of As in Tamn rice (66 ng/g) is noteworthy. Furthermore, the mean As concentration in Tamn rice is nearly 9 times lower than the mean As value for the other Iraq rice (780 ng/g). In any case, the sum of the As concentration washed away and that remaining in the cooked rice matched the As concentration in unwashed raw rice according to a Student's t-test at the 95% confidence level (Table 10).

3.3 Bio-accessibility of As in rice

The bio-accessibility of As in unwashed raw and washed cooked rice was determined by both batch and on-line methods, as described in Section 2.4. For realistic risk assessment, each method should mimic human gastrointestinal conditions. In this thesis, artificial saliva, gastric juice and intestinal fluid were used as extracting agents to provide a good approximation of the bio-accessibility of As in rice. In this study, all surveyed rice samples (n = 5) were of white color, whereas Saudi and Sudan rice samples (n = 5) were of brown color.

The on-line leaching profile, where analyte signal is monitored as a function of time by ICP-MS, provides access to real-time information on the leaching of analyte from the samples by different reagents. As will be seen, this work confirms that on-line leaching is advantageous compared to the batch procedure, as it provides reproducible and similar results in a much more reasonable amount of time. For the first time, a simple on-line continuous leaching method was used to assess the bio-accessibility of As in

Arabic area rice. Compared with a conventional batch method, the proposed on-line method rapidly provides more representative data on bio-accessibility, as the digestion in the human body is a dynamic process.

Figure 8 shows some examples of continuous on-line leaching profiles for a rice sample. As can be seen, leaching with saliva resulted in the largest peak followed by gastric and intestinal reagents, as reported in a previous study.[3,4] This indicates that a significant amount of As is weakly bound to the rice grain, as had been reported for North American rice,[3,4] the majority of the bio-accessible As being released by saliva. The leaching profiles of As in unwashed raw rice in this work are very similar to those obtained with a certified reference material of rice flour, SRM 1568a from NIST, by Nolan and Beauchemin, 2012 using the same procedure.[3] In particular, the onset of As release by gastric juice takes three times as much time as with saliva, indicating that digestion is likely taking place. The fact that a significant amount of As is released by gastric juice is contrary to the report of Dufailly *et al.* for As in seafood.[6] While the sample matrix is different, it is also possible that using the CRI mode to alleviate the large interference from $^{40}Ar^{35}Cl^+$ helped make the $^{75}As^+$ signal distinguishable from the background signal.[6] For the intestinal profile, only a small peak is present compared to with other reagents.
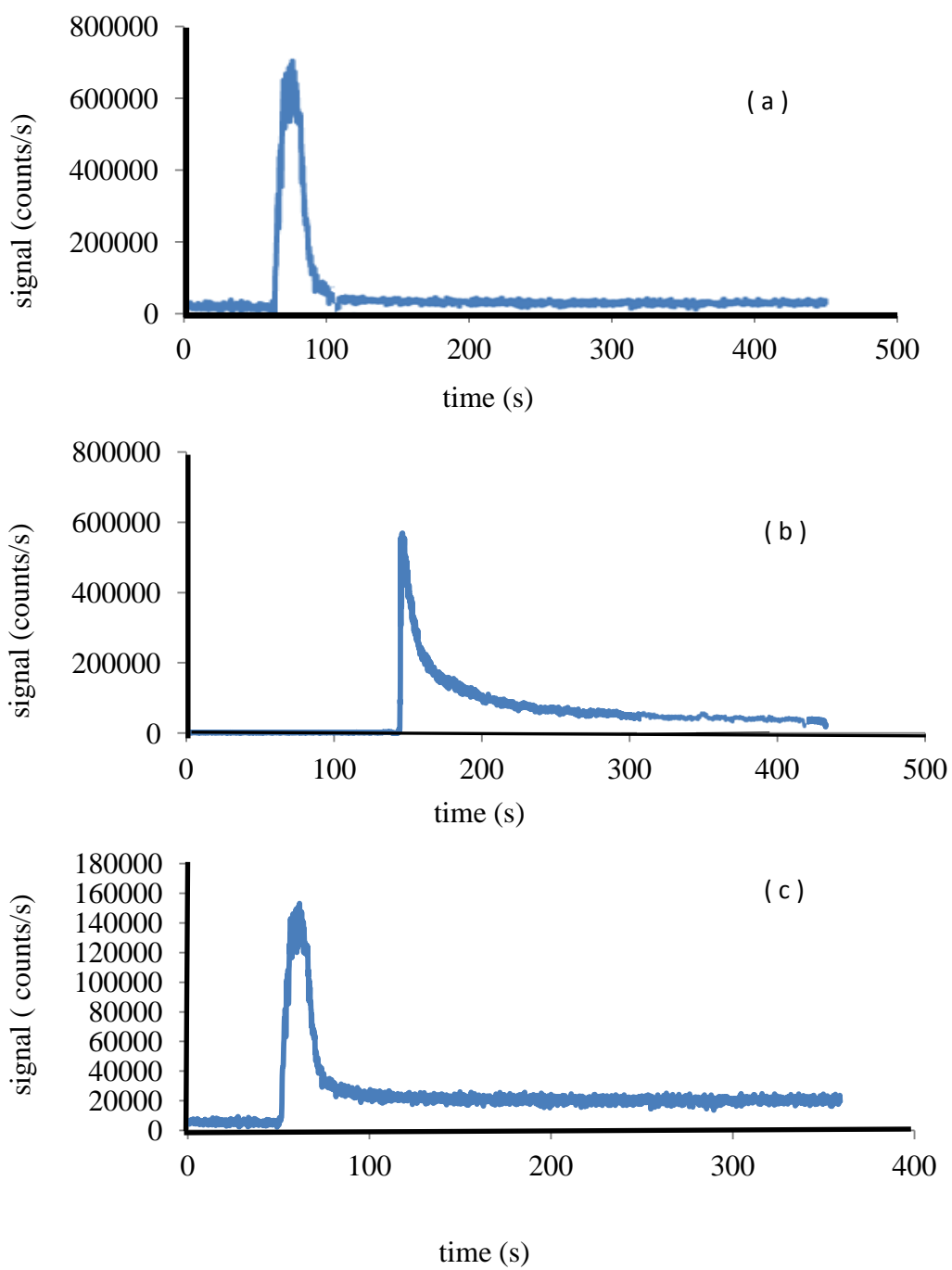
Figure 8. Examples of As leaching profiles of Iran rice by (a) saliva, (b) gastric, and (c) intestinal juice

Tables 11 and 12 shows that, using the on-line leaching and batch models, the concentration of As leached by the artificial gastrointestinal reagents are similar within error. Moreover, the fraction extracted by each fluid varied for the different unwashed raw rice samples, as shown in Figure 9 with on-line leaching.

Despite the high total As concentration in Lebanon, Sudan and Turkey rice, only a relatively small portion was found to be bio-accessible, which is in contrast to rice from Iraq, Morocco, Yemen and Iran, where approximately 90% of the As was bio-accessible. This is not surprising because it is well known that the bio-accessibility of As in rice varies greatly between samples. The global range for As concentration in rice clearly shows the effect of locations of the rice produced. For instance, rice from Egypt contained a mean As concentration of 0.05 mg/kg while rice from China had a mean As concentrations of 0.41 mg/kg.[21]

Figure 9. Bio-accessibility percentage of As in unwashed raw rice samples (n=5) by on-line leaching.

Table 11. Concentrations in ng g^{-1} of leached and total As obtained by the on-line leaching and batch methods for unwashed raw samples

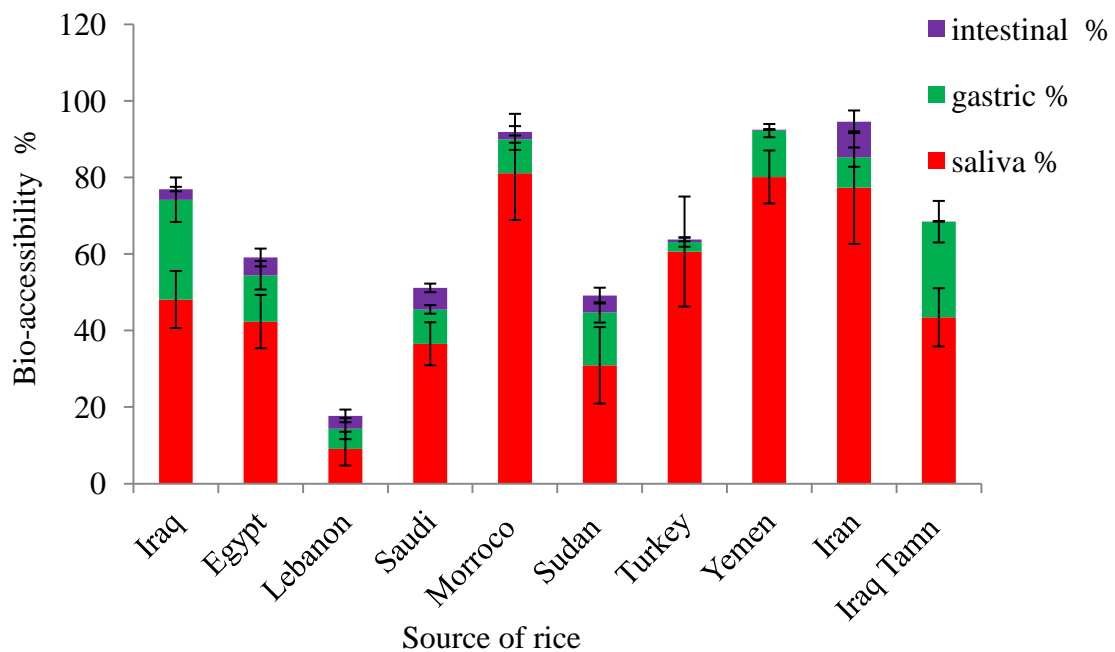| Country	Leaching method	Saliva	Gastric juice	Intestinal juice	Total leached	Leached + residue	Total expected	Student's t-test* Found	Table
Iraq	On-line	301±52	298±90	20±6	620±100	850±110	989±29	1.70	2.36
	Batch	413±64	224±50	24±5	661±81	859±84		2.36	2.57
Egypt	On-line	85±12	19±10	12±8	116±18	212±20	254±50	1.23	2.36
	Batch	91±15	26±8	10±5	127±18	215±27		1.50	2.36
Lebanon	On-line	45±16	8±5	3±2	56±17	350±59	454±60	2.08	2.57
	Batch	33±16	19±10	12±6	64±20	362±61		1.84	2.30
Saudi Arabia	On-line	77±10	9±2	2±1	88±10	156±18	153±29	1.4	2.36
	Batch	65±10	16±2	10±2	91±10	178±18		1.38	2.57
Morocco	On-line	58±14	7±1	6±1	71±14	89±14	100±20	1	2.26
	Batch	86±13	10±1	2±5	97±14	106±14		0.54	2.36
Sudan	On-line	91±21	34±2	9±1	134±21	309±21	312±60	0.10	2.57
	Batch	105±34	47±9	15±7	167±36	340±36		0.90	2.26
Turkey	On-line	120±5	9.0±0.2	9±1	138±5	317±8	298±35	1.11	2.57
	Batch	194±46	8±4	2±2	204±46	320±46		0.81	2.36
Yemen	On-line	97±9	16±2	9±2	122±9	201±9	220±27	0.93	2.30
	Batch	139±12	21±3	0.5±0.1	161±12	174±12		2.21	2.26
Iran	On-line	118±6	19±1	8±2	145±7	164±7	153±37	0.44	2.30
	Batch	184±35	19±6	22±7	225±36	238±36		2.42	2.57
Iraq Tamn	On-line	25±2	22±2	9±2	56±3	100±3	93±11	1.40	2.57
	Batch	40±7	23±5	0.08±0.04	63±9	92±9		0.03	2.57

(n=5)

* Comparing Leached + residue to Total expected

Table 12. Bio-accessible As concentrations in ng g^{-1} obtained by the on-line leaching and batch methods for washed cooked rice samples

Country	Leaching method	water	Saliva juice	Gastric juice	Intestinal juice	Leached + residue	Expected	Found	Student's t-test	
									Table	Table
Iraq	On-line	190±75	258±15	189±4	46±6	668±17	858±99	988±100	2.06	2.26
	Batch	230±50	298±75	230±15	16±4	544±77	960±134	988±100	0.37	2.22
Egypt	On-line	5±3	89±3	10.0±0.5	1.7±1.5	146±3	152±11	210±80	1.61	2.26
	Batch	5.0±0.1	93±10	17±3	1.7±0.5	112±10	154±11	210±80	1.55	2.22
Lebanon	On-line	21±14	48±3	5±2	6±1	59±3	320±75	354±29	0.94	2.30
	Batch	25±1	50±14	9±3	6±2	65±14	355±76	354±29	0.02	2.22
Saudi	On-line	45±8	35±3	9±2	5±4	109±5	154±11	167±17	1.44	2.30
	Batch	51±9	45±8	10±3	4±2	59±9	152±22	167±17	1.25	2.26
Morocco	On-line	14±5	8±3	8±1	1.5±0.5	27±3	41±7	62±16	1.95	2.26
	Batch	20±4	10±5	9±3	2±1	21±6	49±13	62±16	1.46	2.26
Sudan	On-line	31±10	77±3	14±1	3.0±0.3	192±3	223±14	255±38	1.77	2.57
	Batch	36±2	69±10	8±3	3±1	80±10	228±12	255±38	1.5	2.30
Turkey	On-line	67±12	73±7	18±3	6±3	188±8	218±30	233±27	0.83	2.22
	Batch	81±2	88±31	4±3	1±1	93±31	271±42	233±27	1.72	2.57
Yemen	On-line	32±4	87±3	24±9	9±4	132±10	164±11	163±5	0.20	2.26
	Batch	24±1	88±4	23±1	7.0±0.5	118±4	167±6	163±5	1.00	2.26
Iran	On-line	29±21	69±4	45±3	16±2	176±5	195±22	210±8	1.50	2.30
	Batch	27±2	76±12	40±7	25±3	141±14	205±14	210±8	0.71	2.26
Iraq Tamn	On-line	32±10	27±8	12±15	2.0±0.2	97±17	129±22	91±11	2.37	2.30
	Batch	25±3	19.0±0.3	7.0±0.3	6.00±0.12	32±1	107±11	91±11	1.82	2.26

It is worthwhile to mention here the importance of performing risk assessment by determining bio-accessible As instead of simply monitoring total As concentrations. This present study provides data for the percentage of As leached by the different artificial gastro-intestinal fluids by the on-line method. After the rice was exposed to the various fluids, the residue was digested to verify if any As remained in the rice. Mass balance was checked by comparing the total amount leached plus that left in the residue with the expected total concentration determined by digestion of the rice, at the 95% confidence level.

The As concentration released by saliva from the Iraq variety of rice was 413 ± 64 ng/g and that in Iraq Tamn was 40 ± 7 ng/g. This significant difference in As concentration reflects the large difference in total As content, which likely arises because the rice came from different districts of Iraq. However, Figure 9 shows that the percentages that are bio-accessible are very similar between these two unwashed raw rice samples.

The As concentrations leached by saliva from polished white Iraq Tamn, Lebanon and Morocco rice were 40 ± 7, 33 ± 16 and 86 ± 13 ng/g, respectively, while those in two brown rice samples from Saudi Arabia and Sudan were 65 ± 10 and 105 ± 34 ng/g, respectively. Generally, the As concentration in brown rice is greater than in polished rice because elimination of the outer bran layer from rice grains during polishing to make the grain color white likely removes some As.[22]

The As concentration released was generally higher in saliva than in gastric and intestinal juices, demonstrating that As extraction gradually decreased upon passing the three successive reagents. This observation suggests that studying bio-accessibility is significant to prevent any deleterious effects on human health. In general, saliva released the largest percentage of As compared to gastric and intestinal juices, as previously reported by Nolan and Beauchemin in rice samples[3,4] and by Dufailly *et al*. in seafood samples.[6]

The comparison between As concentrations using both on-line and batch methods in case of unwashed raw samples did not show any significant difference. However, the sum of bio-accessible As and that in the residue with the on-line method is closer to the total As concentration measured after digestion of rice samples in Table 11. This is not surprising because studies conducted on the measurement of bio-accessibility using different methods to predict As uptake for human health risk assessment have demonstrated that bio-accessibility may change from one method to the next.[22]

While the results presented so far focused on unwashed raw rice, a more realistic risk assessment would compare the total and bio-accessible As in unwashed raw rice with the concentrations found after washing and cooking in order to assess the effect of cooking and washing rice on its As content. Previous observations indicate that cooking had a slight impact on bio-accessibility in both brown and white rice, and that a washing step can release a significant amount of As.[4] However, Meharg *et al*. reported that the concentration of As removed during the washing step was negligible

because the water was contaminated with As, which contributed to elevated As content in rice.[22]

Table 12 shows that the concentration of bio-accessible As in the washed cooked rice samples using the on-line method varied from 493 to 41 ng/g with a mean value of 121 ng/g, which is comparable to the findings with the batch method, which ranged from 535 to 28 ng/g, with a mean value of 144 ng/g.

Compared to unwashed raw rice, the concentration of bio-accessible As in washed cooked rice was decreased. For example, the As concentration observed for Iraq rice sample before washing and cooking (849 ng/g) is higher than that for the same sample after washing and cooking (668 ng/g). Hence, 190 ng/g As was removed during the washing step prior to cooking. The results of the present study suggest that washing 1 − 3 times until the wash water became clear significantly lowered the risk of As exposure from rice consumption by removing some of the As. Therefore, a meaningful assessment of As exposure risk due to consumption of rice should examine washed and cooked rice. For instance, although using safe water for washing rice significantly minimize the As bio-accessibility in cooked rice, consumption of rice is still an important pathway of As exposure to the consumers in Iraq.

In the present study one objective was to evaluate the effect of washing and cooking procedure on the As concentration, to investigate the risk of As exposure to the consumer through washed cooked rice. This study reveals that in order to minimize the total As content in cooked rice, a large volume of As-safe water should be used for

washing and cooking. Hence, people should also be aware that cooking rice without washing can cause a serious health issue, which sometimes happens in some countries.

The determination of As content in the water washes indicates that it is a good way to reduce the As exposure. This study shows that from 7% to 41% of the extractable As in rice can be removed during the washing step, as shown in Figure 10. It further revealed that the percentage of As in water washes varies to some extent according to rice source, which may be because of different interaction between As and the rice grain.
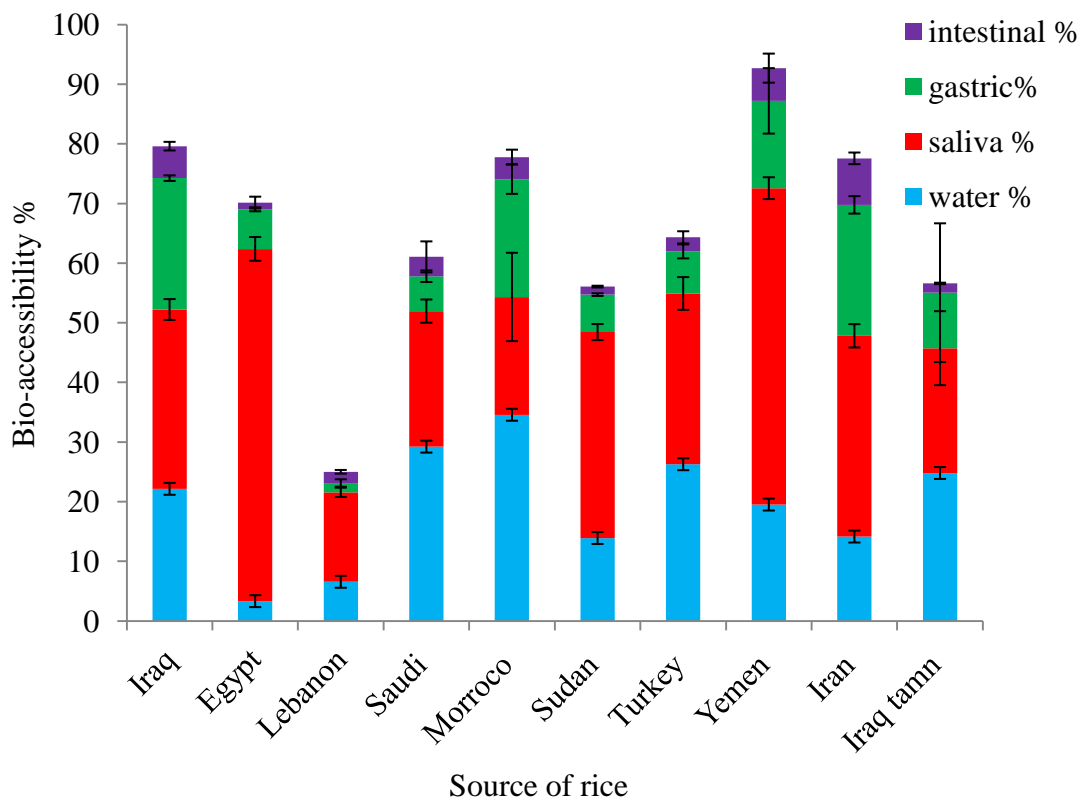

Figure 10. Bio-accessibility percentage of As in washed cooked rice samples (n=5).

It is of great interest also to investigate how much time is needed to remove As from rice grains. The on-line leaching method provides the opportunity to monitor the analyte signal in real-time, as the sample is being washed with DDW. This readily allows one to determine the ideal amount of time for washing rice.[4] The leaching profile in Figure 11 shows how long it typically takes to remove some As during the washing step. It shows that about 100 s is sufficient to help make rice grains safer for human consumption.
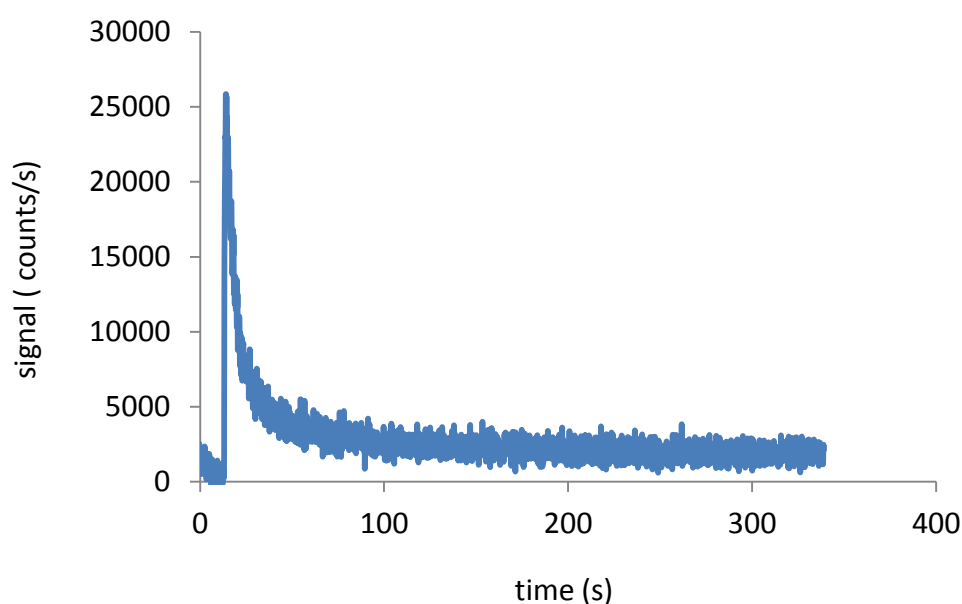

Figure 11. Example of leaching profile of the washing step for Turkey rice.

3.4 Effect of cooking on As bio-accessibility

In the laboratory, rice samples were cooked following the traditional procedure practiced in the world, using As-free water. Measurement of the bio-accessibility of As in cooked rice is an important step because it represents the amount of As in the cooked rice that is available for uptake into the human body. Analysis of As in unwashed raw and washed cooked rice was performed using the same artificial gastrointestinal fluids. To make the risk assessment more relevant, the present study also looked at the wash

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water to provide a better assessment of the bio-accessibility of As in rice. Indeed, despite the high total As concentration, a portion of As can be washed away during the washing procedure. This study revealed that the sum of the bio-accessible fraction and that remaining in the washing water of cooked rice generally agreed with the bio-accessible concentration found for unwashed raw rice at the 95% confidence level using a Student's t-test. These results are in agreement with the previous study by Horner and Beauchemin.[3]

On the other hand, the results proved that the total As concentration in washed cooked rice is less than that in unwashed raw rice because a significant portion was washed away. Although not only the As concentration of the cooking water but also the cooking methods have significant influence on As retention in washed cooked rice compared to unwashed raw rice,[63] washing rice prior to cooking can decrease both total and inorganic As content in cooked rice. Hence the washing step appears more significant than the cooking step for minimizing the As content of cooked rice.

3.5 Speciation analysis of saliva leachates

Different As species, i.e. As(III), As(V), MMA and DMA, in the rice samples (n = 5) were leached out using artificial saliva. The chromatographic separation and subsequent quantification of different As species in saliva leachates were performed as described in Section 2.1.2 The typical positions of the peaks in the chromatogram corresponding to different As species present in a standard solution are shown in Figure 12. As mentioned in a previous work,[3] injection of saliva leachates predominantly resulted in co-elution of As(III) and MMA. Therefore, a five-fold dilution was first

done in order to achieve good resolution of the two peaks. And the analytical column

had to be regularly cleaned with 2 M HNO$_3$. Since the majority of As bio-accessibility

was leached out by saliva in rice samples, speciation analysis was only performed on

saliva leachates. The LOD was 0.02 ng g^{-1} for As(III), 0.04 ng g^{-1} for As(V) and
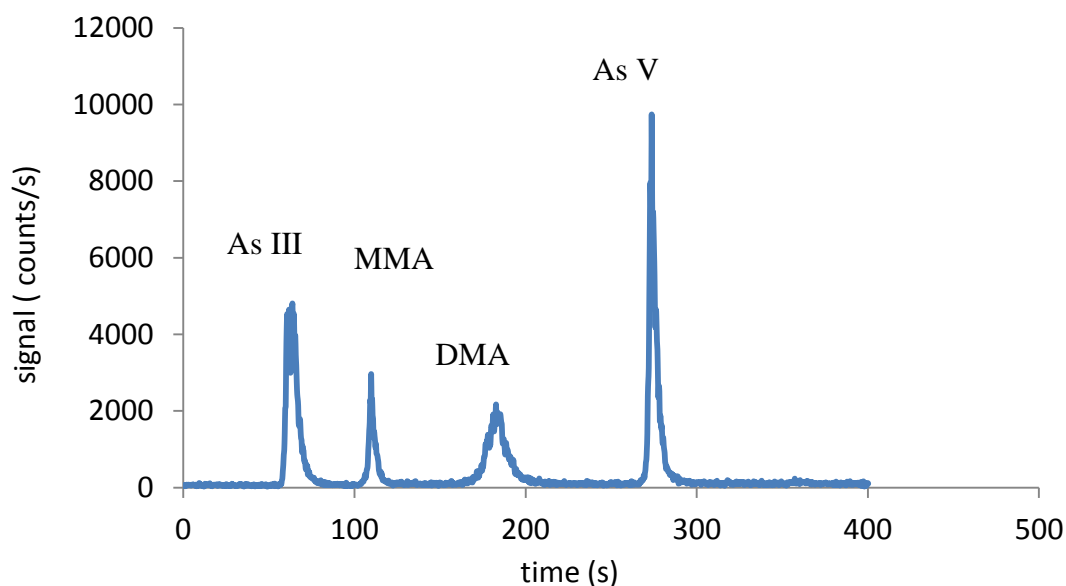
0.03 ng g^{-1} for MMA and DMA.

Figure 12 Chromatogram for a standard solution containing 5 μg L^{-1} of each As species
in fivefold diluted saliva.

Table 13 summarizes the speciation analysis results for saliva leachates, which indicate

that As is mainly present as inorganic species As(III) and As(V). It is noteworthy that

in Iraq, Egypt, Morocco, Sudan, and Iran, the total extractable As by saliva for the

unwashed raw rice samples is mainly represented by As(V) in the range of 13 ± 1.5 to

126 ± 17 ng/g with a rare occurrence of MMA in the range of 0.23 ± 0.03 to 36 ± 7

ng/g. Some DMA was also found in the same samples in the range of 0.016 ± 0.001 to

40 ± 1 g/g. Bio-accessibility concentration of As species in saliva leachates of washed

cooked rice and unwashed raw rice in Figure 13. Unwashed raw rice from Saudi

Arabia, Turkey and Iraq Tamn contains only As(V), the other species not being detectable. The distribution of As species in Lebanon and Yemen rice are very similar to one another: the toxic forms As(V) followed by As(III) predominate, the concentrations of DMA and MMA being low or not detectable. Although As in most rice exists mostly in inorganic forms, a washing step reduces the potential health risk to the population.

Table 13. Concentrations in ng/g obtained for the speciation analysis of bio-accessible As in saliva off-line leachates of unwashed raw ice and washed cooked rice (n=5).

| Country	Washing step	As(III)	MMA	DMA	As(V)	Sum of As species	Total As leached	Student's t test	
								Found	Table
IRAQ	Unwashed	91±14	36±7	40±1	126±17	293±23	301±52	0.46	**2.30**
	Washed	79±7	85±9	46±14	36±7	246±19	258 ±15	0.36	**2.26**
EGYPT	Unwashed	4.1±0.3	16±3	32±4	60±5	112±7	85±12	0.14	**2.26**
	Washed	*N.D.	0.1±0.0	81±12	N.D.	81±12	89±3	0.20	**2.22**
LEBANON	Unwashed	18±3	13±3	N.D.	13±2	44±5	45±16	0.28	**2.57**
	Washed	43±9	N.D.	N.D.	0.02±0.01	43±9	48±3	0.83	**2.30**
SAUDI	Unwashed	N.D.	N.D.	N.D.	46±2	46±2	77±10	1.34	**2.26**
	Washed	N.D.	N.D.	N.D.	36±7	36±7	35±3	1.72	**2.30**
MOROCCO	Unwashed	0.11±0.01	0.23±0.03	0.05±0.01	70±4	70±4	58±14	1.10	**2.30**
	Washed	N.D.	N.D.	N.D.	5±2	5±2	8±3	1.08	**2.22**
SUDAN	Unwashed	0.06±0.02	0.4±0.01	0.016±0.001	88±20	89±20	91±21	0.14	**2.22**
	Washed	N.D.	N.D.	2±1	71±10	73±10	77±3	1.37	**2.57**
TURKEY	Unwashed	N.D.	N.D.	N.D.	100±19	100±19	120±5	2.30	**2.57**
	Washed	N.D.	N.D.	N.D.	75±6	75±6	73±7	1.40	**2.26**
YEMEN	Unwashed	0.03±0.01	N.D.	N.D.	77±2	77±2	97±9	1.53	**2.30**
	Washed	N.D.	N.D.	N.D.	82±12	82±12	87±3	0.68	**2.22**
IRAN	Unwashed	4±1	16±3	32±4	112±60	164±60	118±6	1.69	**2.57**
	Washed	N.D.	N.D.	N.D.	66±6	66±6	69±4	1.00	**2.30**
TAMN	Unwashed	N.D.	N.D.	N.D.	36± 2	36± 2	25±2	2.05	**2.26**
	Washed	N.D.	N.D.	N.D.	31±3	31±3	27±8	1.26	**2.22**

*N.D. not detectable
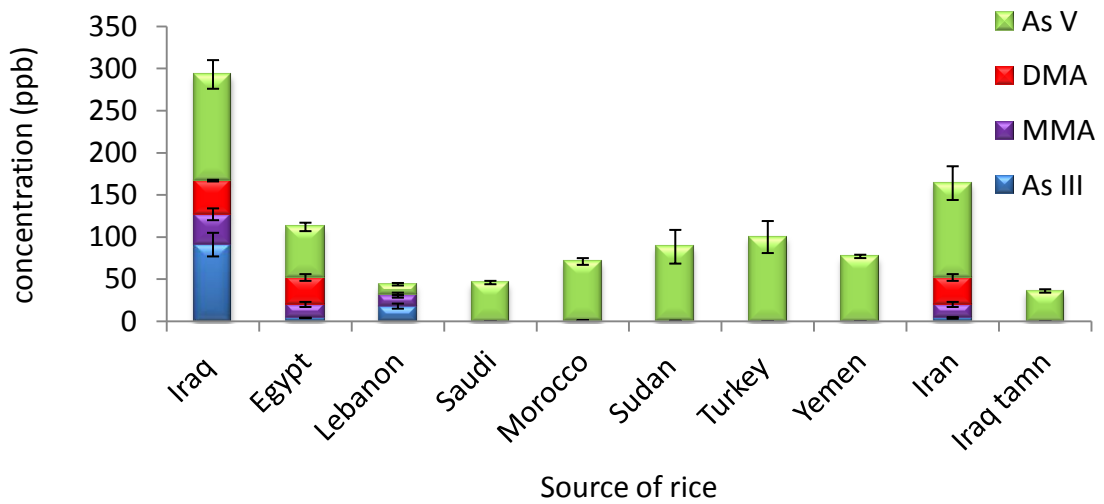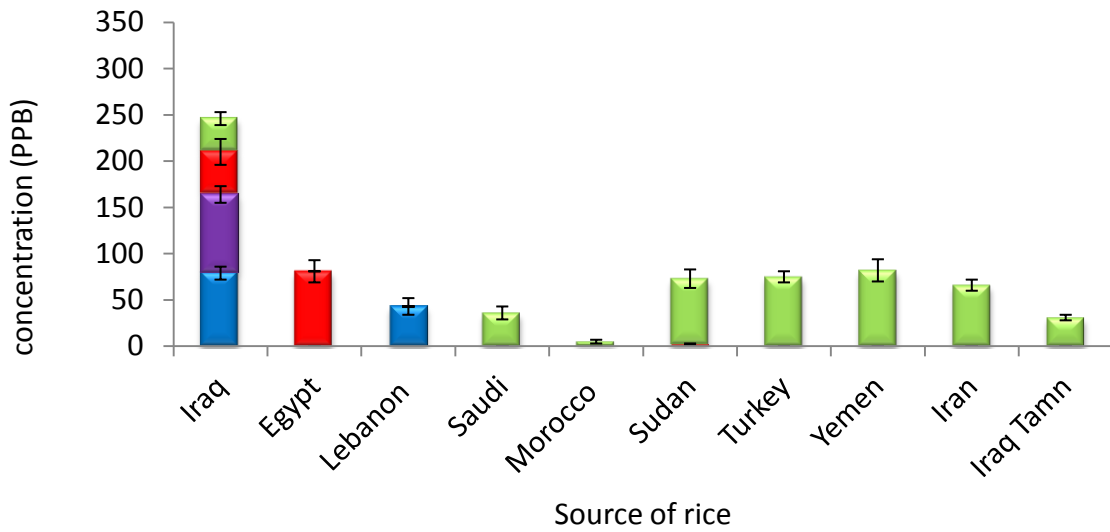
Figure 13. Bio-accessibility concentration of As species in saliva leachates of washed cooked rice (top) and unwashed raw rice (bottom).

Indeed, washing reduced the total concentration of As species in saliva leachates of rice samples from 8 of the 10 countries, the exceptions being Lebanon and Yemen. Washing rice from Saudi Arabia, Turkey and Iraq Tamn reduced the concentration of As(V), which was the only As species detectable in saliva leachates of both unwashed raw rice and washed cooked rice. In the case of Morocco, Sudan and Iran, not only was

the As(V) level reduced but As(III), MMA, DMA, present in unwashed raw rice, were washed away, as they were not detectable in washed cooked samples. In the future, this could be further verified through speciation analysis of the wash water.

Figure 14 shows examples of chromatograms obtained for unwashed raw rice as well as washed cooked rice samples. The results indicate the predominance of As(V) over As(III), MMA, and DMA, as seen in Table 13. Although As(V) is considered to be more toxic than organo-arsenic compounds (see Table 1), this investigation indicates that the concentration of As(V) could be reduced by washing rice, which would reduce the risk of arsenic poisoning. This work further revealed that the concentration of MMA content was low or not detectable. However, in some regions, such as Iraq and Lebanon, As(III) was predominant, while this was the case of DMA in Egypt.

Many studies indicate that 91.7% and 100% of the extractable As is present as inorganic species in rice.[22] The sum of the concentrations of each species leached by saliva agrees with the total bio-accessible concentration of As for both the on-line and the batch methods according to a Student's t-test at the 95% confidence level.
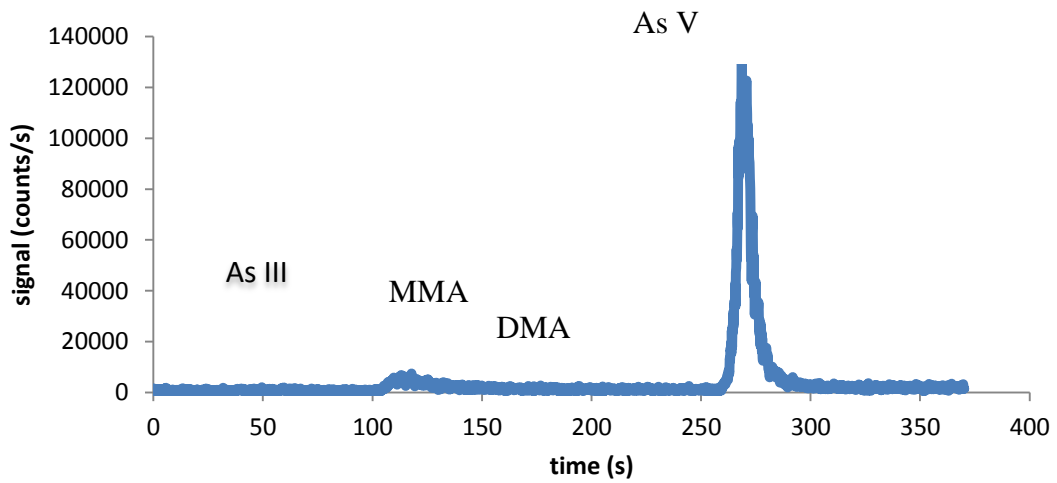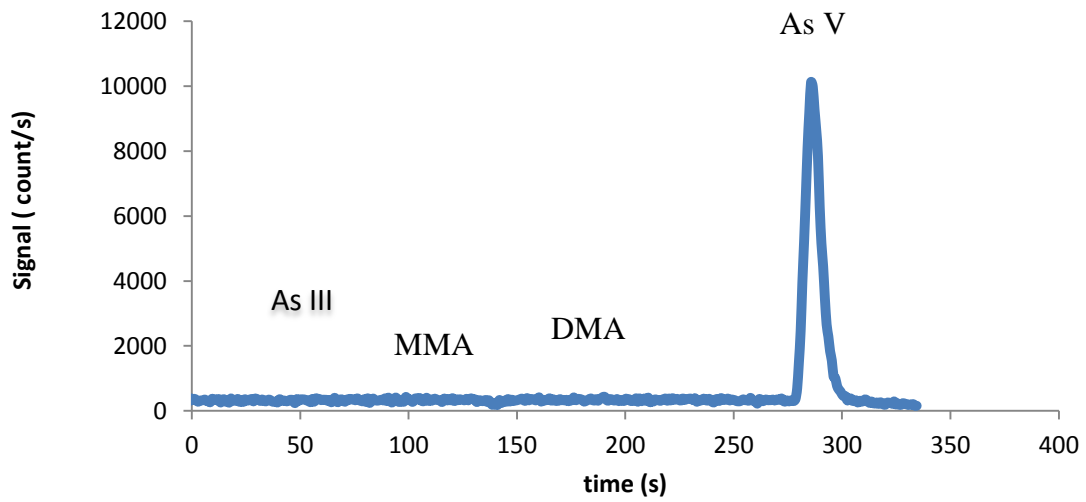
Figure 14. Chromatograms of saliva leachates from Morocco rice samples, i.e. washed cooked (top) and unwashed raw (bottom).

3.6 Effect of cooking on As speciation

The results of As speciation analysis in unwashed raw and washed cooked rice samples in Table 13 show that the distribution of As species in washed cooked rice is very similar to that in unwashed raw rice, except in three cases, Egypt, Lebanon and Iran, where the As species content in washed cooked rice changed compared to unwashed raw rice. There was also an increase in the level of As(V) in saliva leachates of rice from Yemen after cooking. However, it is evident that the change (both increase and decrease) in the relative distribution of As species concentrations because of cooking is not significantly changing the As bio-accessibility.

In Egyptian rice, the increase in DMA observed after cooking may be a consequence of transformation of other arsenic species (inorganic arsenic and MMA) present in unwashed raw rice. For Lebanon rice, cooking rice appears to essentially convert As(V) and MMA into the most toxic form, As(III). In the case of Iran rice, the contents of As(III), As(V), MMA and DMA in unwashed raw and corresponding washed cooked rice were different too. Only As(V) was left after washing and cooking. The process responsible for the observed decrease in some species and increase in others in cooked rice is unknown. [62]

Chapter 4

4.1 Concluding remarks

In this work, even though a high total concentration of As was found in several rice samples, because As bio-accessibility was low, this made the rice less harmful to most consumers, with the exception of people who consume rice in extremely large quantities, or cook their rice in As-contaminated water. These outcomes highlight the importance of taking bio-accessibility into consideration in order to gain an accurate measure of the potential damage to humans. The procedure proposed in this thesis, for total arsenic determination in rice by full digestion followed by ICP-MS analysis, is an accurate method that was previously validated with the analysis of SRM 1568a (rice flour).[3]

The accumulation of As in rice grains varies with the type of grain, being highest in brown rice and lowest in white rice. This is due to the removal of the outer bran layer of the rice grain during the refining process to produce white rice.

An accurate and precise method was used for the quantification of bio-accessible As in rice grains. The results confirmed the effectiveness of the on-line leaching method in comparison to the batch method. In addition, it requires less time for sample preparation and analysis, while also providing valuable real-time leaching data. Furthermore, this procedure reduces the potential contaminations because it is

conducted in a closed system. This work showed that the total As concentration and fraction of bio-accessible As in rice can differ largely between countries.

In this project, a washing step was added to the on-line leaching method to provide significant extra health safety data. In fact, this step helps to pick the "ideal" washing time for rice samples, which minimizes the loss of essential nutrients while maximizing the extraction of toxic elements. This study revealed that washing rice with As-free water decreased the total as well as inorganic As contents in cooked rice by 20-40%. On the other hand, the cooking procedure had little effect on As bio-accessibility.

Arsenate, arsenite, DMA and MMA were also quantified in this work in order to assess the toxicity of As accurately in rice, as it depends on the chemical form present in rice samples. Therefore, it may not be enough to only use accurate and precise methods for the determination of total As and of As bio-accessibility, but reliable methods for the determination of As species may also be warranted if the bio-accessible fraction could be problematic if all in toxic form. Ion-exchange chromatography coupled to ICP-MS with a gradient elution program was used to fully separate As species within 5 min, after a 6-min stabilization. The speciation analysis results showed that arsenate was the most abundant As species found in these samples. Also, the distribution of As species in washed cooked rice is very similar to that in unwashed raw rice, with the exception of rice from Egypt, Lebanon and Iran.

4.2 Future Work

Accurate methods for the determination of multi-element content in rice need to be addressed further in order to establish reliable toxicity studies in an attempt to produce national recommendations and regulations for the toxicity content of foodstuffs, particularly rice.

The bio-accessibility of different elements (toxic and essential) in rice samples should also be studied, along with the effect of washing time on their release. In this way, the ideal washing time could be established to maximize the removal of toxic elements while minimizing the washing out of essential elements. As well, artificial saliva containing enzyme should be studied to see if the enzyme has a significant effect on bio-accessibility. Using this enzyme would indeed make the artificial saliva more representative of actual human saliva. In addition, the method should be applied to a broader range of samples, including brown and white rice, as well as long and short grain rice from each country to establish if one type of rice is inherently safer, irrespective of its origin, and if washing rice prior to cooking is a simple safety measure that is similarly effective for all rice types.

An improvement of the reproducibility of the procedure would be beneficial. Therefore, a study of the effect of the different parameters of the mini-column preparation, such as length and diameter of the mini-column, leaching flow rate, and amount of sample, will be conducted.

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