Results of the 2nd Annual International Interlaboratory Comparison Study for Arsenic Speciation in Food

Tamas Ugrai, Annie Carter, Michelle Briscoe
Brooks Rand Labs, 3958 6th Avenue NW, Seattle WA, 98107, USA | tamas@brooksrand.com

Abstract

Arsenic (As) is a naturally occurring contaminant in the environment and can be absorbed into food. Arsenic in food can be in different species that vary in toxicity necessitating the ability to differentiate and quantify them. Brooks Rand Labs (BRL) has initiated an intercomparison study, now in it's second year as a way for laboratories to assess the intercomparability of their results. Participants were asked to report results for the following analytes, based on their analytical methodology: total As, inorganic As {or the sum of As(III) + As(V)}, dimethylarsinic acid (DMA), monomethyarsonic acid (MMA), Arsenobetaine (AsB), and any other species that is not one of the above five quantified as "other". In addition, participants were asked to measure and report the total As concentration in their speciation extract, for the purpose of determining extraction efficiency. This year the study materials included cocoa powder, tuna fish tissue, seasoned seaweed snack, shellfish tissue, and white rice flour. Twenty eight laboratories participated from Canada, China, England, France, Germany, Malaysia, New Zealand, Norway, Sweden, and the United States.

Partcipation Methods

Overwhelmingly the digestion methods were hotblock or oven based; however, leaches, sonication, and microwave digestions were also employed by some labs. The most prominent extractant used was HNO_3, with methanol, enzymatic, HCl, H_2O, and $HClO_4$ accounting for a significantly smaller fraction of reagents employed. The most favored technique for separation was HPLC-ICP-MS, with a few laboratories separating by hydride generation. The most favored HPLC column used was the Hamilton (PRPX-100).

Separation	Lab Count
Hamilton PRPX	18
As7	5
Hydride	2
Chemical Extraction	1
Agilent	1
ICSep	1

Reagent	Lab Count
HNO_3	19
Methanol	2
Enzymatic	2
H_2O	1
HCl-reduction	1
$HClO_4$	1

Method	Lab Count
Hotblock/Oven	18
Leach	7
Microwave	2
Sonication	1

Fewer laboratories reported results for cocoa powder and seaweed snack than other matrices. Cocoa powder also had the lowest average extraction efficiency. The matrix with the most data submitted was the white rice flour standard reference material (SRM).

Submitted Results by Matrix

Cocoa powder	18
Seasoned seaweed	17
Shellfish tissue	19
Tuna fish (SRM)	20
White rice flour (SRM)	29

Though participating laboratories were not informed of this before analyzing the samples, two SRMs were included in this year's study. SRMs were provided so laboratory results could be compared to certified values rather than a MPV.

Results

The results of the study show the most probable values (MPV) using the Cenfit method (Ref 1). Scoring was performed following the method favored by the United States Geological Society's Standard Reference Sample Project. Data is evaluated using nonparametric statistics. The statistical approach was chosen because it is resistant to undue influence of outliers on the median.

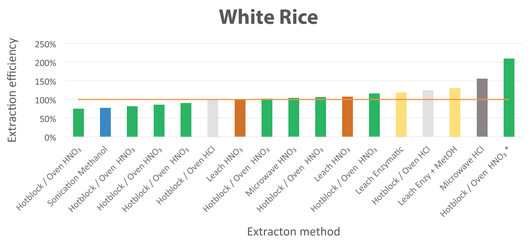
Summary of Most Probable Values (µg/kg)

Matrix	Inorg As	MMA	DMA	AsB	Total As (Extract)	Total As (Sample)	Sum As Spec	Spec Rec
Cocoa Powder	19	ISD	ISD	ISD	27	45	19	42%
Tuna Fish Tissue	39	6.1	140	4000	4400	4800	4185	87%
Certified Values	-	-	150	3896	-	4800	4046	84%
Seaweed Snack	11	ISD	140	560	12000	13000	711	5%
Shellfish Tissue	120	11	640	1100	5500	6800	1871	28%
White Rice Flour	110	11	180	ISD	320	310	301	97%
Certified Values	92	12	180	-	-	285	284	100%

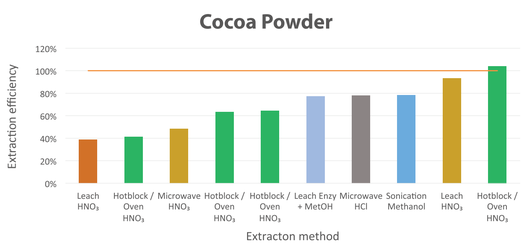
Extraction Efficiency

For speciation analysis it is important to extract all the relevant species from the solid matrix, while keeping the species in their original form.

After removal of Grubbs outliers at 1% risk of false rejection*, the total As in the sample was compared to the total As in the extraction for speciation analysis provided by each individual laboratory.



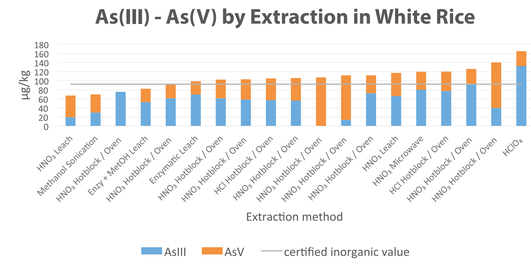
White rice flour showed good extraction efficiency with a 105% average, and 20% RSD regardless of preparation method. Most of the samples were prepared by a hotblock / HNO_3 method, which is a method well suited for rice samples.



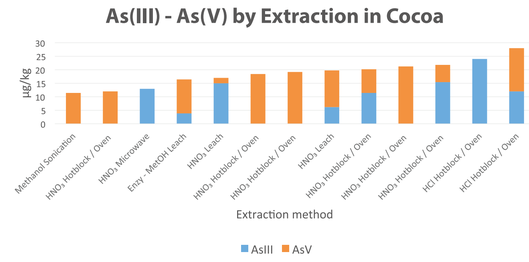
The cocoa powder had the lowest extraction efficiency of 69% with a 22% RSD. However the results were closer to the detection limits for some labs, increasing the uncertainty in measurements.

Inorganic Species Preservation

As(III) and As(V) are known to be prone to conversion due to changes in pH and oxidative properties of the extraction reagents (Ref 2,3). Only laboratories that reported As(III) and As(V) separately are shown in the charts below.



The average ratio of As(III)/As(V) in the white rice sample was 55%. With complete or near complete conversion of the species only occurring in a few HNO_3 preparations resulting in a 43% RSD for the As(III)/As(V) ratio.

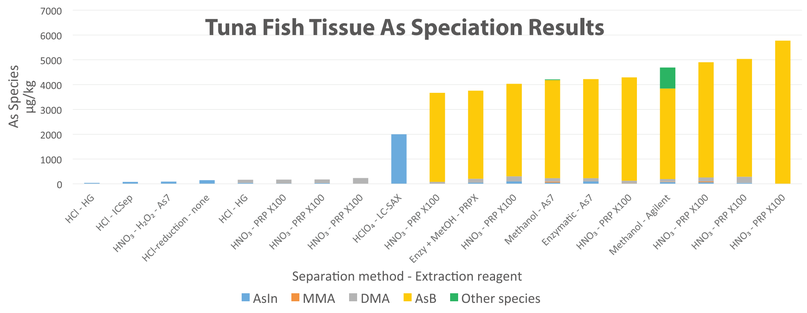


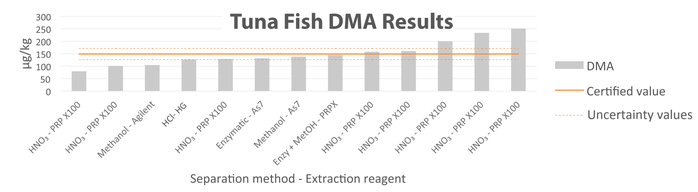
The ratio of As(III)/As(V) varied greatly in the cocoa powder regardless of preparation techniques with an RSD of 93%.

SRM Testing

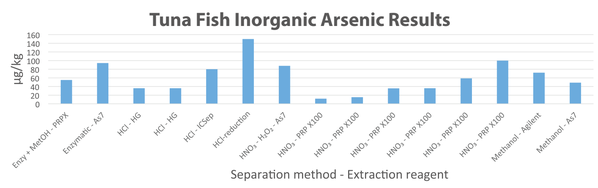
Tuna Fish Tissue

BCR 627 is a tuna fish tissue SRM available from the European IRMM. It is certified for AsB at 3800 µg/kg, DMA at 150 µg/kg, and total As at 4800 µg/kg. The main composition of the SRM submitted by laboratories reflected these values well.

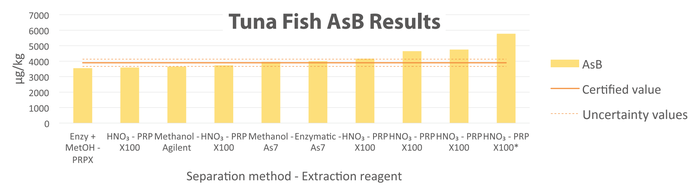




For laboratories that reported detectable DMA, results varied with the average of 150 µg/kg, (MPV 140 µg/kg) and a 34% RSD.



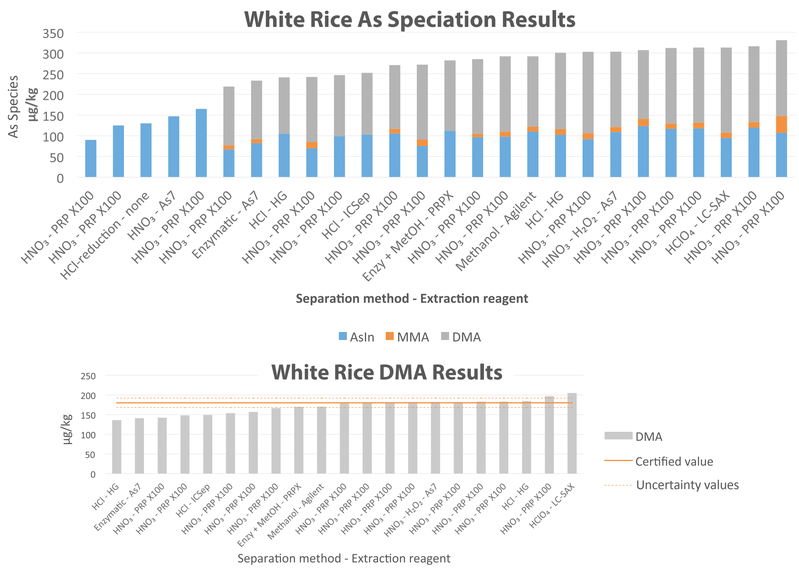
Although not certified for inorganic As, the average results for the tuna fish were **55 µg/kg (MPV 39 µg/kg)** with a **RSD of 53%**, after the omission of Grubbs outliers (not shown). The variation is significantly higher than the results submitted for the white rice.

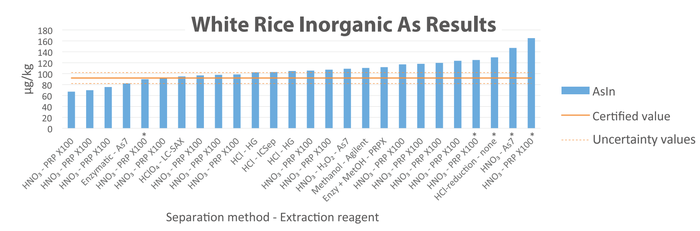


The results were much more consistent for laboratories that submitted results for AsB, with an average of 4200 µg/kg (MPV 4000 µg/kg) and a 11% RSD, after removal of Grubbs outlier at 1% risk of false rejection*.

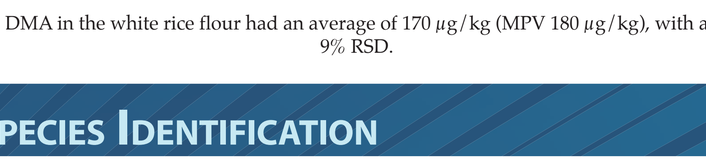
White Rice Flour

NIST 1568b is a rice flour standard reference material certified for inorganic As at 92 µg/kg, DMA 180 µg/kg, MMA 12 µg/kg and total As at 285 µg/kg.

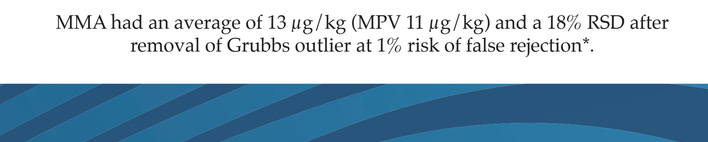




Laboratories submitting data for just inorganic As results appear to have slightly higher inorganic As (average 131 µg/kg)* compared to laboratories submitting data for all species (average 100 µg/kg). Only seven laboratories were within the uncertainty range given by the SRM. With a 106 µg/kg average (MPV 110 µg/kg) with a 21% RSD.



DMA in the white rice flour had an average of 170 µg/kg (MPV 180 µg/kg), with a 9% RSD.



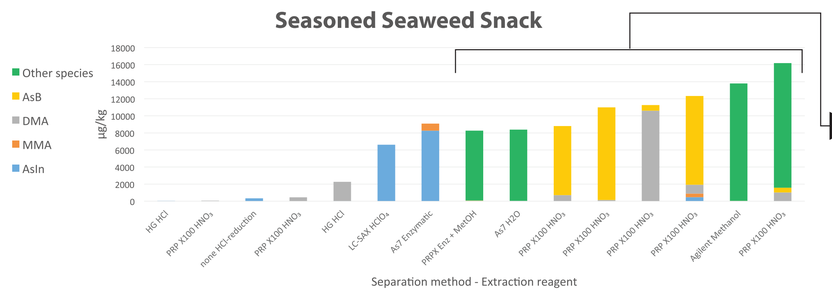
MMA had an average of 13 µg/kg (MPV 11 µg/kg) and a 18% RSD after removal of Grubbs outlier at 1% risk of false rejection*.

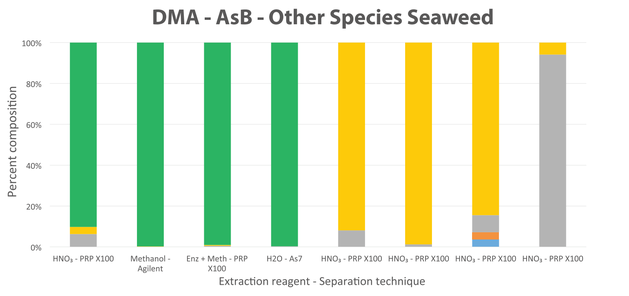
Species Identification

The seasoned seaweed snack and shellfish tissues are mainly composed of AsB, DMA, and "other" species. Although, a few laboratories reported a significant amount of inorganic As.

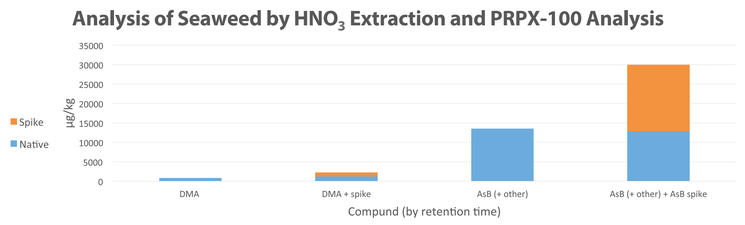
Seasoned Seaweed Snack

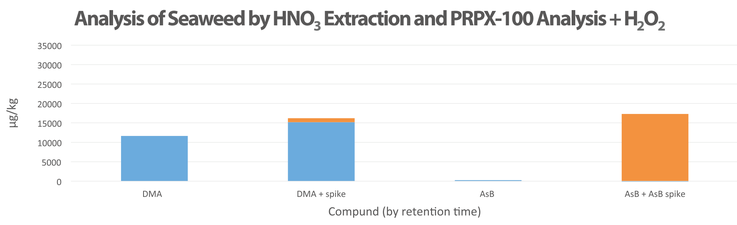
Seasoned seaweed snacks that were digested with HNO_3 and analyzed by the PRP-X100 column appear to have mainly DMA and AsB. The methanol and H_2O extractions have significant "other" species, while the $HClO_4$ and enzymatic only preparation showed significant amounts of inorganic As.





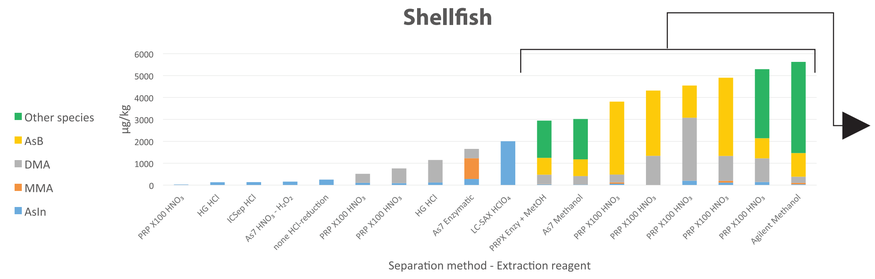
An internal study was conducted at BRL where the seaweed was prepared by HNO_3 and analyzed by a PRP-X100 column. The DMA and AsB matrix spikes yielded acceptable recoveries. After the initial analysis, H_2O_2 was added to the same sample preparation and analyzed again. There was an apparent conversion from AsB to DMA, however the species specific spikes did not show the same conversion. This may be because an organo-arsenic compound co-eluting with AsB is broken down to DMA by the addition of H_2O_2 but AsB is not.

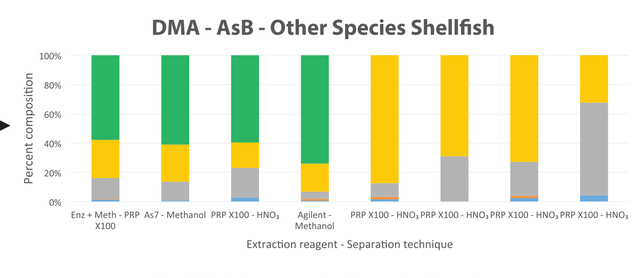




Shellfish Tissue

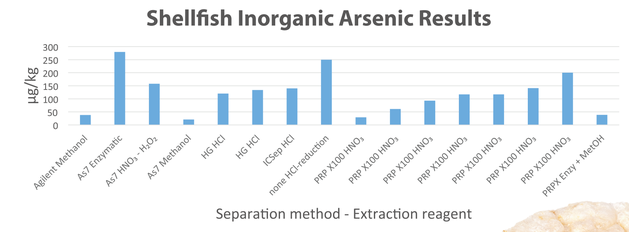
A similar trend can be seen even more vividly where methanol digestions show similar AsB/DMA/Other ratios on three separate analytical columns. The HNO_3 preparations analyzed on the PRP-X100 column show predominantly varying DMA and AsB peaks.





Analyzing an oxidative check on the shellfish samples showed similar results to that of the seaweed snack. DMA was lower in the native HNO_3 digestion while AsB was higher. However, upon an oxidative check the concentrations shifted.

Another interesting aspect of the shellfish sample was the variability of the inorganic arsenic results, with an average of 121 µg/kg (MPV 121 µg/kg) and **63% RSD**, after the omission of Grubbs outliers at 1% risk of false rejection.



Ref 1 : Dennis R Helsel. "Summing Nondetects: Incorporating Low-Level Contaminants in Risk Assessment." Integrated Environmental Assessment and Management Volume 6, Number 3 Pages 361–366
Ref 2: Elemental Analysis Manual: Section 4.11 Version 1.1 (November 2012)
Ref 3: Mélanie Giral, Gérald J. Zagury, Louise Deschênes, Jean-Pierre Blouin.
Comparison of four extraction procedures to assess arsenate and arsenite species in contaminated soils". Environmental Pollution Volume 158, Issue 5, May 2010, Pages 1890.sci8