

ASSESSING THE BIOAVAILABILITY AND BIOACCESSIBILITY OF TOTAL ARSENIC AND ARSENIC SPECIES IN ENVIRONMENTAL SAMPLES

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ABSTRACT

Studies on the bioavailability of As, of impacted vegetables; and As bioaccessibility in the edible parts of carrots (*daucus carota*), beets (*beta vulgaris*) and quinoa (*chenopodium*) growing in polluted soils through “*in vitro*” gastrointestinal process; the As speciation both in the edible parts of vegetables and in their gastrointestinal extracts have been performed. Elemental analysis and As speciation have been performed by ICP-MS and LC-ICP-MS, respectively. The Arsenic recovery after the *in vitro* gastrointestinal digestion was from 98, 90 and 40% for carrots, beets and quinoa, respectively; with no significant transformation of original As species. These studies provide a clearer understanding of the impact that As and other contaminant elements may present in the population of this high polluted Chilean region.

Keywords: arsenic speciation, bioaccessibility, bioavailability, vegetables, gastrointestinal digestion.

INTRODUCTION

Since 1915, the Northern Chilean economy has been mainly supported by the exploitation of copper mining resources around the Atacama Desert. This mining activity produces heavy metals contamination of the water and aquifers of the Loa river basin and its tributaries such as the San Pedro, Salado and San Salvador rivers^{1,2}.

Associated with copper and the other heavy metals from the mines, is arsenic, a very dangerous environmental element. This As is present in the *enargita* (Cu_3AsS_4), one of the sulphur minerals of the mines. In this area, there are also copper mineral smelting plants and during the pyrometallurgical process, the As is released as As_2O_3 both in the gas phase and in fine particles^{3,4}. It is believed that suspended dust particles and intake through the drinking water are the major sources of As exposure for living beings in the area. The concentration of As in the water of the Loa river's hydrographic basin is very different depending on place, and changes from no contamination in the Copiapó river (snow melting, concentration $<0.005 \text{ mg L}^{-1}$) to extreme contamination in the San Salvador river ($2\text{-}2.5 \text{ mg L}^{-1}$)⁵.

Between 1958 to 1970, the Antofagasta population had drinking water from the Loa river with a total As concentration of about $1000 \mu\text{g L}^{-1}$ (most probably in the form of As(V))⁶. Successive water treatment has been decreasing the water concentration to an actual annual average of $50 \mu\text{g L}^{-1}$, but not in all places. This is far from the WHO's recommended amount of $10 \mu\text{g L}^{-1}$. A study in 1992, showed the contamination of As in water and vegetables growing in the Antofagasta region^{8,9}.

Therefore, natural geographic contamination, anthropological action, the zone's desert climate, and the exposure of vegetables, animal and human being communities to these extremes in environmental conditions, makes for a very delicate ecological equilibrium similar to what can be seen in other well-known parts of the world such as India, China, New Zealand^{10,11}, etc. Cancerous and non-cancerous diseases associated with As contamination reach alarming levels in the Antofagasta region, as evidenced by the incidence of cardiovascular^{6,12} bronchial lung, bladder diseases and renal cancer¹³.

In addition to As, the characteristics of the mines themselves result in high concentrations of other heavy metals, which also have a high environmental and human impact. Indeed, the possible contaminating elements in the region (Cu, Cr, Mn, Hg, Pb and Cd), are included on the list of priority toxic pollutants published by the World Health Organization (WHO)⁷ and the International Register of Potentially Toxic Chemicals (IRPTC)¹⁴.

The evaluation of food from the point of view of arsenic and trace element contribution to human diet requires knowledge not only of the total element content but also their absorption rates in the gastrointestinal tract. A way to quantify the absorbable fraction in trace element bioaccessibility is the “*in vitro*” simulation of digestion processes¹⁵.

It is also recognized that inorganic arsenic are probably the most dangerous forms of arsenic in food, being As (III) more toxic than As(V)¹⁶. The formation of As (III) from the As(V) species present in natural environmental conditions, obeys in some case to an initial reduction before the methylation or other bigger species formation (arsenobetain, arsenosugar, etc.), in the detoxification

mechanism that happens in microorganisms and also in more complex living beings¹⁷⁻¹⁹. The methylation process first produces monomethylarsonic acid (MMA) and in a second step dimethylarsinic acid (DMA) both less toxic than inorganic forms because their interaction with tissues is weaker²⁰.

There have been several reports of arsenic speciation in vegetables growing in natural or contaminated soils²¹. According Johnson et al²², rice contains the highest concentrations of inorganic arsenic species compared with other tested products. Broccoli, lettuce, potato, carrots, etc. can concentrate arsenic when the soil or the irrigation water contains As (V). In most of the vegetables, arsenic is taken up by plant roots via macro-nutrient transporters^{23,24}. The coupling of the liquid chromatography (LC) to high sensitive detectors like ICP-MS²⁵ and CV-AFS²⁶, are the most frequent techniques for the detection and quantification of these inorganic and methylated As species at trace level after their smooth extraction from the samples²⁷.

The objectives of this study have been centralized on the polluted Chiu Chiu region located Northern Chile that is about 10 Km from Calama and 235 Km from Antofagasta city. The study performed which addressed the following points: i) The total As content in the edible parts of vegetables growing in the area where the indigenous population live. ii) The As species present in the edible parts of lyophilized vegetables. iii) The bioaccessibility of the total As in the edible parts of these vegetables under “*in vitro*” reproduction of gastrointestinal process and finally. iv) The possible transformation of original As species during the gastrointestinal process.

As far as we know, such global studies have not been performed yet in the area under investigation.

MATERIALS AND METHODS

Instrumentation

An inductively coupled plasma mass spectrometer, (HP-4500, Agilent Technologies, Analytical System, Tokio, Japan) operating under normal multi-element tuning conditions was used for total arsenic and metals analysis. The main analytical parameters of the ICP-MS are the following (R.F. power: forward 1350 W; reflected 2.2 W; coolant argon flow rate 14 L min^{-1} ; auxiliary argon flow rate 0.9 L min^{-1} ; integration time 0.1 s per point, point per peak 3).

For As speciation the ICP-MS has been used as the detector system after LC species separation. The column effluent was directly introduced into a Meinhard-type concentration glass nebulizer and a double-pass Scott-type spray chamber with a surrounding water jacket maintained at 5°C . Single ion monitoring at m/z 75 was used to collect the data. For chromatographic separation, a high-pressure pump (LDC Division, Riviera Beach, Florida, USA) was used as a sample delivery system. Samples of $100 \mu\text{L}$ were introduced through a $0.45\text{-}\mu\text{m}$ nylon syringe filter into the injection valve Rheodyne 9125 (USA). The connections between the HPLC and the ICP-MS were made of polytetrafluoroethylene tubing (i.d. 0.5 mm). The HPLC column was a Hamilton PRP-X100 ($10\mu\text{m}$, $250 \text{ mm} \times 4.1 \text{ mm}$, Torrance, CA, USA) and Phenomenex precolumn ($25 \times 2.3 \text{ mm}$ 12-20 μm).

All signal quantification was performed in the peak area mode. The

peaks were integrated using either ICP-MS Plasma Lab software or Grams/32 software (Galactic Industries, Salem, NY, USA).

Sonopuls Ultrasonic Homogenizer (HD 2200 Bandelin Electronic Berlin, Germany) and a Selecta Vibromatic shaker were also used in the sample extraction process.

Arsenic Species Solutions:

The Standard of As (III) of $1000 \pm 2 \text{ mg L}^{-1}$ was prepared in HNO_3 (2%, m/m), TraceCERT™ Ultra (Fluka, Sigma-Aldrich, Steinheim, Germany). The standard of As(V) 1000 mg L^{-1} , was prepared in HNO_3 (2% m/m), CertiPUR (Merck, Darmstadt, Germany). Dimethylarsinic acid (DMA) and methylarsonic acid (MMA) standard of 1000 mg L^{-1} , were prepared from methyl disodium arseniate ($\text{Na}_2\text{CH}_3\text{AsO}_3$, 99% Supelco, Bellefonte, PA, USA) and cacodilic sodium trihydro ((CH_3)₂AsNaO₃·3H₂O) 98%, Fluka) TraceCERT™. These solutions were kept at 4°C in the dark. Working solutions were daily prepared and then diluted with water to the final concentration.

Reagents

All reagent were of analytical or reagent grade. HNO_3 60% (Scharlau), HCl (15%) (Scharlau), HClO_4 (Scharlau), (acetic acid 99.8% (Panreac), hydroxylamine hydrochloride 99% (Carlo Erba), hydrogen peroxide 33% (Panreac), ammonium acetate 97% (Scharlau), ammonium phosphate 98% (Scharlau), alpha-amylase (Sigma Aldrich, Spain 11800 USP Units), pepsine (Sigma Aldrich, Spain, enzymatic activity 944 U/mg protein), pancreatin (Sigma Aldrich, Spain, activity equivalent to 4 x US Pharmacopoeia specifications/mg pancreatin), lipase (Sigma Aldrich, Spain, 944 USP units), protease (Sigma Aldrich, Spain, 11800 USP units), bile extracts (glycine and taurine conjugates of hydoxycholic and other bile salts Sigma Aldrich, Spain). Deionized water (18.2 MΩ cm), obtained with a Milli-Q water system (Millipore, Inc., Spain) was used in the solutions preparation. Plastic and glassware was maintained in 10% HNO_3 for 24 h before use.

Simulated gastrointestinal juices

Salivary juice (pH 6.8 ± 0.1) was prepared by dissolving 0.15 g NaH_2PO_4 , 0.029 g amylase, 0.1 g KCl and 0.1 g NaCl and make up to 100 mL with water. Gastric juice (pH 1.8 ± 0.1) was prepared by dissolving 6.0 g of pepsin and 0.88 g sodium chloride (Sigma) in water, adjusting pH 1.8 with HCl and making up to 100 mL with water. Intestinal juice was prepared just before use by mixing equal volumes of A and B solutions. (Solution A: 1.5 g pancreatin, 0.44 g sodium chloride, 30 mg alpha-amylase and water up to 50 mL. Solution B: 2 g bile salts, 0.44 g sodium chloride and water up to 50 mL)²⁸.

Vegetable samples

Chilean vegetables samples were collected from 3 different places of the Chiu Chiu Chilean area. About 10 Kg of carrots (*daucus carota*), beets (*beta vulgaris*) and quinoa (chenopodium) were taken. For comparison about 2 Kg of the same type of vegetables of Spanish origin were purchased. The samples were sent fresh from origin to laboratory and kept frozen under nitrogen atmosphere. The Figure 1 shows the places were Chilean carrots, beets and quinoa.

Certified Reference Materials (CRMs):

Rice flour (1568a NIST), white cabbage (BCR 679) and tomato leaves (NIST 1573a) were used as certified reference materials. The concentration of As and other heavy metals was determined on these CRMs following the same method as the one described below for the vegetables under study.

Sample treatment for determination of the total As content in lyophilized vegetable samples by ICP-MS analysis

The Carrots and beets were peeled with a plastic knife and the edible part was blended in a titanium blender until a homogeneous mash was achieved. A fraction of about 2 Kg of flesh were lyophilized, bottled and maintained at 4°C until analysis. No other treatment apart from drying at 80°C and maintaining it at 4°C was performed on the quinoa.

For mineralization of vegetables about 0.5 g, were digested in PTFE vessels adding 5 mL of concentrated HNO_3 and 2 mL of H_2O_2 . The mixture was maintained for about 4 hours in open air until vapor stoppage before microwave digestion under the following conditions: 600 W, 200 °C, 20 min and 150 psi^{29,30}. The digested samples were filtered, transferred to polyethylene containers diluted with 0.2 M HCl to appropriate volumes and stored at 4°C until analysis. The solid residue was discarded. Reagent blank did not show any significant contamination.



Figure 1: Chiu Chiu Region

The analytical characteristics of the method were the following: detection limits (DL) in $\mu\text{g Kg}^{-1}$, 5.0. Relative standard deviation (RSD), 2%. Method validation was performed by triplicate with the above mentioned CRMs.

“*In vitro*” gastrointestinal digestion of vegetables for arsenic bioaccessibility by ICP-MS:

Samples were digested following a simulated digestion process with three steps²⁸.

Salivary digestion:

About 1.0 g of lyophilized sample was placed in a 25 mL erlenmeyer with 5 mL of salivary juice and 2 mL water and shaken in a vibromatic Pselecta (508 U/min) for 5 min for degassing. The mix was heated for 5 min in a water bath (37-39 °C), shaken 5 min, heated again for 5 min and centrifuged at 5000 rpm during 20 min to separate salivary and solid phases.

Gastric digestion:

10 mL of the prepared gastric juice, was added to the solid fraction after salivary digestion, heated for 1 h in a water bath, shaken for 30 min in the vibromatic Pselecta (508 U/min), adjusting the pH to 1.8 with HCl (15%), and heated 3 h more at 36-39 °C. The mixture was centrifuged at 5000 rpm for 20 min to separate the soluble and solid fractions.

Intestinal digestion:

10 mL of the prepared intestinal juice was added to the solid fraction obtained before and the procedure was repeated as in the gastric digestion. The remained solid extract was discarded.¹⁵

The three liquid extracts after digestion were mineralized in a microwave oven and appropriately diluted and analyzed by ICP-MS.

Extraction procedures for As(III), As(V), MMA and DMA speciation by LC-ICP-MS in vegetables

Procedure 1: Methanol: water extraction. To 0.5 g of the fresh homogeneous mesh of edible part of vegetables, was added 5 mL of methanol: water (1:1) solution. The mixture was heated for 30 min, at 55°C, focalized ultrasonic probe for 5 min and centrifuged at 5000 rpm for 20 min at ambient temperature. The procedure was repeated in the solid fraction. The two liquid extracts were mixed and evaporated to a final volume of 2 mL.

Procedure 2: Enzymatic extraction. To 0.3 g of the fresh homogeneous mesh of edible part of vegetables, was added, 10 mg alpha-amylase and 3 mL water. The mixture was focalized ultrasonic probe for 60 s in a bath ice and 30% power. After that, was added 30 mg protease, focalized ultrasonic probe for 120s at 30% power and centrifuged for 10 min at 4000 rpm. The solution was made up to 2 mL for analysis³¹.

The two procedures were performed in triplicate. Table 1 shows the chromatographic conditions for As species separation.

Table 1: Chromatographic conditions for As species separation

PROCEDURE 1	MOBILE PHASE	(NH ₄)H ₂ PO ₄ (A: 5mM; B:25mM), pH 6
	GRADIENT	0-15 MIN (A: 100-0 Y B: 0-100) 15-25 MIN (A: 0-100 AND B: 100-0) FLOW RATE: 1 ML X MIN. RECONDITIONS: 25-30 MIN (A:100, B:0)
PROCEDURE 2	MOBILE PHASE	(NH ₄)H ₂ PO ₄ (10mM), PH 6
	ISOCRATIC	FLOW RATE 1 ML X MIN. RECONDITIONS: 25 MIN (A:100)

Statistical analysis

All experiments were performed at least in triplicate. Differences were considered significant when $p < 0.05$ following the t Student's test³².

RESULTS AND DISCUSSION**Determination of total arsenic content and bioaccessibility in carrots (*Daucus carota*) beets (*Beta vulgaris*) and quinoa (*Chenopodium quinoa*)**

Table 2 shows the total concentration of As in the lyophilized flesh of carrots and beets and the fresh quinoa growing in contaminated soils; the concentration of the same vegetables bought in the Spanish market, and the As concentration rate between Chilean and Spanish samples. The concentrations of As tested in the Spanish samples fall within those levels reported for uncontaminated vegetables³³.

Table 2: Total As content in Chilean and Spanish lyophilized vegetables. Results expressed as mean \pm SD (mg kg⁻¹) for n = 3 determinations.

Vegetables		As
Carrots	Flesh-C ⁽¹⁾	0,52 \pm 0,04
	Flesh-C ⁽²⁾	0,54 \pm 0,06
	Flesh-S ⁽¹⁾	0,02 \pm 0,01
	Factor	24
	Peel-C ⁽¹⁾	1,62 \pm 0,02
	Peel-S ⁽¹⁾	0,15 \pm 0,01
Factor	11	
Beets	Flesh-C ⁽¹⁾	0,62 \pm 0,05
	Flesh-C ⁽²⁾	0,64 \pm 0,07
	Flesh-S ⁽¹⁾	0,02 \pm 0,00
	Factor	26
	Peel-C ⁽¹⁾	3,20 \pm 0,06
	Peel-S ⁽¹⁾	0,22 \pm 0,01
Factor	14	
Quinoa -C ⁽¹⁾		0,20 \pm 0,02
Quinoa -C ⁽²⁾		0,21 \pm 0,04
Quinoa -S ⁽¹⁾		0,01 \pm 0,01
Factor		20

C: Chilean; S: Spanish. Factor: Metal concentration in Chilean /Spanish samples.⁽¹⁾

Table 3: shows results for the White Cabbage, Rice Flour 1568 and Tomato Leaves (NIST 1573a) CRMs, used to validate the total As determination.

	Certified value (mg K ⁻¹)	Experimental value (mg K ⁻¹)	% Error
White Cabbage BCR 679 ⁽¹⁾	7,0 \pm 3,8	8,5 \pm 2,0	21
	Certified value (mg K ⁻¹)	Experimental value (mg K ⁻¹)	
Rice Flour 1568 ⁽¹⁾	0,29 \pm 0,03	0,29 \pm 0,04	2
Tomato Leaves (NIST 1573a) ⁽¹⁾	0,112 \pm 0,004	0,110 \pm 0,006	2

Table 3: Quality control and traceability of the total As concentrations, using standard reference materials (SRM).

Analysis performed by ICPMS⁽¹⁾

Table 4, shows the total As content and the As concentration in the different extracts of the "in vitro" gastrointestinal digestion process. In order to check the possible interference of chloride in the ⁷⁵As signal due to the formation of ⁴⁰Ar³⁵Cl⁺ during ICP-MS analyses, the signal at m/z 75 of salivary, gastric and intestinal juices were registered. It was observed that the signal intensity for the three extracts was negligible and did not differ from that of a blank solution, which indicates that the procedure is free of interference by chloride.

The comparison of the total As content and total extracted As shows that practically the whole As is bioaccessible from carrots and beets (98% and 90% respectively). However, for quinoa, only about 40% of As is bioaccessible. Fresh carrots and beets have a water content of about 85%, while in quinoa it is only about 10%. It has been reported that the average consumption per inhabitant of quinoa in the Andean countries is about 1.15-2.35 Kg per year³⁴ and about 2-6 Kg per year for carrots and beets. Considering: the maximum intake of 2.5 Kg per year of quinoa and of 6 Kg per year of carrots and beets; the As concentration in the lyophilized vegetables; the water content and the As bioaccessibility, the calculated accessible doses of As expressed as μ g As per year, is about 470 for carrots, 550 for beets and 180 for quinoa. Therefore, quinoa seems to be the vegetable with the lower toxicological risk from the bioaccessible point of view.

Table 4: Bioaccessibility of As in the digestion process of Chilean samples by ICP-MS. Results expressed in lyophilized vegetables as mean \pm SD (μ g kg⁻¹) for n = 3 determinations.

	Carrots	Beets	Quinoa	
Extracted As (μ g Kg ⁻¹)	Salivary	313 \pm 18	289 \pm 28	34 \pm 4
	Gastric	135 \pm 12	186 \pm 21	31 \pm 4
	Intestinal	65 \pm 9	84 \pm 10	13 \pm 6
Total extracted As (μ g K ⁻¹)	513 \pm 45	559 \pm 38	78 \pm 15	
Total As (μ g K ⁻¹)	520 \pm 41	616 \pm 57	196 \pm 24	
Recovery (%)	98 \pm 3	90 \pm 4	40 \pm 4	

As speciation in carrots, beets and quinoa:

In the speciation studies, an important step is the extraction efficiency of the species present in the sample. Table 5 shows the total As concentration in the vegetables under study referred to fresh flesh and the extraction efficiency in the methanol water 1:1 extract and in the enzymatic extracts, by ICP-MS procedures. As can be seen, methanolic extraction is appropriate for extraction of the total As species in carrots and quinoa, but not for beets, in which the enzymatic extraction is necessary.

Table 5: Methanolic and enzymatic extraction efficiency for total As by ICP-MS in carrots, beets and quinoa, and As species contribution (%) to total As content in both extracts. n= 3.

Vegetables	Methanolic Extraction Efficiency (%)	Enzymatic Extraction Efficiency (%)
Carrots	Total As: 97 \pm 5	Total As: 12 \pm 5
	As(III): 45 \pm 7	As(III): <DL
	As(V): 43 \pm 5	As(V): <DL
Beets	Total As: 10 \pm 2	Total As: 95 \pm 9
	As(III): <DL	As(V): 20 \pm 5
	As(V): <DL	Unknown: 70 \pm 3
Quinoa	Total As: 99 \pm 6	Total As: 7.4 \pm 0.9
	As(III): 15 \pm 4	As(III): <DL
	As(V): 82 \pm 9	As(V): <DL

Table 5 shows the species As(III), DMA, MMA and As(V) detected in the methanolic (for carrot and quinoa) and enzymatic (for beet) extracts by LC-ICP-MS. Extracts were appropriately diluted in each case. The species for beets, were characterized by spiking the sample with 5 μ g L⁻¹ of As(III) and As(V). For carrots, only As(III) and As(V) are present. Relative peaks quantification, show about a 50% distribution between the two inorganic As.

These results agree with results obtained in previous work²⁷. For quinoa, 90% of the As is present as As(V), been the As(V) concentration of As(III) about 10 %. No other species are found in the methanolic fraction. Beets show a different behavior. As species present in this vegetable is only slightly extracted in methanol - water but almost 100% in the enzymatic hydrolysis.

As speciation in the salivary, gastric and intestinal extracts for carrots and quinoa.

In order to know if the digestion process could change the original species present in the food, changes in their bioaccessibility, a speciation analysis has been performed in the salivary, gastric and intestinal juice of carrots and quinoa.

The analysis was performed by LC-ICPMS under the same conditions than performed in original samples. Table 6 shows the results obtained for carrots. Similar results were obtained in quinoa sample. The percentages are given respect to the whole content in the corresponding extract.

Table 6: Comparison of the As species distribution in the methanolic extract for carrots and quinoa and enzymatic extract for beets and the As species distribution in the whole gastrointestinal extract with respect to the total As extracted.

Vegetables	Species distribution% Methanolic or enzymatic extract	Whole Gastrointestinal extract
Carrots	As(III): 45 ± 7 As(V): 43 ± 5	As(III): 50 ± 10 As(V): 36 ± 15
Beets	As(III): <DL As(V): 20 ± 5 Unknown: 70 ± 3	As(III): <DL As(V): 33 ± 7 Unknown: 70 ± 12
Quinoa	As(III): 15 ± 4 As(V): 82 ± 9	As(III): <DL As(V): 75 ± 7

When vegetables grow in contaminated soils, the bioavailable part of the elements present in the soil can pass to the vegetable through intake mechanisms in which the microorganisms present in the soil are frequently involved, and the proper structure of the vegetal^{35,36}. The Spanish samples are in the level reported for plants growing in non-contaminated soils^{37,38}. It is relevant that only As(III) and As(V) are present in carrots and quinoa, this is not the case of beets, were As(V) and mainly an unknown As species are presented (Table 6). Sacarose is the sugar extracted from the beets; therefore an As-sacarose or derivatives could be the unknown species. Considering that inorganic species are the most toxic ones, beet could be the vegetable with the lower concern³⁹. It is known than the terrestrial plants in general have very low efficient mechanisms of biometilation⁴⁰. Probably also the drastic conditions of these soils from the environmental point of view make it difficult for the microorganisms present in the soil, to develop biomethylation mechanisms giving methylated species able to be absorbed through the roots of these vegetables as proposed by Ye and col.⁴¹

It is well known than the main contribution of contaminant (and also nutrient) elements to living beings arise from consumption of food and water. This happens also for arsenic. As can be seen, the “*in vitro*” digestion process carried out in the studied vegetables liberates the whole As from carrots and beets (Table 4), and therefore this vegetables intake could be dangerous if concentration in the samples is high enough to be a toxic doses. Quinoa behavior is different and only about 40% of As is liberated. For the three vegetables, about 50-60% of the total concentration extracted is released in the salivary juice. It is important to highlight that for beets and carrots, results about total concentration of As, and the sum of the bioaccessibility fractions of As under the digestion process are very similar. However, the quinoa behavior is different and only is recovered after the whole digestion process about a 40%. Fresh carrots and beets have a water content of about 85%, while quinoa is only of about 10%. Concentration of As referred to fresh vegetables shows that quinoa pre-concentrate about 3 times more As that the others, but its bioaccessibility is about half of the carrots and beets, therefore not mayor concern than carrots and beets can be expected for this vegetable considering a similar intake.

Knowledge of oral bioaccessibility of a contaminant is useful for estimating potential human health risks. However, it is even more important to know the species under which the contaminant is liberate to bloodstream. Table 6 shows that not significant transformation of As species after “*in vitro*” gastrointestinal digestion occurs in comparison to the original species. As(III) is extracted

mainly in the two first extracts and As(V) mainly in the last for quinoa and carrots. Previous studies of Calatayud M et al.⁴² show the transformation of As(V) to As(III) after gastrointestinal digest when other vegetables, different to that of this study, are contaminated with As(V) after soaking or boiling.

Although some others studies can be found about the determination of As in soils and vegetable in the II Chilean Region, the diversity of the places where different levels of contamination can be found, the different sources of contamination and the different As water concentration depending on the river and the section, made difficult to compare our results with the content in the Chilean bibliography. A precedent work performed by us ten years ago, showed a higher concentration of As in carrots growing in the same area⁴³. The studies of Ferreccio C. et al.⁴⁴ reports concentrations of As slightly higher than reported in this study for vegetables in the region.

CONCLUSION

The “*in vitro*” digestion process has shown that As species and As bioaccessibility in the carrots, beets and quinoa growing in the same soil contaminated by the element differ between vegetables. Inorganic As (III) and As(V) are present in carrots and quinoa. Unknown species are the main components of As in beets, probably arsenosugars derivatives. Bioaccessibility of As in carrots and beets was almost, 100%, while for quinoa was about 40%. Considering the maximum intake of 2.5 Kg per year of quinoa and of 6 Kg per year of carrots and beets; the As concentration in the lyophilized vegetables; the water content of 10% in the quinoa and 85% en beets and carrots, the As bioaccessibility obtained and the main As species present in the vegetables, the accessible doses of As is about 470 µg per year for carrots, about 550 µg per year for beets and about 180 µg per year for quinoa. Therefore, quinoa seems to be the vegetable with lower toxicological implication. The As speciation in the salivary, gastric and intestinal extracts of carrots showed that no significant transformation of As species after “*in vitro*” gastrointestinal digestion occurs. As(III) is mainly found in the salivary and gastric extracts and As(V) mainly in intestinal extract for the tested vegetables.

More research is necessary to determine the species present in the beets where identification is limited by both the availability of standards and the complexity of the matrix.

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