

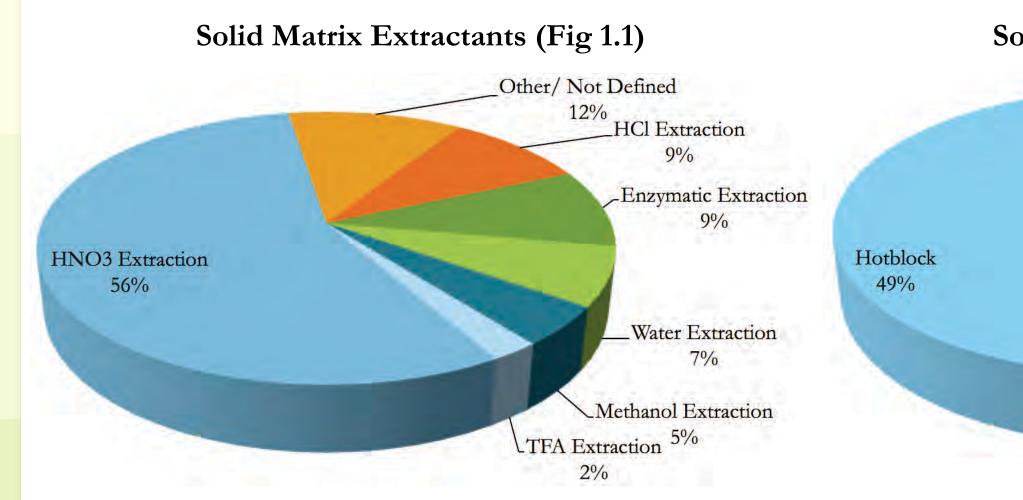
Abstract

Arsenic is naturally occurring and is a common contaminant in the environment that can be absorbed by plants, ending up in the food supply. Recent advances in the research community have further defined the detrimental effect of different forms of arsenic. In response, many regulatory agencies, such as the US Food and Drug Administration, have considered placing limits on the allowable level of various contaminants, including inorganic arsenic, that food may contain. The toxicity of arsenic is strongly dependant on oxidation state and complexation. Therefore, it is necessary to not only quantify the total amount of arsenic in the sample but to also quantify the different forms of arsenic in a sample. With the variety of analytical methods being followed by various laboratories, it is important to validate that the arsenic speciation data that is being produced is intercomparable. Although some certified reference materials do exist, they are not completely representative of raw food matrices. In response to this need, Brooks Rand Labs organized an intercomparison study for arsenic speciation in food matrices. The study included brown rice flour, white rice flour, kelp powder, and apple juice.

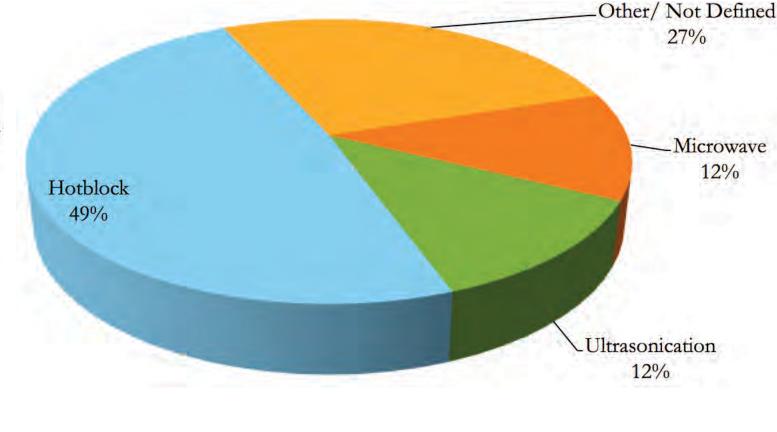
Study Participation

Four matrices were supplied to laboratories participating in the study; brown rice flour, white rice flour, kelp powder and apple juice. Participating laboratories were asked to report arsenite (AsIII), arsonate (AsV), monomethlyarsonic acid (MMA), dimethylarsinic acid (DMA), and arsenobetaine (AsB) the sum of As(III), As(V) could also be reported as inorganic arsenic. Forty-one sets of results were received from 39 participating laboratories from over 15 countries.

For the digestion of the solid matrices a variety of extractants and digestion schemes were used. The majority of sample preparations were performed using a HNO3 hot block digestion.



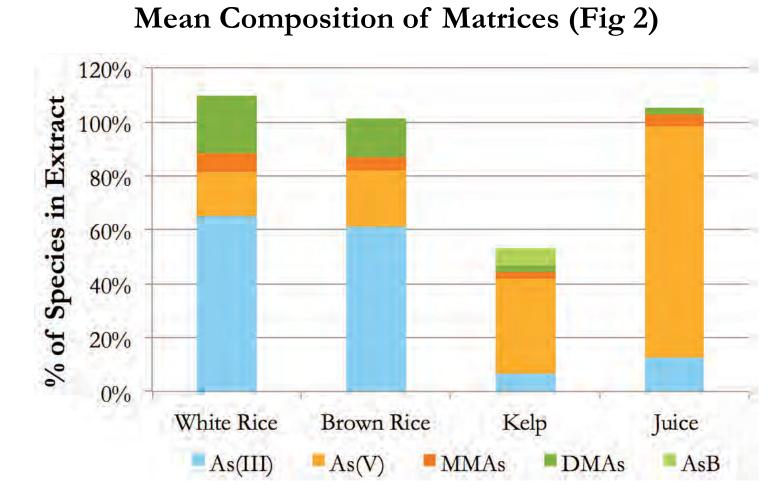
Solid Matrix Digestion (Fig 1.2)



Study Results

Statistical data analysis was performed on the results received from the participating laboratories according to the US Geological Survey Standard Reference Sample Project (Ref 1). According to data from the study, the samples were found to have the following composition (Fig 2).

The results and characteristics for white rice flour and brown rice flour were found to be very similar; therefore, the white rice data is not is shown separately in the technical evaluation section.



Study Material	Total As in Sample	Total As in Extract	As(III)	As(V)	Inorg As	MMAs	DMAs	AsB
White Rice Flour	40.7	39.9	25.91	(6.49)	30.15	(2.86)	8.49	(ND)
Brown Rice Flour	52.46	54	33	11.15	38.9	(2.75)	7.71	(ND)
Kelp Powder	63700	59335	(3790)	20924	23763	(1530)	(1467)	(3837)
Apple Juice	16.64	14.57	1.84	12.5	13.95	(0.6)	(0.35)	(ND)

(Table 1) The most probable values (median) for each parameter and study material is shown. Results for parameters highlighted in red (parenthesized) should not be considered valid most probable values, as either the variability of the results was too high or an insufficient number of data points above the associated detection limits was reported. A reported result of "ND" (non detect) means that all reported values were less than their associated detection limits.

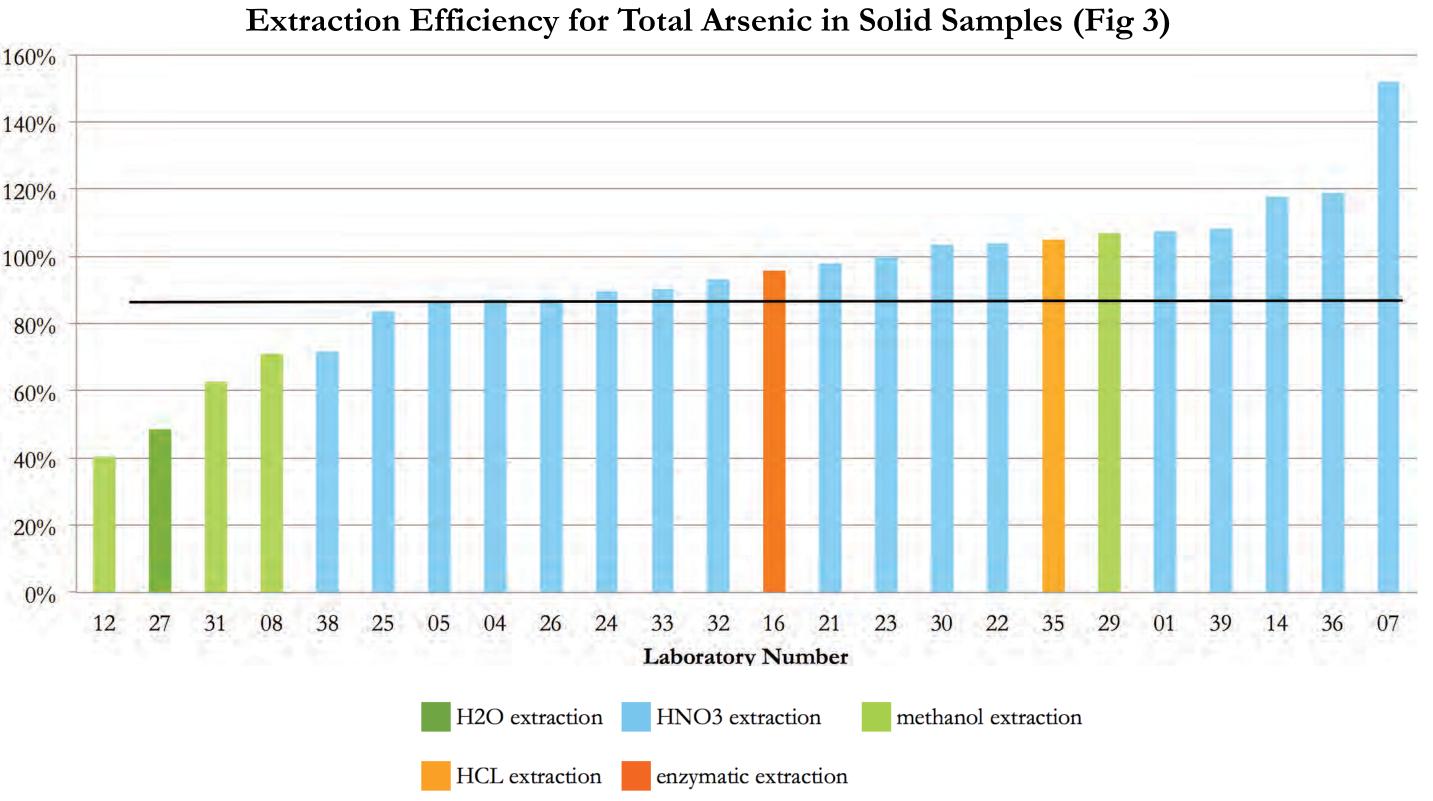
Technical Evaluation

Extraction:

criteria:

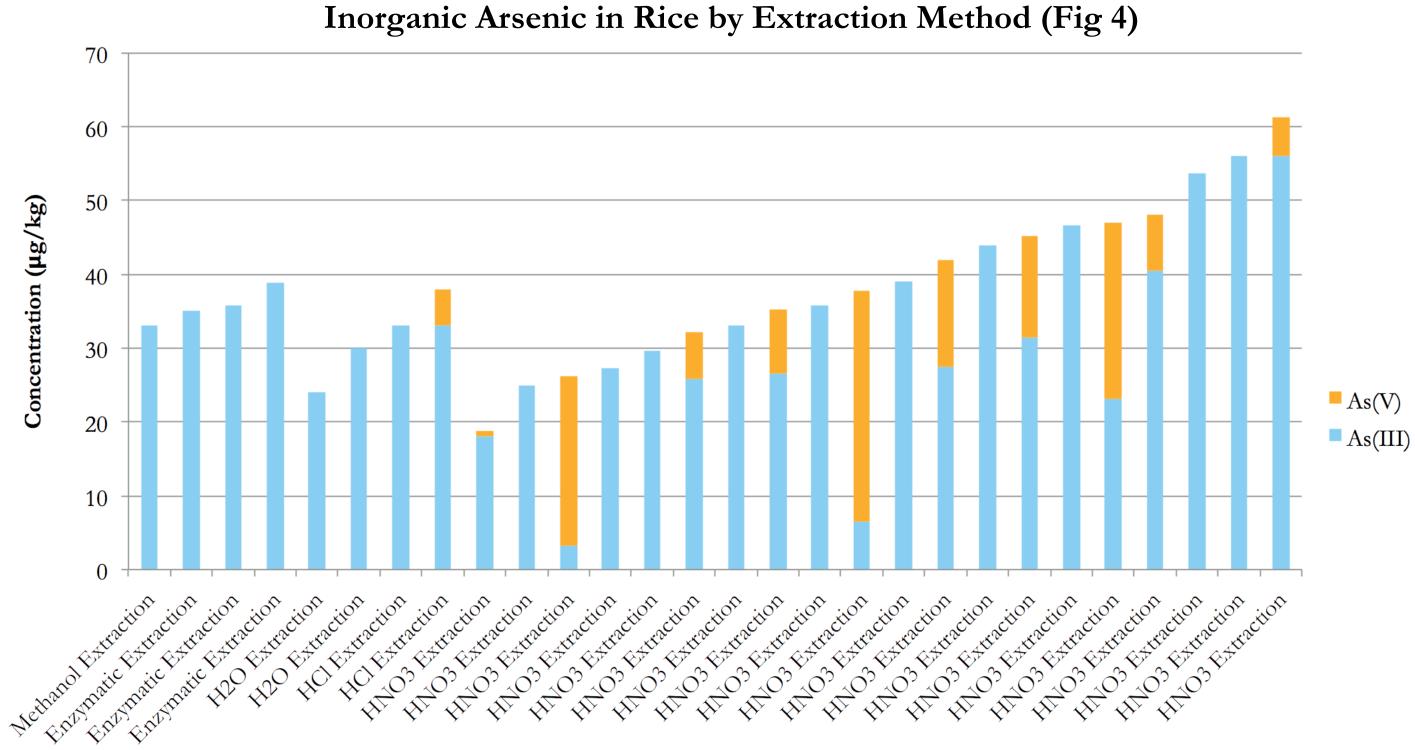
- analytical methodologies.

Extraction Efficiency:



(Fig 3) Only 59% of the laboratories reported extraction efficiency data. Most of the labs used a nitric acid based extraction method, and of those labs, most reported extraction efficiencies of 80-120%.

Species-Specific Results for Rice: limits, the data is not shown.



(Fig 4) The median result for As(III) in brown rice was found to be 33 µg/kg. The laboratories that reported As(III) results that were within 20% of the median value were well distributed between those that used methanol, water, enzymatic, and nitric acid extraction methods. Only 11 laboratories reported As(V) results at quantifiable levels, 8 did not report As(V), and 22 reported results that were below their detection limits. Most of the laboratories that reported As(V) used a nitric acid digestion technique. The median value of the results that were quantifiable was 11 µg/kg, and the RSD was 70% from those laboratories. With the large number of non-detect results, it is difficult to assess the true value of As(V) in the rice sample.

WP26 An International Intercomparison Study For **Arsenic Speciation In Food**

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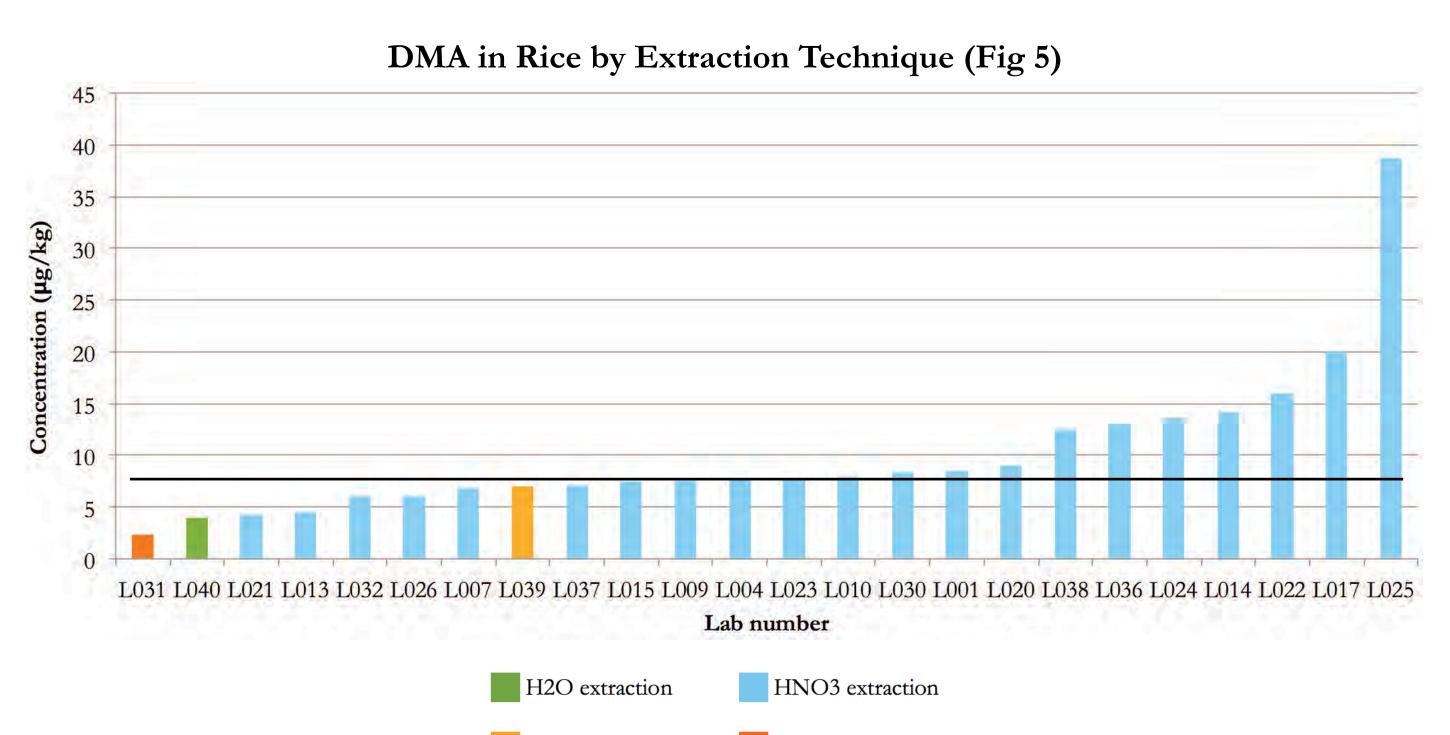
For arsenic speciation of solid food matrices, a successful extraction method must meet three important

The extraction technique used must be capable of extracting all the arsenic species from the matrix. The extraction technique must preserve oxidation and complexation states, except where only the sum of inorganic species is of interest (then only complexation states must be preserved). The extraction technique must be able to produce a solution that is compatible with the separation and

Typically, samples can be prepared for total arsenic analysis using aggressive reagents that completely liberate all arsenic from the matrix. However, for speciation analysis, less aggressive reagents are typically used to preserve species oxidation states and complexation. Different extractant/matrix combinations can have very different extraction efficiencies. Therefore, it is important to compare the total amount of arsenic found in the extract for speciation analysis to the total amount of arsenic in the sample. In this study the majority of the laboratories used a dilute nitric acid extraction method for the preparation of speciation samples.

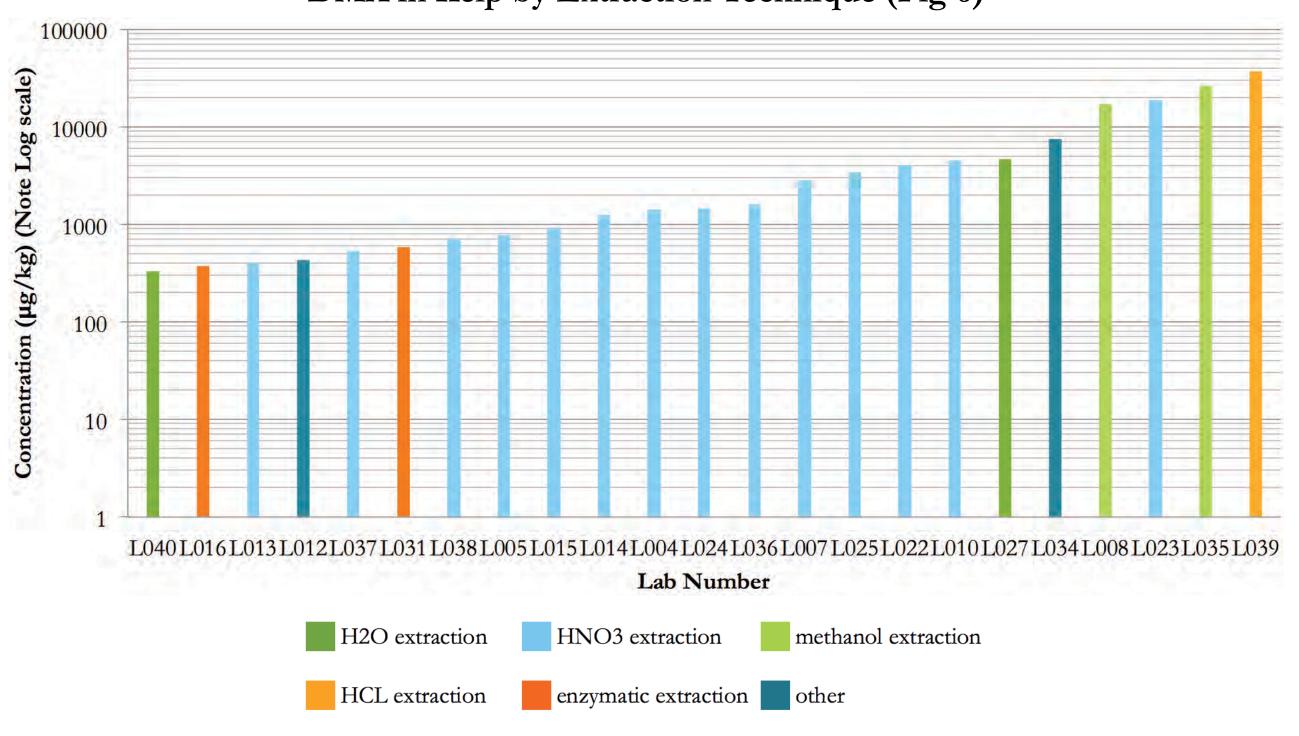
The MMA results in rice were negligible, with very few labs reporting results and all results near their detection

Extraction technique

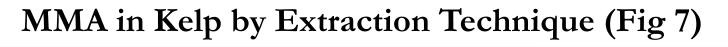


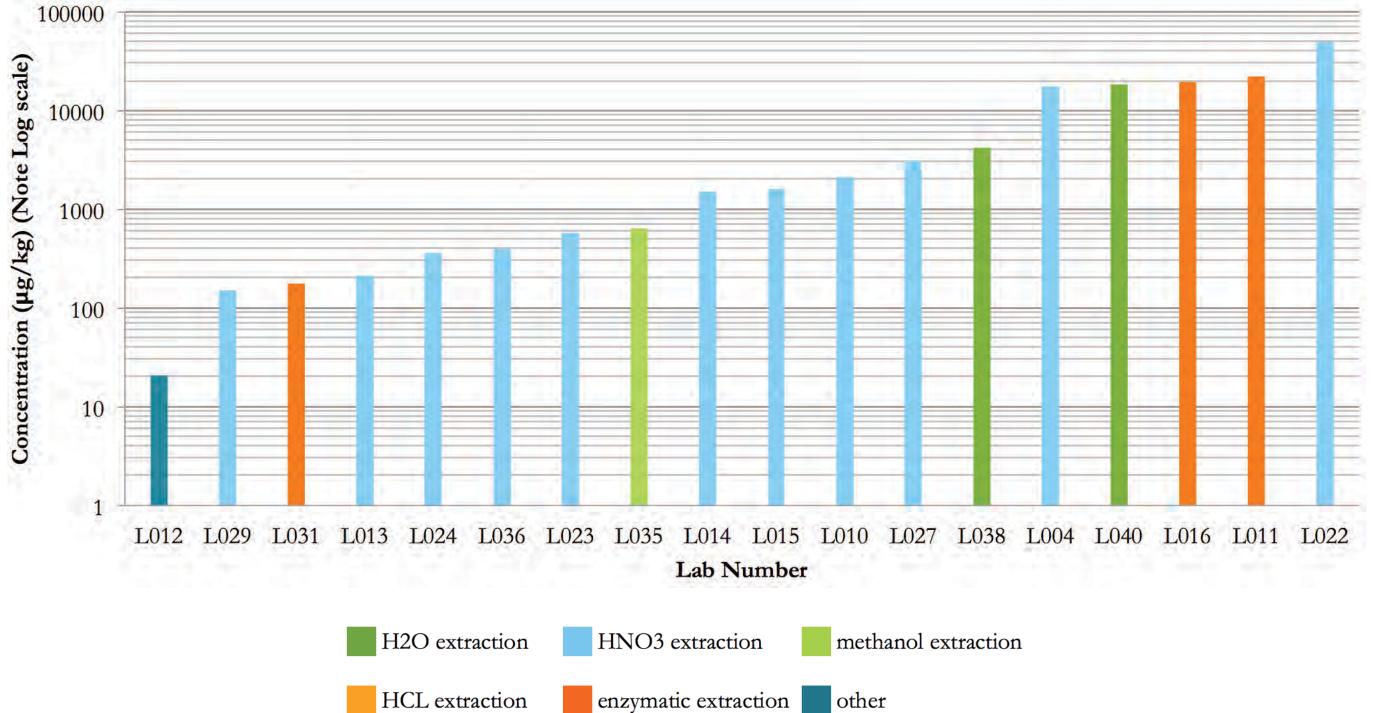
(Fig 5) Only half of the laboratories participating in the study were able to quantify DMA in rice with a median concentration of 7.7 µg/kg. Most laboratories that reported DMA in the rice flour used a nitric acid digestion.

Species specific extraction efficiency in Kelp samples: The kelp matrix was included in the study due to the high level of arsenic found in the matrix in addition to the presence of many less common organic arsenic species.



(Fig 6) Note: logarithmic scale used. The DMA results varied widely with a range of 331 – 37,068 µg/kg. Of the 23 labs that reported DMA, 9 reported results of less than 1000 μ g/kg, 10 reported results in the 1,000 – 10,000 μ g/kg range, and 4 reported results over 10,000 μ g/kg.





(Fig 7) Note: logarithmic scale used. The MMA results varied widely with a range of 21 – 48,186 µg/kg. Of the 18 labs that reported MMA, 8 reported results of less than 1000 $\mu g/kg$, 5 reported results in the 1,000 – 10,000 $\mu g/kg$ range, and 5 reported results over 10,000 µg/kg.

HCL extraction enzymatic extraction

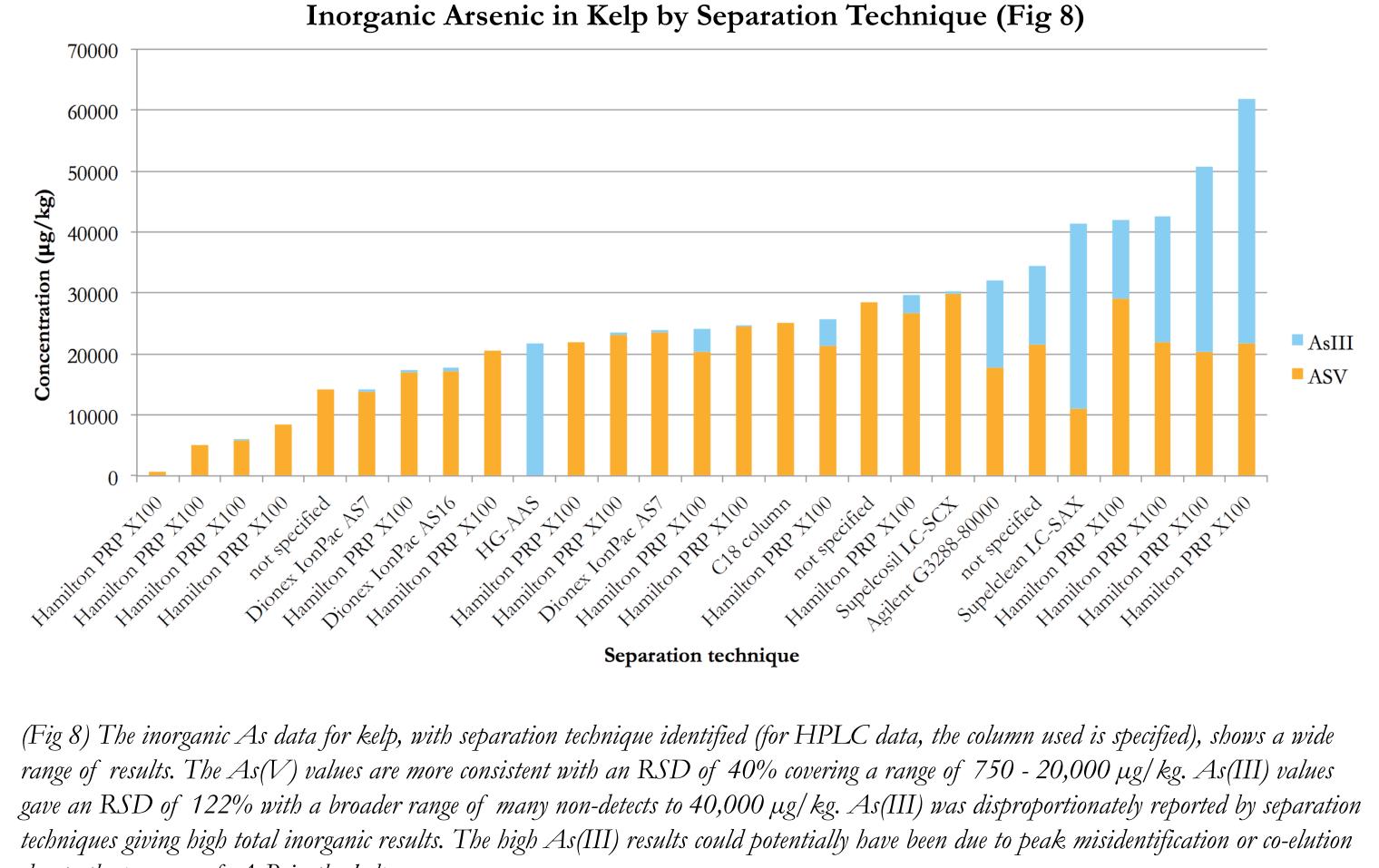
DMA in Kelp by Extraction Technique (Fig 6)

Species separation and quantification:

After arsenic is extracted from the matrix, the oxidation and complexation states are typically separated by gas or liquid chromatography and is then quantified using a time-resolved detection technique. The challenges of this technique are achieving good chromatographic separation of the individual species, preventing co-elution, and preventing shifting retention times due to matrix effects.

Separation and quantification in Kelp sample:

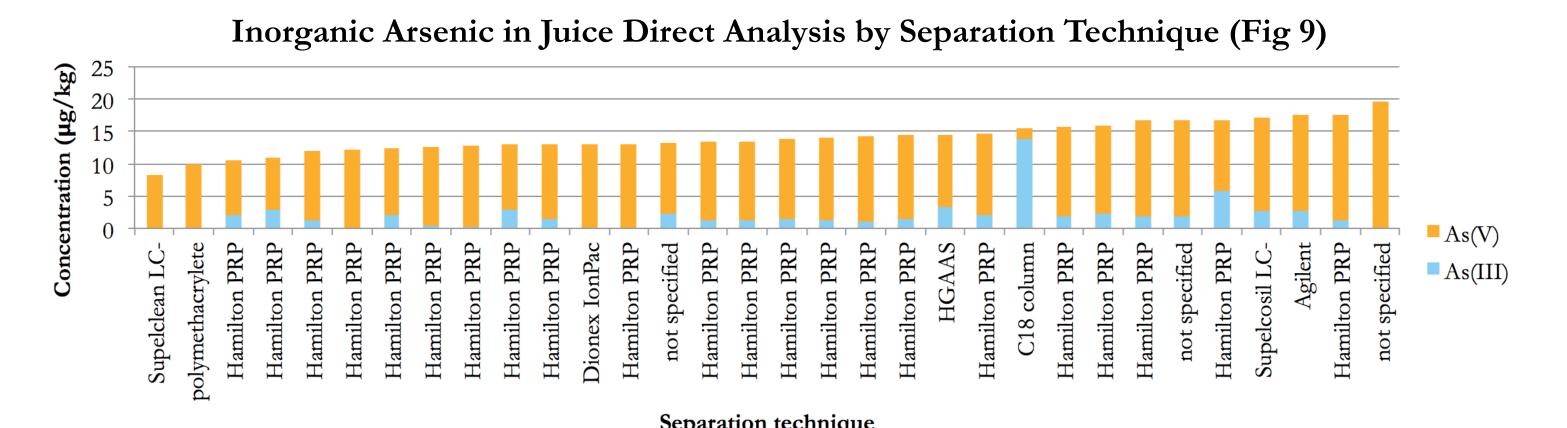
Many of the participating laboratories followed the FDA 4.11 method (Ref 3). The method itself states there is a potential for species co-elution of As(III) and AsB. Although the method attempts to resolve the two species chromatographically and requires checks to identify co-elution, there is still a possibility of quantifying organic forms of arsenic as inorganic.



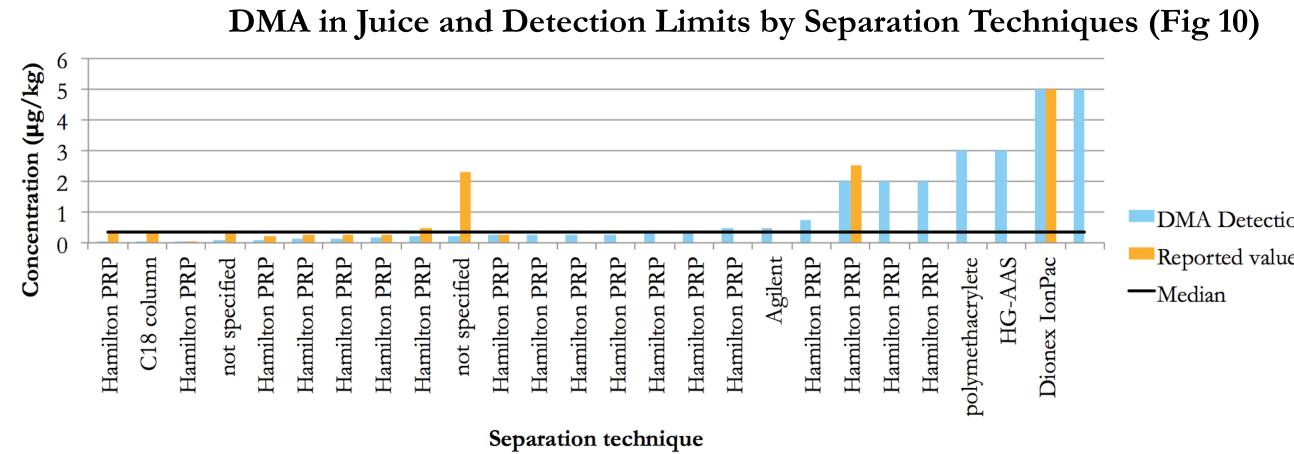
due to the presence of AsB in the kelp.

Separation and quantification juice sample:

Seventy percent of the labs reported that they did not perform a preparation procedure. This gives insight on the reported method detection limits. The MMA composition results in juice were negligible, with very few labs reporting results and all results near their detection limits, the data is not shown.



(Fig 9) Of the 26 labs that reported As(III) in the undigested juice samples, 6 reported results below their detection limit, 13 reported results of 1-2 μ g/kg, 7 reported results of 2 – 6 μ g/kg. The detection limits varied but 77% of the labs reported detection limits that were 1 $\mu g/kg$ or lower. As(V) showed consistent results with 80% of the labs reporting results within ± 1 standard deviation of the median and the achieved detection limits well below the median value. By direct injection it is shown most separation techniques compare well for inorganic As. Note the laboratory using the C18 column have inverse As(III) and As(V) results compared to the median values in the study.



(Fig 10) Only half of the labs reported DMA results that were above their detection limits and only a quarter were more than 2x their detection limits.

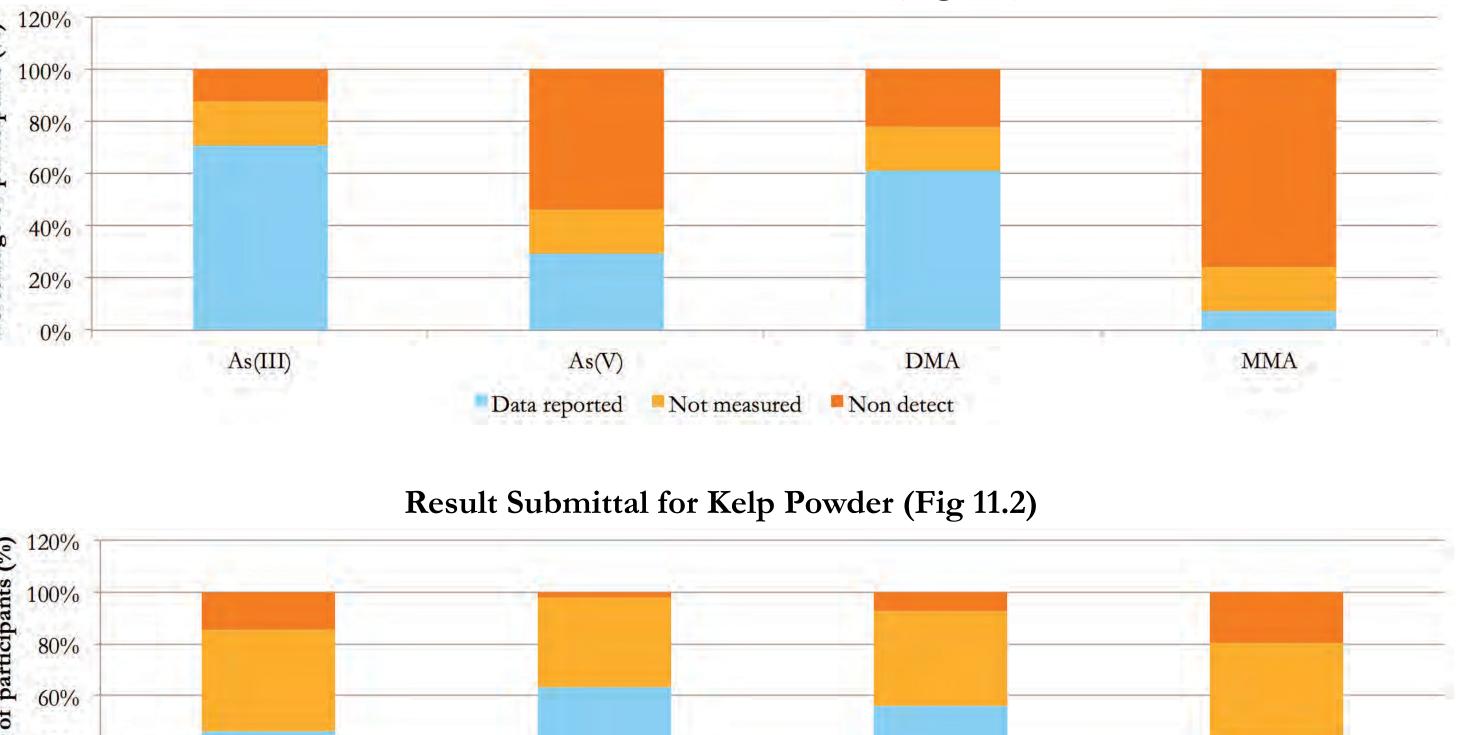


DMA Detection Limits Reported values for DMA in juice

Summary

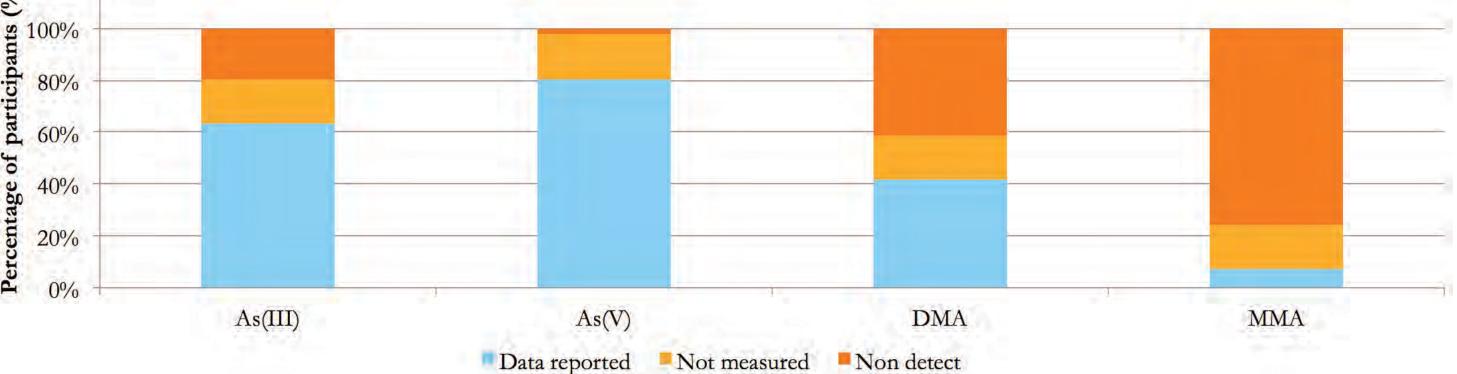
Results submitted varied from detectable results, non-detects, and not measured. A non-detect result is indicative of arsenic levels in the samples too low for the technique used for the quantification, whereas "not measured" indicates limitations of the specific speciation technique used or the preference of the participating laboratory (Fig 11.1-11.3).

Result Submittal for Brown Rice (Fig 11.1)



Data reported Not measured Non detect

Result Submittal for Apple Juice (Fig 11.3)



The FDA 4.11 method, which includes a nitric acid hot block assisted extraction and a Hamilton PRP-X100 column, was the predominant method used comprising over half of the data reported. There was no other method that was followed by more than 10% of the laboratories. The predominance of the FDA method could bias mean values of the study to that method. However, there was often nearly as much variability within the FDA method as there was within all other methods.

Rice: In rice samples, the nitric acid hot block extraction gives a greater spread in inorganic arsenic than the combination of other preparation techniques. The sum of inorganic arsenic shows a smaller standard deviation than of both As(III) and As(V) potentially showing some level of species conversion

Kelp: The median of each species of arsenic in the kelp sample was high, it showed a greater variability both by column and separation technique. As(III), DMA, MMA and AsB all had variable results potentially due to peak co-elution, species conversion and possible peak misidentification.

Juice: Most laboratories did not prepare the samples allowing the evaluation of separation technique parameters such as raw detection limits. As(III), MMA, and DMA were low in the sample resulting in many non-detects. As(V) was the predominant arsenic species in the juice samples, most separation techniques were able to quantify the arsenic species well.

References

1) Standard Reference Sample Project, Office of Water Quality, Branch of Quality Systems, United States Geological Survey (USGS) http://bqs.usgs.gov/srs/SRS_Spr04/statrate.htm 2) Hoaglin, D.C., Mosteller, F. and Tukey, J.W. (eds.) (1993) Understanding Robust and Exploratory Data Analysis, Wiley, New York, NY.

b) United States Food and Drug Administration (FDA), Elemental Analysis Manual for Food and Related Products, 4.11 Version 1.1 (November 2012).