ABSTRACT

Title of Dissertation: HUMAN DIETARY EXPOSURE TO ARSENICALS IN SEAFOOD: OCCURRENCE AND ANALYTICAL CONSIDERATIONS.

Caleb Luvonga, Doctor of Philosophy, 2021

Dissertation directed by: Prof. Sang Bok Lee
Department of Chemistry and Biochemistry.

Consumption of seafood has been on a steady rise based on reports of associated health benefits. Marine organisms are sources of staple and functional food. However, they are also the main source of total arsenic exposure in humans. Most food safety regulations are based on total arsenic. Unfortunately, total arsenic as an indicator for risk from dietary intake is inadequate. Knowledge of arsenic speciation is important as the chemical form of arsenic controls its bioavailability, mobility, and toxicity. Therefore, an accurate account for the myriads of arsenic species, naturally occurring in seafood, is required. However, this present considerable analytical challenge. Arsenic speciation in seafood is challenging owing to its existence in diverse chemical forms and oxidation states, interconversions between chemical forms, matrix complexity, lack of widely accepted analytical methods, and lack of commercially available standards and certified reference materials. Identification and quantification

of the toxic arsenic species is imperative to understanding the risk associated with exposure to arsenic from dietary intake, which in turn underscores the need for speciation analysis. Setting of standards for arsenic in food is complicated, owing to the enormous metabolic diversity of organic arsenic species in humans, lack of knowledge about their toxicity, and lack of reliable speciation data on seafood. To establish human exposure to arsenic from seafood, five proxy seafood samples were selected to represent the entire food chain. The selection was based on their high consumption rate, which makes them economically important and a significant route for arsenic exposure. The seafood samples are either candidate reference materials or materials meant for interlaboratory comparisons, which require measurements for certification purposes or property values assignment. This work contributed towards that effort. Analytical methods for the determination of total arsenic and hydrophilic arsenic species were developed and optimized for analysis of the seafood samples. A structural library was developed based on *in silico* fragmentation data of extant lipophilic arsenicals that are reported in literature. The library aims to enhance the screening and identification of the novel lipophilic arsenicals for which very little is known, standards are not available, and whose toxicological profiles are of interest.

HUMAN DIETARY EXPOSURE TO ARSENICALS IN SEAFOOD: OCCURRENCE AND ANALYTICAL CONSIDERATIONS.

by

Caleb Luvonga

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2021

Advisory Committee:

Professor Sang Bok Lee, Chair

Professor Neil Blough

Professor Alice Mignerey

Professor Akua Asa-Awuku

Dr. Catherine A. Rimmer

Dedication

To the late Eleanor Mataga, my mother, and the woman who always believed in me and pushed me hard to pursue excellence. I hope this will make you proud. It is unfortunate that you are not here to witness it all. To my wife Elizabeth N. Luvonga and our daughters Bianca, Beulah and Barachelle. I have shown you the way. I hope when the time comes that you will follow in my footsteps.

Acknowledgements

I am grateful to all who have been instrumental to the success of this project. The timely completion of this great task would not have been possible without your support, particularly the following: My advisors, Prof. Sang Bok Lee at University of Maryland (UMD), College Park and Dr. Catherine Rimmer at the National Institute of Standards and Technology (NIST) for your guidance, support and encouragement. Both Dr. Katrice Lippa, the leader of Organic Chemical Metrology Group (OCMG) and Dr. Michael Winchester, the leader of Inorganic Chemical Metrology Group (ICMG) for providing excellent work facilities. Dr. Lee L. Yu for sharing his wealth of knowledge and experience in trace element analysis, reading through manuscripts, and constructive criticism. Dr. Benjamin Place and Niksa Blonder for your guidance and assistance in designing the arsenolipids database. Dr. Willie E. May for being more than a mentor and making it possible for me to achieve my lifelong dream. Dr. Bryant Nelson and Dr. Rose Kimani for proofreading my work. Members of OCMG and ICMG for the positive and inclusive work environment. The Mays and Amanuels families for great hospitality and always welcoming me to your homes to enjoy great company and home-cooked meals. I would like to thank my dear friends and family for your great help and support to my family, especially in my absence. I am looking forward to spending more time with you all. My lovely daughters Bianca, Beulah and Barachelle, thank you for your patience and understanding throughout my lengthy absence, I'm finally coming home! Thanks to my dear wife Elizabeth for taking care of everything at home, for your patience, encouragement, and support. Most of all, my heartfelt thanks to the Almighty God, in whom there is no shadow of turning.

Table of Contents

Dedication ii

Acknowledgements iii

Table of Contents iv

List of Tables x

List of Figures xi

List of Abbreviations xiii

Chapter 1: Introduction 1

1.1. Nature and occurrence of arsenic in marine environment 6

1.2. Sources and distribution of organic arsenic species in marine organisms 11

1.2.1. Inorganic Arsenic 14

1.2.2. Methylated Arsenic Species 15

1.2.3. Arsenobetaine 17

1.2.4. Arsenosugars 19

1.2.5. Arsenolipids 20

1.2.6. “Residual” Arsenic 23

1.3. Consumption Patterns 24

1.4. Analytical Considerations for Risk Assessment 25

Chapter 2: Organoarsenicals in Seafood: Occurrence, Dietary Exposure, Toxicity and Risk Assessment Considerations. 35

2.1 Introduction 35

2.1.1. Microalgae (Spirulina) 35

2.1.2. Macroalgae (Seaweed) 41

2.1.3. Seafood 49

2.2. Arsenic speciation in Seafood 58

2.3. Metabolic transformation and toxicity of Arsenic 60

2.3.1. Methylated Arsenicals 65

2.3.2. Trimethylarsine oxide (TMAO) 68
2.3.3. Arsenocholine (AsC) 69
2.3.4. Arsenobetaine (AsB) 70
2.3.5. Tetramethyl arsonium ion (TETRA) 71
2.3.6. Thioarsenicals 72
2.3.7. Arsenosugars (AsSugars) 74
2.3.8. Arsenolipids (AsLipids) 80
2.4. Considerations for Risk assessment 85
2.4.1. Risk assessment 85
2.4.2. Instrumental needs 88
2.4.3. Standards and Reference Materials needs 89
Chapter 3: Analytical Methodologies for Determination of Organic Arsenic Species in Edible Marine Species. 92
3.1. Introduction 92
3.2. Sample handling and pretreatment 96
3.2.1. Cleaning 97
3.2.2. Freeze drying 98
3.2.3. Homogenization 98
3.3. Extraction 99
3.3.1. Extraction Techniques 100
3.3.1.2. Supercritical fluid extraction (SFE) 105
3.3.1.3. Accelerated solvent extraction (ASE) 108
3.3.1.4. Microwave-assisted extraction (MAE) 108
3.3.1.5. Ultrasound-assisted extraction and ultrasound probe sonication. 109
3.3.1.6. Solid-phase microextraction (SPME) 110
3.3.2. Extraction efficiency and arsenic species transformation 111
3.4. Sample cleanup procedures 113
3.4.1. Silica gel cleanup 113
3.4.2. Solid-phase extraction (SPE) 114
3.4.3. Size exclusion chromatography (SEC) 115
3.5. Separation 116

3.5.1. Capillary Zone Electrophoresis (CZE) 118
3.5.2. Gas chromatography (GC) 119
3.5.3. Liquid chromatography (LC) 119
3.5.3.2. Reversed-Phase Liquid Chromatography (RP-LC) 125
3.5.3.3. Ion-Pair Chromatography 126
3.5.3.4. Micellar Liquid Chromatography (MLC) 127
3.5.4. Multidimensional Chromatographic Techniques 127
3.6. Detection Techniques 128
3.6.1. Atomic Absorption and Atomic Fluorescence Spectrometry 129
3.6.2. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) 130
3.6.2.1. ICP-MS signal interferences 130
3.6.2.2. Sensitivity improvement by Hydride Generation 132
3.6.3. Hydride Generation- Atomic Fluorescence Spectrometry (HG-AFS) 133
3.7. Characterization 135
3.7.1. Identification using LC-ICP-MS coupled to X-ray Absorption Spectroscopy (XAS) 136
3.7.2. Simultaneous identification and quantification by LC-ICPMS/HRMS . 137
3.7.2.2. Orbitrap™ Mass Spectrometry 143
3.7.2.3. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. 144
3.7.3. Identification by tandem mass spectrometry fragmentation 145
3.8. Standards and Reference materials 148
Chapter 4: Method Development for the Determination of Total and Hydrophilic Arsenicals in Seafood. 152
4.1. Introduction 152
4.2. Experimental 154
4.2.1. Sample preparation 154
4.2.2. Reagents and standards 154
4.2.2.1. Safety warning 155
4.2.3. Instrumentation 155
4.3. Procedure 157
4.3.1. Determination of arsenic species 157

4.3.1.1. Extraction 157
4.3.1.2. Measurement 158
4.3.1.3. Determination of moisture content in rice flour and geoduck clam 158
4.3.2. Determination of total arsenic 158
4.3.2.1. Digestion 158
4.3.2.2. Addition of Internal Standard and Spiking Standard 159
4.3.2.3. Measurement 159
4.3.2.4. Determination of moisture content in Oyster tissue 160
4.4. Results and discussion 160
4.4.1. Identification of arsenic species in geoduck and kelp 160
4.4.2. Determination of Arsenic species in geoduck and kelp 163
4.4.3. Development of in-house standards 166
4.4.4. Optimization of Extraction Method 167
4.4.4.1. Effect of solvent composition on extractable arsenic 167
4.4.4.2. Effect of extraction methods on extraction efficiency 170
4.4.4.3. Effect of temperature on extraction efficiency 172
4.4.4.4. Effect of time on extraction efficiency 173
4.4.4.5. Effect of bead beating on extraction efficiency 173
4.4.4.6. Effect of surfactant (SDS) on extraction efficiency 175
4.5. Risk assessment associated with consumption of geoduck and kelp 175
4.6. Conclusion 176
Chapter 5: Determination of Total Arsenic and Hydrophilic Arsenic Species in Seafood. 178
5.1. Introduction 178
5.1. Experimental 181
5.1.1. Sample Collection and Preparation 181
5.1.2. Reagents and standards 183
5.1.2.1. Safety warning 184
5.1.3. Instrumentation 184
5.2. Procedure 185
5.2.1. Determination of Total Arsenic 186

5.2.1.1. Digestion 186
5.2.1.2. Addition of Internal Standard and Spiking Standard 187
5.2.1.3. Measurement 187
5.1.1. Determination of moisture content in Oyster tissue 187
5.1.1. Arsenic speciation analysis 188
5.1.1.1. Extraction 188
5.1.1.2. Addition of Internal Standard and Spiking Standard 188
5.1.2. Determination of AsSugars 189
5.1.3. Measurement 189
5.1.4. Determination of moisture content in rice flour and geoduck tissue 190
5.1. Results and discussion 190
5.1.1. Arsenic species stability 190
5.1.2. Mass fraction of total Arsenic 197
5.1.3. Mass fraction of arsenic species 201
5.1.3.1. Inorganic arsenic 203
5.1.1.1. Mass fraction of other arsenic species 206
5.2. Conclusion 210
Chapter 6: Structural elucidation and annotation of arsenolipids in seafood based on in silico tandem mass spectrometry. 212
6.1. Introduction 212
6.2. Methods 217
6.2.1. Literature search and structure determination 218
6.2.2. *in silico* fragmentation mass spectrum 218
6.2.3. Comparison to literature spectra 219
6.2.4. Analysis of fragmentation mass spectral patterns 219
6.2.5. AsLipids structure prediction 220
6.2.6. Development of AsLipids *in silico* library search engine 222
6.3. Results and Discussion 223
6.3.1. Literature search and mass spectrum extraction of extant AsLipids 223
6.3.2. Generation of predicted spectra for extant AsLipids 231
6.3.3. Creation of the searchable molecular database 233

6.3.4. Searching the spectral library 237
6.3.4.1. Search by Compound 238
6.3.4.2. Search by Spectrum 239
6.3.4.2.1. Ionization Mode 240
6.3.4.2.2. Molecular Ion Resolution 240
6.3.4.2.3. Precursor Ion 240
6.3.4.2.4. Precursor Ion Resolution 240
6.4. Conclusion 242
Chapter 7: Future Perspectives. 244
7.1. Analytical Considerations 244
7.1.1. Extraction recovery 244
7.1.2. Identification tool for novel arsenic species 246
7.2. Standards and certified reference materials (CRMs) needs 248
7.2.1. Synthetic and isotopically labeled standards needs 248
7.2.2. Matrix-matched CRMs needs 249
7.3. Risk Assessment considerations 249
7.3.1. Toxicity studies 250
7.3.2. Regulatory framework 251
7.4. Conclusion 251
Bibliography 253

List of Tables

Table 1: Important nutritional elements in seaweeds..... 44

Table 2: Global Seafood Production from Aquaculture in 2018.. 52

Table 3: Experimental biological activities of different arsenicals..... 69

Table 4: Comparison of sample extraction techniques 106

Table 5: Figure of merit for common mass analyzers 142

Table 6: Mobile phase compositions and conditions for separation of arsenic species..... 156

Table 7: Mobile phase compositions and conditions for separation of arsenic species..... 185

Table 8: Total arsenic and arsenic species in common seafood..... 202

Table 9: Generic Structure for the development of predicted AsLipids structures..... 221

Table 10: Arsenolipids identification in Seafood 229

List of Figures

Figure 1: Arsenic compounds found in Seafood..... 13
Figure 2: Estimated distributions of Arsenic Species found in different seafood types 14
Figure 3: Three proposed arsenic metabolic pathways. 17
Figure 4: Seafood Consumption by country and hypothetical intake of arsenic species..... 25
Figure 5: Classification of arsenic species in seafood based on their toxicities. 30
Figure 6: Schematic representation of the project work-flow. 34
Figure 7: Health benefits for taking spirulina 39
Figure 8: Market drivers for growth of demand for spirulina 40
Figure 9: Phylogenetic tree of three classes of marine macroalgae: green, red and brown. ... 41
Figure 10: World Aquaculture Production of Aquatic Algae. 47
Figure 11: Global Capture Fisheries and Aquaculture Production, 1980 – 2030. 53
Figure 12: Main groups of species in seafood trade in terms of value, 2018. 54
Figure 13: Trade of Seafood and Fisheries Products. Source: FAO 2020. 56
Figure 14: World Employment for Fishers and Fish Farmers by Region in 2018..... 57
Figure 15: Structures of common arsenic compounds. 59
Figure 16: Structures of deprotonated oxo-anions of arsenate and phosphate..... 63
Figure 17: Structures, names, and abbreviations of methylated thioarsenical compounds..... 73
Figure 18: Structures, names, and abbreviations of Arsenosugars. 75
Figure 19: Chemical structure of AsSugar-PL 958 extracted from brown algae. 76
Figure 20: Structure of AsSugar 254, DMAsSugarHydroxy 77
Figure 21: Structures, names, and abbreviations of arsenolipids..... 81
Figure 22: Analytical procedure for arsenic speciation in seafood. 96
Figure 23: Typical AsSugar and thio-AsSugar tandem mass spectometry fragments..... 146
Figure 24: Anion exchange separation of seven arsenic species. 161
Figure 25: Cation exchange separation of seven arsenic species..... 162
Figure 26: Anion exchange separation of arsenic species in geoduck clam and kelp..... 163
Figure 27: Relative proportions of arsenic species in geoduck and kelp 166
Figure 28: Experimental design for assessing the extraction efficiency of arsenic in kelp .. 167

Figure 29: Effect of solvent composition (methanol/water) on extraction efficiency 169
Figure 30: MMA signal enhancement with increasing methanol content in extractant..... 169
Figure 31: Effect of sonication and extraction time on the extraction efficiency. 171
Figure 32: Effect of extraction method on the extraction efficiency. 171
Figure 33: Effect of temperature on extraction efficiency 172
Figure 34: Effect of extraction time on extraction efficiency. 173
Figure 35: Effect of bead beating on extraction efficiency. 174
Figure 36: Analytical procedure for total and hydrophilic arsenic in seafood. 181
Figure 37: Mass fraction of arsenic species in standards after acid and water extraction 194
Figure 38: Typical AsSugar tandem mass spectrometry fragments after acid digestion 195
Figure 39: Anion exchange LC-ICP-MS of spirulina and geoduck acid and water extracts 196
Figure 40: Mass fraction of total arsenic in common seafood. 198
Figure 41: Control samples for AsB, DMA, iAs and total Arsenic 201
Figure 42: Comparison of extraction of iAs and DMA in geoduck clam and spirulina 203
Figure 43: Mass fraction of control samples after acid and water extractions. 204
Figure 44: Mass fraction of arsenic species in various edible marine species. 205
Figure 45: AE separation of arsenic in seafood, spirulina, and kelp spiked with MMA 209
Figure 46: Workflow for generating AsLipids structure and in silico MS/MS library. 217
Figure 47: Schema of the Relational Database structure. 222
Figure 48: Arsenolipids in silico web-based mass spectral library home page 238
Figure 49: Web-based graphical user interface of search based on compound. 238
Figure 50: Output of partial search text matching. 239
Figure 51: Web-based graphical user interface of search based on unknown spectrum. 240
Figure 52: Output of database search based on spectrum showing the list of compounds. .. 241
Figure 53: Butterfly diagram showing comparison of unknown and *in silico* spectrum 241

List of Abbreviations

Å	Angstrom
AA	Arachidonic acid
AAS	Atomic absorption spectroscopy
ACMP	Apparent charge of mobile phase
ACN	Acetonitrile
ACS	American Chemical Society
ADHD	Attention-deficit hyperactivity disorder
ADI	Allowable dietary intake
ADP	Adenosine diphosphate
AE	Anion-exchange
AES	Atomic emission spectroscopy
AFS	Atomic Fluorescence Spectroscopy
AIF	All-ion fragmentation
amu	Atomic mass units
API	Atmospheric pressure ionization
Ar	Argon
As	Arsenic
As^{0}	Elemental arsenic
As3MT	Arsenite methyltransferase
As^{III}	Arsenite (H_3AsO_3)
As^{-III}	Arsine (H_3As)
As^{V}	Arsenate (H_3AsO_4)
AsB	Arsenobetaine
AsC	Arsenocholine

AsDAGs	Arsenic-containing diacylglycerols
ASE	Accelerate Solvent Extraction
AsFOHs	Arsenic-containing fatty alcohols
AsFAs	Arsenic-containing fatty acids
AsFA 362	[15-(dimethylarsinyl)pentadecanoic acid]
AsFA 388	[17-dimethylarsinyl-9-heptadecenoic acid]
As-GSH	Arsenic-containing glutathione
AsHCs	Arsenic-containing hydrocarbons
AsHC 332	[1-(dimethylarsinyl)pentadecane)]
AsHC 360	[1-(dimethylarsinyl)heptadecane)]
AsHC 444	[1-(dimethylarsinyl)tricosane)]
AsLipids	Arsenolipids
AsPCs	Arsenic-containing phosphatidylcholines
AsPEs	Arsenic-containing phosphatidylethanolamines
AsPGs	Arsenic-containing phosphatidylglycerols
AsPIs	Arsenic-containing phosphatidylinositols
AsSugars	Arsenosugars
AsSugar-254	Hydroxy-dimethylarsinoylribosides
AsSugar-328	Glycerol-arsinoylribosides (also AsSugar-OH)
AsSugar-392	Sulfonate-arsinoylribosides (also AsSugar-SO_3)
AsSugar-408	Sulfate-arsinoylribosides (also AsSugar-SO_4)
AsSugar-482	Phosphate-arsinoylribosides (also AsSugar-PO_4)
AsSugar-Phytols	Phytol-arsinoylribosides
AsSugar-PLs	Arsenosugar phospholipids
AsTAGs	Arsenic-containing triacylglycerols

ATP	Adenosine triphosphate
BBB	Blood-brain barrier
bw	Body weight
C	Carbon atom
^{13}C	Labelled carbon atom
CAGR	Compound annual growth rate
CAS	Chemical Abstracts Service
Caco-2	Human colorectal adenocarcinoma cells
°C	Degree Celsius
CE	Cation-exchange
CFM-ID	Competitive fragmentation modelling for metabolite identification.
CH_3^+	Methyl cation
CH_3OH	Methanol
$(CH_3)_2OAs$	Dimethylarsinoyl moiety
CI	Confidence Interval
CID	Collision-induced dissociation
Cl	Chlorine
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
cps	Counts per second
CT-HG-AAS	Cryogenic trap hydride generation atomic absorption spectrometry
CRMs	Certified reference materials.
CSV	Comma separated values
CVD	Cardiovascular disease

CZE	Capillary zone electrophoresis
CZE-ESI-MS	Capillary zone electrophoresis electrospray ionization mass spectrometry
DHA	Docosahexaenoic acid
DIA	Data independent acquisition
dil	Dilution
DMA^{III}	Dimethylarsinous acid
DMA^{V}	Dimethylarsinic acid
DMAA	Dimethylarsinoylacetic acid
DMAB	Dimethylarsenobutanoic acid
DMAE	Dimethylarsinoyl ethanol
DMAP	Dimethylarsenopropanoic acid
DNA	Deoxyribonucleic acid
$DMDTA^{V}$	Dimethyldithioarsinic acid
$DMMTA^{III}$	Dimethylmonothioarsinous acid
$DMMTA^{V}$	Dimethylmonothioarsinic acid
dw	Dry weight
EABA	European Algae Biomass Association
EFSA	European Food Safety Authority
EH	Enzymatic hydrolysis
EI	Electron impact ionization
EPA	Eicosapentaenoic acid
ESI	Electrospray Ionization
$ESI\text{-}IT\text{-}MS^{n}$	Electrospray ionization ion trap multistage mass spectrometry
ESI-MS	Electrospray ionization mass spectrometry
ET-AAS	Electrothermal atomic absorption spectrometry

ETD	Electron-transfer dissociation
EU	European Union
eV	Electron volt
EXAFS	Extended X-ray absorption fine structure
FAB	Fast atom bombardment
FAIMS	Field asymmetric waveform ion mobility spectrometry
FAME	Fatty acid methyl ester
FAO	United Nations Food and Agriculture Organization
FDA	Food and Drug Administration
fg	Femtogram
FT	Fourier transform
FT-ICR	Fourier transform ion-cyclotron
FT-ICR-MS	Fourier transform ion cyclotron resonance mass spectrometry
fw	Fresh weight
FWHM	Full Width at Half Maximum
g	Gram
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometry
GC-ICP-MS	Gas chromatography inductively coupled plasma mass spectrometry
GF-AAS	Graphite furnace atomic absorption spectrometry
GLA	γ-linolenic acid
GRAS	Generally recognized as safe
H	Hydrogen atom
^{2}H	Labelled hydrogen atom
h	Hour

HAMQAP	Health Assessment Measurements Quality Assurance Program
$H_2AsO_4^{2-}$	Arsenate ion
H_2O	Water
H_2O_2	Hydrogen peroxide
$H_2PO_4^{2-}$	Phosphate ion
H_2S	Hydrogen sulfide
HCD	High-energy collisional dissociation
HCl	Hydrochloric acid
HDL	High density lipoproteins
He	Helium
HepG2	Human liver cancer cell line.
HG	Hydride generation
HG-AAS	Hydride generation atomic absorption spectrometry
HG-AFS	Hydride generation atomic fluorescence spectrometry
HML	Hollings Marine Laboratory
HMOX-1	Heme oxygenase-1
HNO_3	Nitric acid
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
IARC	International Agency for Research on Cancer
iAs	Inorganic arsenic
IC_{50}	Concentration at which the cell viability is reduced by 50%
ICP	Inductively coupled plasma
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry

INAA	Instrumental Neutron Activation Analysis
InChI	International Chemical Identification
IS	Internal standard
IT-TOF	Ion-trap time-of-flight
JAFC	Journal of Agricultural and Food Chemistry
JFCA	Journal of Food Composition and Analysis
°K	Kelvin (Thermodynamic temperature)
K^+	Potassium ion
kcal	Kilocalorie
Kg	Kilogram
L	Liter
LC	Liquid chromatography
LC-ICP-MS	Liquid chromatography inductively coupled plasma mass spectrometry
LC-PUFAs	Long-chain polyunsaturated fatty acids.
LD_{50}	Concentration at which 50% of the population dies.
LDCs	Least developed countries
LDPE	Low-density polyethylene
LIFDCs	Low-income food-deficient countries
LTQ	Linear-trap quadrupole
m	Meter
MAE	Microwave-Assisted Extraction
MALDI	Matrix-Assisted Laser Desorption Ionization
MALDI-TOF-MS	Matrix-assisted laser desorption ionization time-of-flight mass spectrometry
MeOH	Methanol

METLIN	Metabolite and Chemical Entity Database
mg	Milligram
$Mg(ClO_4)_2$	Magnesium perchlorate
MLC	Micellar liquid chromatography
mm	Millimeter
mM	Millimolar
mmol/L	Millimole per liter
MMA^{III}	Monomethylarsonous acid
MMA^{V}	Monomethylarsonic acid
$MMDTA^{V}$	Monomethyldithioarsonic acid
MMT	Million metric tons
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MS^n	Multistage mass spectrometry
MSPD	Matrix solid-phase dispersion
MW	Microwave
m/z	Mass to charge ratio
N	Nitrogen atom
N_2	Nitrogen gas
Na	Sodium atom
Na^+	Sodium ion
$NaBH_4$	Sodium borohydride
NaOH	Sodium hydroxide
NASA	National Aeronautics and Space Administration

ng	Nanogram
ng/g	Nanogram per gram
NH_3	Ammonia
$NH_4H_2PO_4$	Ammonium dihydrogen phosphate
NH_4NO_3	Ammonium nitrate
$(NH_4)_2SO_4$	Ammonium sulfate
NIST	National Institute of Standards and Technology
nm	Nanometer
NMI	National Metrology Institute
NMIJ	National Metrology Institute of Japan
NMR	Nuclear magnetic resonance
NOAA	National Oceanic and Atmospheric Administration
O	Oxygen atom
O_2	Oxygen gas
Orbitrap	Ion-trap mass analyzer
Orbitrap-MS	Orbitrap mass spectrometry
Pc	c-phycocyanin
PDH	Pyruvate dehydrogenase
PDMS	Polydimethylsiloxane
pg	Picogram
pg/g	Picogram per gram
pH	Potential/Power of hydrogen
pKa	Acid dissociation constant
PLE	Pressurized liquid extraction
ppm	Parts per million

ppb	Parts per billion
ppt	Parts per trillion
PRM	Parallel Reaction Monitoring
PT	Proficiency testing
PTFE	Polytetrafluoroethylene
PTWI	Provisional tolerable weekly intake
Q1	First quadrupole
Q_{app}	Apparent charge
QITMS	Quadrupole ion trap mass spectrometry
Q-TOF	Quadrupole time-of-flight
Q-TOF-MS	Quadrupole time-of-flight mass spectrometry
QqQ	Triple quadrupole
RF	Radio frequency
Rh	Rhodium
ROS	Reactive oxygen species
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
RP-LC	Reversed-phase liquid chromatography
s	second
SAM	S-Adenosylmethionine
SDS	Sodium dodecyl sulfate
SEC	Size-exclusion chromatography
SFE	Supercritical fluid extraction
SIDS	Small island developing states
SIM	Selected ion monitoring

Sm^{2+}	Samarium ion
SMILES	Simplified molecular-input line-entry system
SO_2	Sulfur dioxide
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SRM®	Standard Reference Material
Std	Standard
TBAH	Tetrabutylammonium hydroxide
TEAH	Tetraethylammonium hydroxide
TETRA	Tetramethylarsonium ion
Thio-DMA^V	Thio-dimethylarsinic acid
TMAO	Trimethylarsine oxide
TMAS	Trimethylarsine sulfide
TMAB	Tetramethylammonium hydroxide
TOF	Time-of-flight
UAE	Ultrasound-Assisted Extraction
µg	Microgram
µg/g	Microgram per gram
µg/L	Microgram per litre
µM	Micromolar
UPS	Ultrasound probe sonication
UROtsa	Human bladder (Urothelial) cell line.
UK	United Kingdom
UN	United Nations
UN DESA	United Nations Department of Economic and Social Affairs

UN SDGs	United Nations’ sustainable development goals
U.S	United States of America
UVPD	Ultraviolet photodissociation
UV/vis	Ultraviolet and visible range of the light spectrum
ω-3	Omega-3
ω-6	Omega-6
WHO	World Health Organization
XAS	X-Ray absorbance spectroscopy
XANES	X-Ray absorption near-edge spectroscopy

Chapter 1: Introduction

Arsenic is a ubiquitous notoriously toxic element, which occurs naturally in the earth's crust, soil, freshwater and marine environments with no known biological roles in humans.[1,2] Arsenic has a complex chemistry, and a diverse group of arsenicals exist in the marine environment. Arsenic is primarily released into the environment through various anthropogenic sources.[2–4] Humans are exposed to arsenic through dermal contact, inhalation, water and dietary intake, but for most, dietary exposure is the most significant.[5] Diet, especially seafood, is the main source of arsenic exposure for humans,[6] except for areas with endemic high drinking water contamination.[7–9]

Inorganic arsenic (iAs) is a well-characterized non-threshold category I carcinogen.[10] However, knowledge related to the abundance, identity and toxicity of organic arsenic species is limited.[11] Therefore, knowledge of arsenic speciation is important as the chemical form of arsenic controls its bioavailability, mobility and toxicity.[12] Arsenobetaine (AsB) is bioavailable to humans (i.e., it is taken up from the gut), however, it is not metabolized but is quickly excreted unchanged in the urine.[13] The toxicity of most arsenolipids (AsLipids) is currently not known. In this case, it is important to acquire more knowledge regarding these lipophilic arsenic species, as noted by the European Food Safety Authority (EFSA) in their scientific opinion on arsenic in food.[14] Therefore, more data on arsenic speciation, including the AsLipids is needed for future risk assessments of arsenic in food.

Toxicity studies are primarily based on iAs and AsB, a tetraalkylated and predominant organoarsenical in seafood, that is considered benign. Paucity of toxicity data for

organoarsenicals, the dominant species in seafood, has led to conjecture of their innocuous nature. However, toxicokinetic studies show that some organoarsenic species, arsenic-containing hydrocarbons (AsHCs), are bioaccessible and cytotoxic with toxicities similar to those of pernicious trivalent inorganic arsenic (iAs^{III}), which underpins the need for quantification of such arsenic species.

As early as the 1960s, it was shown that marine oils were concentrated in arsenic, as oils extracted from marine samples, *e.g.* herring (*Clupea* harengus), mackerel (*Scomber scomber*) and liver of cod (*Gadus morhua*) contained arsenic levels up to 19 µg/g.[15] However, no chemical structures of the AsLipids were identified. Research on lipophilic arsenicals remained negligible compared to the hydrophilic arsenic species. The main reason was challenges with the methodology, since the preferred instrumentation i.e., inductively coupled plasma mass spectrometry (ICP-MS) is not compatible with organic solvents, which are needed to solubilize the lipids.[16]

This trend continued for four decades, until in 2008, following the modification of the ICP-MS and using it concurrently with electrospray ionization mass spectrometry (ESI-MS), the chemical structures of two groups of intact AsLipids were identified in marine oils.[17,18] They were characterized as arsenic-containing hydrocarbons (AsHCs) [17] and arsenic-containing fatty acids (AsFAs).[18] These studies initiated a larger research focus regarding the AsLipids.

Insufficient data on the organoarsenic species in seafood remains the crucial missing link to understanding human exposure and informing regulatory practices. Lack of regulatory limits for arsenic in seafood is at least partly attributable to the multiplicity

of species and gaps in our knowledge of their toxicity and metabolism. Currently, there is no reliable information on the toxicity of organoarsenicals like arsenosugars (AsSugars) and arsenolipids (AsLipids). Estimation of arsenic toxicity requires the identification and quantification of the individual arsenic species. Therefore, efforts have been focused on the development of methods for the separation and detection of arsenic compounds. Unfortunately, there is no widely accepted analytical protocol for arsenic speciation analysis.

Analytical methods are a prerequisite for obtaining quantitative and qualitative information on the occurrence of organoarsenicals, especially AsLipids, in marine samples. The development of such analytical methods for determination of the organic arsenic species is therefore an essential first step towards further studies of these species. With analytical methods developed for the determination of lipophilic arsenicals, marine samples can be studied, and data on their occurrence in samples related to both food and feed safety can be acquired.

It is not yet possible to extract all the arsenic species from all the sample matrices using a single set of conditions. Therefore, extraction conditions must be optimized considering the target arsenic species. The objective usually is to extract arsenic species quantitatively without their degradation or chemical transformation. Stability of arsenic species is critically dependent on the sample matrix, preservation and extraction techniques employed. The most common extractants for hydrophilic arsenicals are water, methanol, and methanol/water mixtures with different compositions. Extractants must be chosen based on arsenic species present in the sample. Extraction efficiencies

typically increase by applying more chemically aggressive extraction conditions. This calls for a delicate balance between maximum extractability and species integrity to avoid the undesirable chemical transformations of the native arsenic species.

Arsenic speciation in seafood is challenging owing to their existence in myriads of chemical forms and oxidation states, interconversions that occur between species, the complexity of the seafood sample matrix, lack of well-characterized and commercially available standards to be used as calibrants and certified reference materials (CRMs) for method validation, and a lack of widely accepted measurement protocols.

The aim of this work was to (1) develop analytical methods for arsenic speciation in seafood, (2) improve sample preparation methods, (3) identify and quantify arsenic species in seafood using liquid chromatography coupled to inductively coupled plasma mass spectrometry and electrospray ionization mass spectrometry (LC-ICP-MS/ESI-MS), (4) develop an *in silico* database for structural elucidation and annotation.

The structure of the dissertation is presented as follows: Chapter 1 outlines the different classes of seafood highlighting their importance as a source of nutrients and their impact on human health and the global economy. Also, the different arsenic species present in seafood as contaminants of concern are introduced. A justification for the selection of proxy seafood samples is provided and an overview of the analytical considerations for risk assessment with regards to seafood is given as an introduction to Chapters 2 and 3, which serve as literature review on the research topic.

Chapter 2 reports a comprehensive summary of the current state of understanding of the various aspects of organoarsenic formation in the marine food chain in terms of

occurrence, exposure, metabolic transformation, and toxicity of smaller-molecule oxo-arsenicals, AsSugars, AsLipids, and thio-arsenicals. A brief discussion about the mechanism of toxicity of inorganic arsenic is also presented because it is the arsenic species with well-understood adverse effects. This chapter is published in the Journal of Agricultural and Food Chemistry (JAFC). *J. Agric. Food Chem. 2020, 68, 943-960.* https://dx.doi.org/10.1021/acs.jafc.9b07532.

Chapter 3 aspires to provide an overview of the current state of practice in arsenic speciation analysis of edible marine species, which include seafood and seaweed from extraction to detection, quantification, and characterization, while highlighting the general analytical considerations. The discussions in this chapter are focused on hydrophilic organoarsenicals, like methylated arsenicals and AsSugars, lipophilic organoarsenicals, like AsHCs, AsFAs, AsSugar phospholipids (AsSugar-PLs), collectively called arsenolipids. This chapter is published in the Journal of Agricultural and Food Chemistry (JAFC). *J. Agric. Food Chem. 2020, 68, 1910-1934.* https://dx.doi.org/10.1021/acs.jafc.9b04525.

Chapter 4 discusses the work performed in the development and optimization of the analytical methods. The optimized method was used to characterize the seafood samples and was the basis for the research work on the determination of total and hydrophilic arsenic species in seafood presented in Chapter 5, which was published in the Journal of Food Composition and Analysis (JFCA). *J. Food Comp. Anal. 2021, 96, 1-11*. https://doi.org/1016/j.jfca.2020.103729. This work also contributed to the value assignment for total arsenic and arsenic species in seafood samples used in the 'Health

Assessment Measurements Quality Assurance Program: Exercise 5' (HAMQAP) that was offered by the National Institute of Standards and Technology (NIST) in 2020. The work is published as NISTIR 8343, HAMQAP, Exercise 5 Final Report available at https://doi.org/10.6028/NIST.IR.8343.

Lack of authentic standards and reference materials has impeded the development of databases necessary for the identification of AsLipids. The current identification methods for AsLipids are very expensive in terms of instrumentation and require high level of technical competencies, which is beyond the reach of many laboratories. Therefore, new approaches are required for the identification and structural elucidation of AsLipids. In this work, a structural database for the identification and annotation of AsLipids has been presented, based on *in silico* fragmentation data of extant AsLipids, using competitive fragmentation modeling for metabolite identification (CFM-ID). This tool will provide a cheap, fast, robust, convenient, customizable, and readily accessible method of identification for AsLipids. The development and implementation of the tool is described in detail in Chapter 6.

Chapter 7 gives a discussion of the future perspectives.

1.1. Nature and occurrence of arsenic in marine environment

Even though there is consistent evidence of health benefits from modest consumption of seafood, there exist possible risks and adverse effects associated with the consumption of seafood, especially arsenic that is inherently present as a contaminant, particularly organic arsenic species.[19] Occurrence of high content of arsenic in seafood has been known since early 1900s. However, the first systematic investigation of this

phenomenon in seafood was made in the 1920s, and it took another 50 years before the unknown arsenic compound in lobster was identified as AsB.[1] Toxicological studies demonstrated that AsB was harmless and its occurrence in seafood presented no health concerns.[20]

Since the identification of AsB, over 200 additional arsenic species have been reported in many marine organisms, which serve as common types of seafood.[7,21] The arsenic species of great significance, either because they occur at high levels (e.g., AsSugars and AsLipids) or because they have toxic properties (e.g., iAs and AsHCs) or are important metabolites (e.g., DMA), are discussed in depth in Chapter 2.

There is great diversity in the level of arsenic in seafood, but arsenic in most seafood samples falls within the mass fraction range of about 5 µg/g to 100 µg/g dry mass.[21] Terrestrial foods, on the contrary have almost invariably low levels of arsenic with most samples having less than 0.05 µg/g dry mass,[22] with the exception of rice, which typically contain about 0.1 µg/g to 0.4 µg/g.[21]

Arsenic has similar chemistry with members in the same group i.e., phosphorus and nitrogen, which might explain how they end up in marine organisms. In normal seawater of pH 8.1, arsenic exists primarily as the diprotonated oxo-anion $[H_2AsO_4]^-$ (pK_{2a} = 6.8, ionic radius 0.248 nm). Under the same conditions, phosphate, a major seawater nutrient, also exists as the diprotonated oxo-anion $[H_2PO_4]^-$ (pK2a = 7.2, ionic radius 0.238 nm). Marine algae have membrane transport systems that take up the essential phosphate from seawater, but these systems cannot discriminate against the structurally similar arsenate species. This allows easy access for arsenic into the algal

cell.[23] Within the cell, As^V is again mistaken for phosphate leading to disruption (decoupling) of oxidative phosphorylation processes with resultant toxic effects. Further discussions on the mechanism will be presented in Chapter 2. To alleviate the potential toxicity of As^V, algae have developed a process of converting it to AsSugars through successive oxidative alkylation steps.[24]

AsB is the most widespread and abundant hydrophilic organoarsenic species in seafood, though its biosynthetic origin remains unknown. The dominance of AsB in seafood is likely related to the similar chemistry of arsenic and nitrogen. AsB is structurally similar to glycine betaine, which serves as an osmolyte protecting osmoconforming organisms from changes in the salinity of their ambient water.[25] Thus, when salinity is high the cells actively takes up small charged organic molecules, including glycine betaine, to counteract osmotic pressure difference within and outside the cells. Glycine betaines are favored over inorganic ions because at high concentrations they are less damaging to proteins in the cell.

The similarity of structure of AsB and glycine betaine, see structures in Chapter 2, points to the possibility that the cell may be unable to distinguish between these ions and takes up AsB by the same transporter. This hypothesis gains credence from the fact that mussels maintained in seawater of varying salinities show a marked decrease in their arsenic content at lower salinities.[26] Furthermore, the efficiency of uptake of AsB from seawater by these mussels decreased with increasing levels of glycine betaine, a result consistent with competitive uptake.[27] On the other hand, fish of the same species

taken from adjacent waters of varying salinities showed a clear direct relationship between arsenic content and salinity.[28]

The fact that organoarsenic species are found in marine organisms at high concentrations raises the possibility that they may actually be of importance to the organism, which could be incidental use or directed use.[21] Incidentally, the organism uses some compounds containing arsenic because they, by chance, mimic and provide the same service as other commonly used natural products e.g., arsenobetaine for glycine betaine. The lower quantities of the arsenic compounds in organisms relative to the compounds they might mimic, however, suggests that such a role for arsenic is of little biological consequence for the organism. The possibility remains that the arsenic analog is very effective in its role, and that its presence even at modest concentrations might provide a significant advantage to the organism.[21] However, there is no data so far to support this view.

Of more fundamental interest is that the organoarsenic compounds serve a particular role in the organism, and that their biosynthesis is directed by biochemical requirements of the organism. For example, products derived from fatty fish, like cod liver oil, have high levels of arsenolipids, which are arsenic analogues of lipid natural products present at high levels in fish. The origin of the usual lipid is algae, and it appears likely that the AsLipids are also synthesized by algae.[29] Work by De Mooy et al.[30] demonstrated that unicellular algae utilize nitrogen and sulfur, rather than phosphorus, in the biosynthesis of their membrane lipids in low-phosphate waters. This strategy to preserve phosphorus when the phosphate supply is limited raises the possibility that

arsenic may also be used in membranes in a similar way, and that the AsLipids result from directed synthesis by a process activated when phosphate levels are low.

Marine algae and shellfish are the seafood exposure sources with the greatest diversity of arsenicals.[31] Current guidelines for arsenic exposure exists only for iAs,[14] which is the major form of arsenic in drinking water, and can be present at appreciable levels in rice.[32] The reference doses for iAs are based on studies of high exposure from contaminated drinking water sources, but more recent studies have found effects of iAs from lower drinking water concentrations and from food.[33–36] Regulators are now focusing on identifying the impacts of arsenic at concentrations relevant to those populations without a specific contaminant source of arsenic. It is therefore critical from a risk-based approach to conduct arsenic speciation analysis in order to determine the arsenic species present and their relative proportions consumed by humans and thus enable more accurate risk assessment.[21,37,38]

Toxicity, bioaccumulation, bioavailability and mobility of arsenic are largely dependent on the chemical form and the extent of methylation.[39] Typically, the lower the oxidation number, the higher the toxicity and the higher the methylation, the lower the toxicity. iAs species are non-threshold Class 1 carcinogens, while simple methylated arsenicals such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are Class 2B with intermediary toxicity.[10] AsB is nontoxic.[20] Studies suggest that AsSugars exhibit no acute cytotoxicity or mutagenicity, even though these compounds may be metabolized within the human body to form potentially toxic metabolites like DMA.[40]

Other more complex organic arsenic compounds in the form of AsSugars and AsLipids, are also present at significant quantities in some types of seafood and have been shown to be taken up and metabolized in humans. These classes of arsenic compounds produce the same urinary metabolite as iAs, namely DMA,[40] therefore their presence in diet could be confusing to studies of iAs using biomarkers such as urine and blood, or in studies evaluating the contribution of other dietary sources to biomarker concentrations of arsenic. Recent findings have also shown that some forms of organic arsenic species and their intermediate metabolites display cytotoxicity in cell cultures,[41–45] suggesting that further studies of toxicity and metabolic pathway are needed.[8]

1.2. Sources and distribution of organic arsenic species in marine organisms

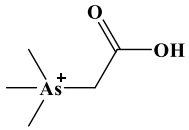
Levels of arsenic in seawater are low and uniform (1–5 μg/L),[46] with arsenic mainly present as As^V. In freshwater, arsenic is also present primarily as iAs, but levels can be much higher than those in seawater. Despite the lower arsenic seawater levels, much higher concentrations of arsenic are found in the marine food chain compared with freshwater systems. This apparent anomaly may be explained by the transformation of iAs to organoarsenicals at the base of the marine food chain, and the higher accumulation and retention of these organic compounds in marine organisms.[31]

Microalgae are the primary iAs accumulators in the marine environment and represent an important stage in arsenic migration through the food chain.[47] It is generally understood that iAs, which is mainly absorbed in the form of As^V from seawater, is metabolized by the algae to a variety of organic arsenic species, especially AsSugars.[48] Seaweed has some of the highest total arsenic concentrations in the marine food web,[14]

shellfish generally have higher arsenic content than finfish, and demersal fish often contain more arsenic than pelagic fish, although differences between and within species can be large.[31,49–51] Arsenic compounds found in seafood are divided into classes depending on their structure and properties (see Fig. 1).

Arsenobetaine

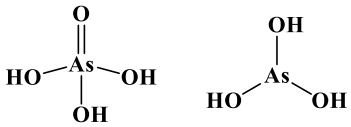
Most abundant Arsenic species in shellfish and finfish, non-toxic



Inorganic arsenic (iAs)

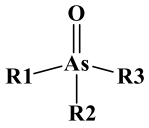
Minor component in most seafood, known carcinogen

Arsenate (As^{V}) **Arsenite (As^{III})**



Methylated Arsenicals

Minor constituents of seafood, metabolites of iAs, AsSugars & AsLipids



Examples:

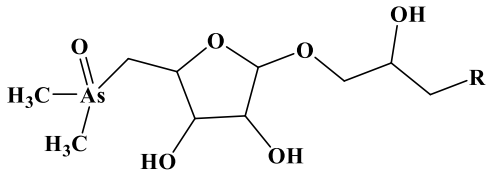
MMA: R_1=CH_3, $R_{2\text{-}3}$=OH

DMA: $R_{1\text{-}2}$=CH_3, R_3=OH

TMAO: $R_{1\text{-}3}$=CH_3

Arsenosugars (AsSugars)

Major arsenic compound in seaweed; metabolized by humans



Examples:

AsSugar 328: R=OH

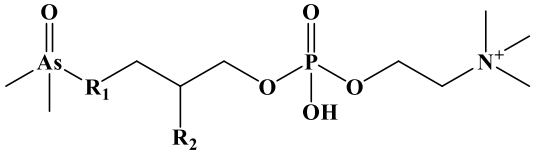
AsSugar 392: R=SO_3

AsSugar 408: R=SO_4

AsSugar 482: R=$OPO_3CH_2CHOHCH_2OH$

Arsenic-containing Phosphatidylcholines (AsPCs)

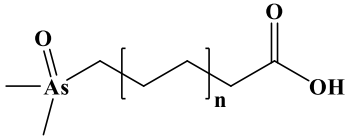
Identified in Roe



R_1 = Fatty Acid; **R_2** = Fatty Acid

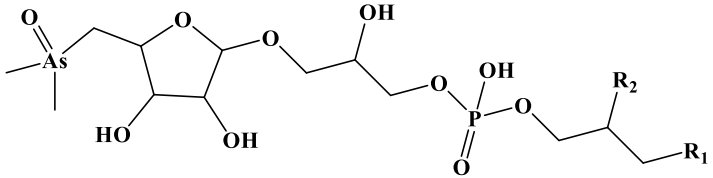
Arsenic-containing Fatty Acids (AsFAs)

Identified in fish oils; metabolized by humans



Arsenic-containing Phospholipids (AsPLs)

Identified in seaweed



R_1 = Fatty Acid

R_2 = Fatty Acid

Arsenic-containing hydrocarbons (AsHCs)

Identified in fish oils; metabolized by humans

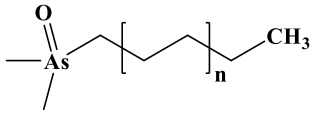


Figure 1: Arsenic compounds found in Seafood

Estimated arsenic species distributions, based on literature reports and mean total arsenic concentrations for different seafood types,[14,52] are depicted below (see Fig. 2).

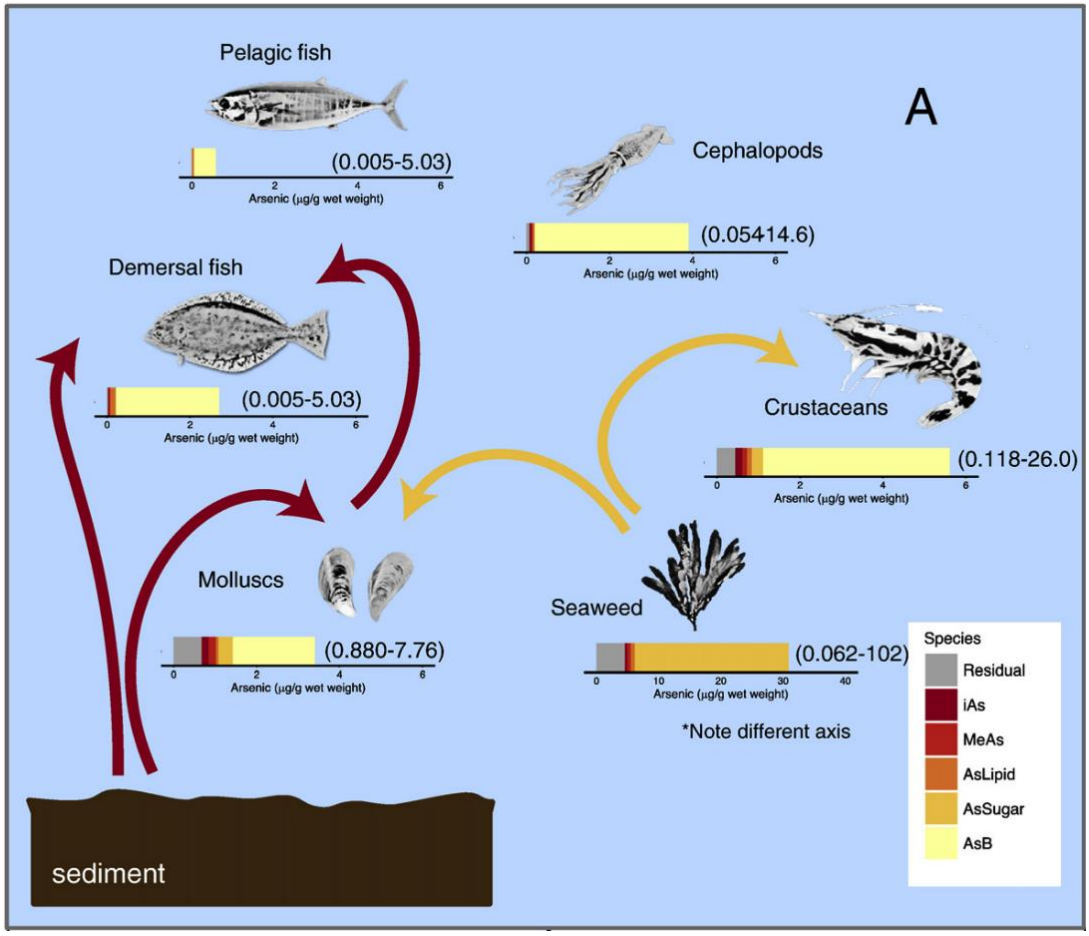


Figure 2: Estimated distributions of Arsenic Species found in different seafood types.[31]

1.2.1. Inorganic Arsenic

The presence of iAs is considered as a component of arsenic exposure from seafood, though this rarely happens. Elevated iAs body burdens may correspond to enhanced arsenic in sediments and the water column, depending on the organisms' feeding mechanism, and can be caused by proximity to a point source of contamination.[53]

Conversely, levels of organic arsenic in seafood are not affected by contaminated sites.[54]

Elevated levels of iAs have been reported in bivalves and gastropods at some sites,[54–56] and have been the basis of consumption guidelines in the Pacific U.S.[57] Pelagic fish, which generally have a low proportion of iAs tend not to accumulate higher concentrations of total arsenic from areas with elevated arsenic levels,[58] whereas benthic-feeding organisms can accrue increased concentrations of iAs.[53]

Compared with organic arsenic, efforts to assess iAs exposure from seafood have become more frequent, but there remains significant uncertainty in predicting iAs levels in different marine-sourced foods,[59,60] again emphasizing the need for routine monitoring. Petursdottir et al.,[61] reviewed the regulations for iAs in seafood as a food source and for use in animal feed.

Concentrations of iAs are negligible in most seafood. However, the brown algae, Hijiki (*Hizikia fusiforme*), an edible seaweed used extensively in Asian cuisine, is well-documented as having high amounts of total arsenic, the majority of which is in inorganic form.[62–65] Some samples of other species of brown algae have also been found to contain high proportions of iAs, suggesting the need for more monitoring.[19,66]

1.2.2. Methylated Arsenic Species

Methylated arsenicals are present in marine ecosystems from enzymatic methylation of iAs to form compounds containing one to four methyl groups. Such arsenicals generally occur as minor arsenic species in seafood, with DMA being the most prominent.

Mollusks can contain DMA at higher proportions (3–46%) than are typically seen in finfish or algae.[54,67,68] MMA is uncommon in marine environments and is generally present in trace amounts only, the same with TMAO, though it has not been found in seaweed but can occur at higher levels in some fish species.[31] High levels of tetramethyl arsonium (TETRA) ion have been found in clams and gastropods.[69]

The biosynthetic pathway for methylation of iAs to TMAO has been studied extensively in fungi and involves reduction of As^V followed by oxidative methylation with S-adenosylmethionine (SAM) acting as the methyl donor. For marine organisms, the involvement of SAM in the methylation of arsenic has been demonstrated for the alga *Polyphysa peniculus* using CD_3^- labeled SAM.[70]

Other pathways proposing the involvement of glutathione (GSH) or protein conjugated intermediaries have been fronted by Hayakawa et al.,[71] and Naranmandura et al.,[72] (see Fig. 3 below) and will be discussed further in Chapter 2.

Other minor arsenic species include arsenocholine (AsC) which is rarely found in seafood, probably because it is effectively metabolized to AsB,[73] although it is the major arsenical in some species of jelly fish.[74] Several arsenic compounds can also occur as thiol analogs, where sulfur replaces the oxygen atom.

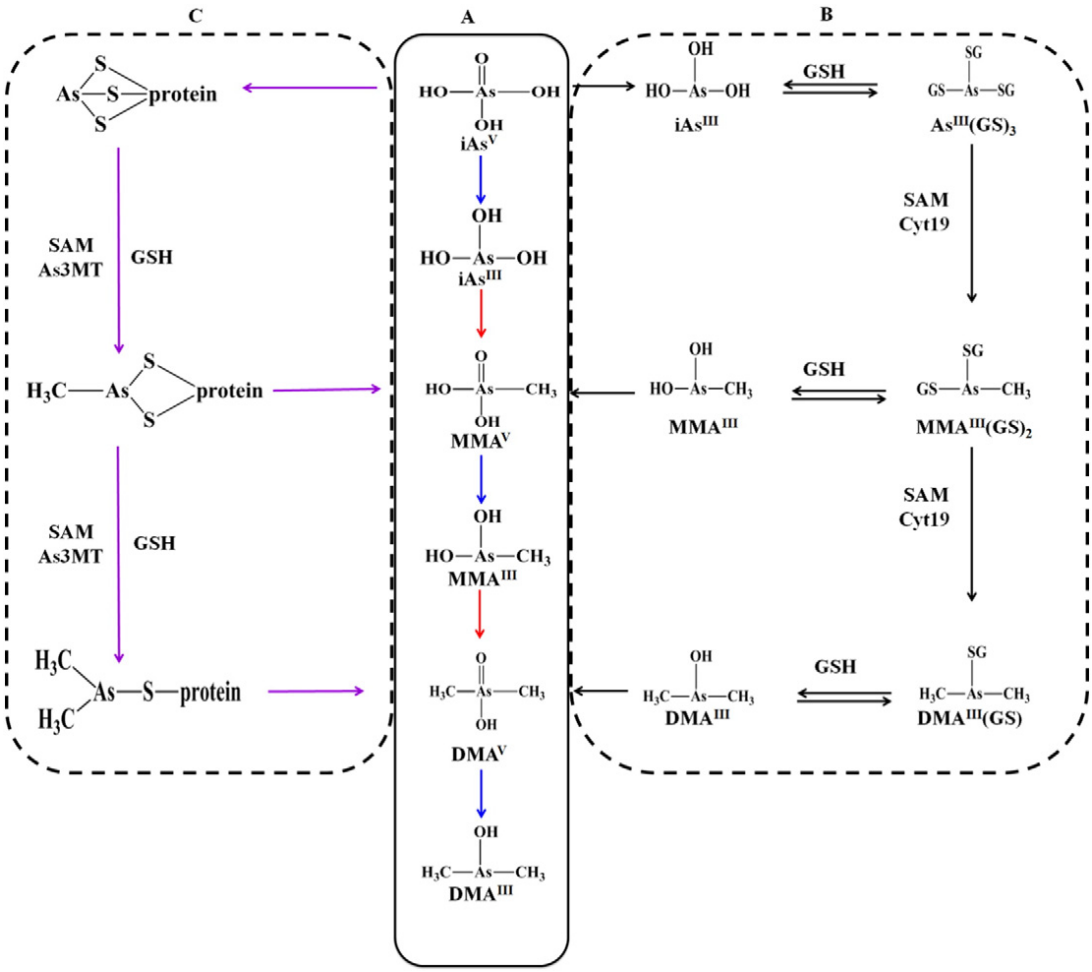


Figure 3: Three proposed arsenic metabolic pathways. (A) Oxidative methylation pathway,[75] where red arrows show methylation while blue for reduction; (B) Reductive methylation through As-GSH complexes;[71] (C) Reductive methylation through As-protein complexes.[72]

1.2.3. Arsenobetaine

Arsenobetaine is an arsenic analog of the amino acid derivative, trimethylglycine (glycine betaine). AsB is recalcitrant to metabolism, discussed in detail in Chapter 2. The source of AsB in the food web is unclear, and there are a number of theories about the biosynthetic pathway of AsB formation.[46] One possible pathway is via the oxidation of AsC following breakdown of the trimethylated AsSugar species. These compounds, however, are usually present at such low concentrations that it is difficult to perceive

them as precursors to the abundant AsB. A second pathway, not involving AsSugars, is via the reaction of DMA^{III} with glycoxylate.[46] This may account for the presence of AsB in terrestrial organisms such as mushrooms.[76]

Whatever the source of AsB, its predominance in seafood is probably due to its similarity to the important osmolyte, glycine betaine, which translates into AsB being bioaccumulated from dietary sources. Experimentally, AsB has been shown to be efficiently absorbed from seawater by mussels,[77] whereas shrimp and fish accumulate AsB efficiently only from food.[73] AsB is not biosynthesized by fish when fed possible AsB precursors dimethylarsinoyl ethanol (DMAE) or dimethylarsinoyl acetate (DMAA).[73] In mussels, the retention of AsB seems to depend on the salinity of their surrounding water supporting the idea that AsB can mimic an osmolyte.[26] Similarly, a trend in increasing total arsenic with salinity was observed across three species of pelagic fish, where AsB is expected to be the dominant arsenic species, also suggesting that AsB uptake and retention is related to salinity.[28]

AsB is the major arsenic species in most finfish and shellfish. AsB is also found in zooplankton and some algae at the base of the food chain, but its presence in algae might possibly be due to epiphytic plankton or symbiotic bacteria on the surface of marine flora.[78] In bivalve mollusks,[79] which have complex arsenic speciation, AsB can be a significant portion of hydrophilic arsenic,[68] whereas in cephalopods and crustaceans, which have much simpler arsenic speciation, AsB is the dominant species.[77,] In finfish arsenic species is also predominantly AsB, although AsLipids can be a significant fraction in some oily fish.[80,81]

1.2.4. Arsenosugars

AsSugars are ribose derivatives, which contain mainly dimethylarsinoyl ($Me_2As(O)$-) moiety bound via the C-5 of the ribose ring, with various substituents at C-1.[1,82,83] They can also contain a trimethylarsenio moiety instead of the dimethylated one, although these are far less prevalent. There are so far at least 20 known AsSugars, of which four of them i.e., AsSugar-OH, AsSugar-PO_4, AsSugar-SO_3 and AsSugar-SO_4 (see Fig. 2) are by far the most widespread in marine organisms. An in depth discussion of these compounds will be presented in Chapter 2.

There is no known biological function for AsSugars, just as their exact biosynthesis is still unknown. None of the enzymes involved in the attachment of the ribose- moiety to DMA have been identified, although it is highly likely that the methyl groups and ribose moiety attached to arsenic are provided by S-adenosylmethionine (SAM).[84,85] It has been demonstrated that AsSugars are directly synthesized by phytoplankton,[86–89] and the brown macroalgae *Fucus serratus*.[24] AsSugars have also been found in deep sea vent mussels,[90,91] which suggests a bacterial source of these compounds. However, neither the *Fucus*-associated fungi *Fusarium oxysporum meloni* isolated from *Fucus gardneri*,[92] nor the culturable bacteria associated with unicellular phytoplankton cultures,[87] were found to transform arsenic species.

AsSugars are associated with marine algae, which accumulate arsenic from seawater and store it largely in the form of AsSugars, usually at high concentrations (20–100 µg/g dry weight), and as the major arsenic species (>80% of total arsenic).[135,161] Mollusks and crustaceans that are predominantly filter feeders or grazers, can also

contain AsSugars from consuming algae or phytoplanktons, though concentrations are generally much lower.[136,144]

The accumulation of AsSugars in seaweed results from the transformation of As^V. Marine algae take up arsenic from seawater presumably due to the chemical similarities between arsenate ($HAsO_4^{2-}$) and the major seawater nutrient, phosphate (HPO_4^{2-}).[11] To alleviate its potential toxicity, most algal species transform As^V to AsSugars and other organoarsenicals. A pathway has been proposed for the transformation which involves enzyme-catalyzed production of methylated arsenic followed by synthesis of AsSugars and AsLipids.[11] The variable distribution of AsSugars among seaweed is believed to be due to difference in enzymatic systems that carry out the biotransformation processes.[94] Certain seaweed such as hijiki, however, accumulate As^V due to lack of genetic capability for its conversion or because the process is energetically expensive.[95] Geiszinger *et al*.[24] showed that the transformation of As^V in a brown alga (*Fucus serratus*) becomes inefficient under high exposure.

1.2.5. Arsenolipids

Lipophilic organoarsenic compounds generally known as arsenolipids (AsLipids) are novel species of interest that have relatively high natural levels in seafood and seaweed.[96] In seafood, AsLipids comprise up to 70% of the total arsenic content,[18] which accounts for between 0.3-3.6 μg As g^{-1} dry weight. The highest amounts were found in fatty fish,[80] like herring (*Clupea harengus*), cod (*Gadus morhua*), capelin (*Mallotus villosus*), and mackerels (*Scomber scombrus*) and seaweed,[96] like Hijiki (*Hizikia fusiformis*) and Wakame (*Undaria pinnatifida*). The existence of arsenic in

lipid extracts of fish and algae was originally reported by Lunde in 1968,[15] but their structures still remained unknown. The first structure of AsLipids was presented by Morita and Shibata in 1988 through their classic study using ^{1}H NMR and identified AsSugar-PL 958 in a marine alga, Wakame (*Undaria pinnatifida*).[97]

AsLipids elicited research interest due to their novel structures, their likely role in membrane biochemistry, and since they exist in common seafood with potential health concern based on arsenic toxicity.[21] AsLipids are of toxicological concern because of the recent discovery that AsHC 332, AsHC 360 and AsHC 444 demonstrated substantial toxicities in various *in vitro* and *in vivo* systems, comparable to that of iAs^{III}, a well-characterized carcinogen.[42,44,98–101] However, the molecular modes of action regarding their toxicity as well as their metabolism in the liver still remain unclear.[98] Among AsHCs, the toxicity is dependent on the structure and polarity of the AsHC.[42]

Since AsHCs are often found to be the dominant AsLipids, especially in fish and fish oil, there is pressure for obtaining more information on AsLipids. Even though their cytotoxic effect in human liver cells are lower than iAs^{III} and they do not show any genotoxic effects, the risk to human health from AsFA 362 and AsFA 388 cannot be excluded.[43,45] The need for more data on AsLipids is evident, and simple methods for the determination of AsLipids are of great importance to accomplish this task. Further discussions on this topic will be presented in Chapter 2.

More than 200 AsLipids have been discovered in various marine samples and distributed between ten lipid classes: As-containing fatty acids (AsFA), first reported in cod liver oil,[17] have been found in a range of fish species [7,80,102–106] in addition to

algae;[107] As-containing hydrocarbons (AsHC), following the first report of their presence in capelin,[18] have also been found in various fish[81,106,108–110] and in two species of algae;[111,112] dimethyl As-containing fatty alcohols (DMAsFOH), reported after 48 h incubation of HepG2 cells with AsHC 332 and AsHC 360;[98] trimethylarsenio fatty alcohols (TMAsFOH), first reported in capelin oil,[113] have been found in capelin, herring and blue whiting fish meals and edible seaweed dulse;[112,114] As-containing phosphatidylethanolamine (AsPE) and As-containing phosphatidylcholine (AsPC), first reported in herring caviar,[115] have been found in capelin, herring and blue whiting fish meals and green alga (*Coccomyxa schmidle*);[114,116,117] arsenosugar phospholipids (AsSugar-PL), first reported in marine alga Wakame,[97] have also been detected in other algae;[107,111,112,118] As-containing phosphatidylinositol (As-PI) and As-containing phosphatidylglycerol (AsPG) have only been reported in green alga;[117] arsenosugar phytols (AsSugar-Phytol), first reported in unicellular green alga (*Dunaliella tertiolecta*),[119] have been found in biota and sediments from lakes;[120,121] and As-containing triacylglycerides (AsTAGs) were first presumed to be present in oil extracts of blue whiting fish (*Micromesistius poutassou*),[122] and later in the Peruvian anchoveta (*Engraulis ringens*),[106] and green alga (*Coccomyxa schmidle*).[27]

The AsLipids are often grouped into categories of polar and non-polar AsLipids,[102,122] and so far, more focus has been on polar AsLipids. Different extraction methods have been used for the determination of AsLipids, including partitioning between heptane and MeOH/H_2O (9:1);[7,109] sequential extraction of hexane followed by MeOH/DCM,[106,107,123] acetone and MeOH/H_2O;[80] and sequential extraction of

MeOH/chloroform, subsequently partitioned between H_2O and MeOH/chloroform and then the AsLipids further partitioned between hexane and MeOH.[81]

Further sample clean up and purification includes partitioning between several solvents of increasing polarity using silica columns.[102,106,124] With a wide choice of extractions, the order of the extraction can have an effect on the stability of AsLipids in the biological sample, since sample preparation can interfere with the identification of AsLipids, presumably due to their stability towards hydrolysis reactions.[81,106]

1.2.6. "Residual" Arsenic

A residual (unextractable) fraction often remains following speciation analysis, and can contain a significant proportion of total arsenic in some samples.[123,125] The form of arsenic in this fraction remains unclear (see Fig. 4). Seaweed can contain variable amounts of unextractable or residual arsenic,[123,126–128] which is thought to be bound to thiol-containing structural compounds.[78] Mollusks frequently have high levels of residual arsenic (8–58%),[54,67] and while arsenic in crustaceans is mostly hydrophilic, the residual fraction can also be significant (9–17%).[129] In mussels, the residual fraction was identified as predominantly thiolated arsenic compounds, suggesting this to be a metallothionein-rich protein-bound fraction rather than a lipid fraction.[54] Very little is known about the metabolic fate of protein-bound arsenic when consumed.

The unextracted arsenic may be attached to cell components and/or proteins [127] and require aggressive extraction conditions that would likely alter their native chemical forms.[130] If the focus of one's analysis is unambiguous identification of native

arsenicals in the samples, preserving the stability of the species takes precedence over quantitative extraction with aggressive conditions.

1.3. Consumption Patterns

Globally, the highest consumers of seafood are populations from Iceland, Maldives, and Japan,[131] while parts of Scandinavia, the North Baltics, and Southeast Asia are also high consumers.[132,133] Japan and Korea are high consumers of pelagic fish and shellfish (mollusks, and crustaceans), whereas pelagic and demersal fish are highest in the North Baltics, and shellfish consumption is a major source of seafood in Southeast Asia.[133]

Seaweed has highly variable consumption rates between countries, and likely between ethnic sub-populations. Seaweed is a staple in Japan, Korea and China, and consumption rates in Japan have been estimated as high as approximately 20 g wet weight per day,[134] with a shift in the prominent type of seaweed consumed from Kombu, which can have higher iAs concentrations,[59] to Wakame and Nori.[135]

The USA and Western Europe are medium consumers of seafood on a global scale,[131] yet seafood consumption is estimated to account for 90% of total As exposure in the U.S. Variation between sub-populations is large, both in terms of total consumption and seafood type. Sex, age and proximity to the coast influence fish consumption, and ethnicity plays a major role in consumption rates, with the “Other” category of ethnicity, which includes Asians, Native Americans, Pacific and Caribbean Islanders and mixed races, having the highest consumption of fish and shellfish.

The consumption of different classes of seafood from several countries with varying diets is shown on the left side of Fig. 4, while the hypothetical intake of arsenic species

by different consumer groups was then estimated as shown on the right side of Fig. 4, based on median seafood concentrations and estimates of arsenic species distribution (see Fig. 2). Exposure is also affected by the concentrations of organoarsenicals in seafood. Levels of total arsenic content varied by greater than two orders of magnitude for most of the seafood types,[14] and the distribution of arsenic species can also vary.

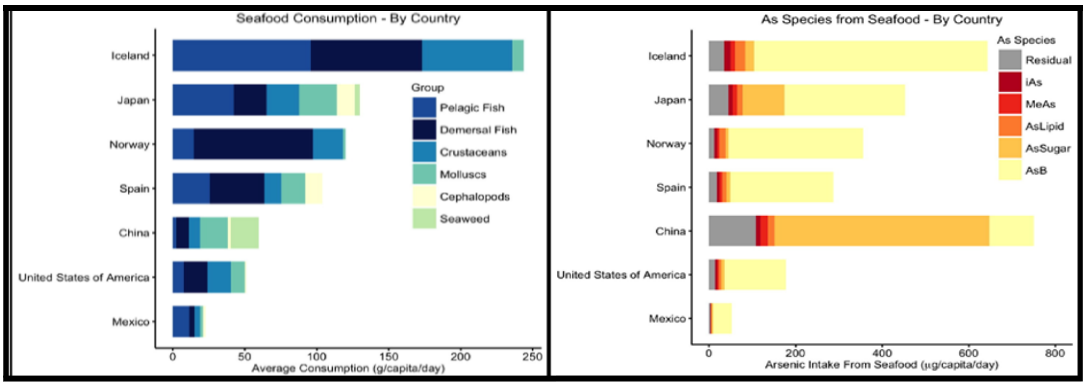


Figure 4: Seafood Consumption by country and hypothetical intake of arsenic species based on estimated distributions of arsenic species and seafood consumption data.[31]

Consumptions were compared using estimates of iAs reported in mussels,[55] and of higher AsLipid concentrations found in oily pelagic fish [81] compared with median estimates. The effect of these reported arsenic species concentrations on intake reinforces the need for further evaluation of arsenic species in seafood.

1.4. Analytical Considerations for Risk Assessment

Total arsenic as an indicator for risk due to dietary exposure is inadequate,[93] and accurate account for the myriads of arsenic species in the seafood present considerable challenge for food safety regulatory authorities.[61,136,137] Since not all the arsenic in seafood is toxic, the complex distribution of arsenic species in marine organisms imply that evaluation of risk focusing primarily on iAs may provide a biased perspective.[93]

Arsenic occurs naturally in seafood in a variety of organic chemical forms. Therefore, considering only the iAs fraction in determining toxicity might underestimate the risk, since the major fraction containing arsenic may be present in a form with potential and/or unknown toxicity.[137] This would contravene the precautionary principle of risk assessment that errs on the side of caution. Focusing only on iAs, especially for seaweed, where AsSugars with unknown toxicities dominate, would distort the level of potential toxicity.[93]

Seafood is considered safe owing to the benign nature of AsB that predominates and the low levels of iAs. However, the recent finding that some AsLipids, especially the AsHCs, have cytotoxic properties like those of iAs has created interest regarding their implications in human health. Current regulations for arsenic exposure focus mainly on iAs, a known carcinogen.[31] Unfortunately, apart from the fact that iAs is 100% bioavailable in drinking water, the regulatory limits for iAs are derived from studies of high exposure from regions with endemic contamination of drinking water.[138,139] Therefore, the mode of action and exposure levels invalidates the significance of these risk assessments with regard to seafood as a source of arsenic exposure.[140]

Setting of standards for arsenic in seafood is intricate owing to the enormous metabolic diversity of organic arsenic species in humans and the lack of reliable speciation data on dietary sources.[93] Knowledge of arsenic speciation in this case is key since the chemical form of arsenic controls its bioavailability, mobility and toxicity.[12] The requirement to establish regulations in relation to toxic iAs in food and to collate additional organic arsenic speciation and toxicity data was underscored by the European Food Safety Authority (EFSA) in their 2009 review.[141]

The challenges encountered while evaluating exposure to organoarsenicals in seafood are largely due to analytical complications in reliably determining their complex distribution in some of these samples. Speciation analysis for organoarsenicals in seafood and seaweed is almost exclusively achieved by liquid chromatography coupled with inductively coupled plasma mass spectrometry and/or electrospray ionization mass spectrometry (LC-ICPMS/ESIMS). This approach consists of four main stages: Extraction, Separation, Detection and Characterization or Identification. A detailed discussion on this approach will be discussed in Chapter 3.

Analytical methods capable of distinguishing and quantifying the various forms of arsenic species in seafood and seaweeds are required. Intrinsic to these methods are sample handling procedures that maintain the arsenic species in their native form as they naturally occur. Factors restricting progress in this area include the need to perform sample preparation before analysis, and the lack of adequate knowledge on the stability of the analytes during common sample preparation steps.

Pétursdóttir et al.,[114] observed that the order of extraction solvents had a major influence on the concentration of each fraction with a large portion of the arsenic in the hydrophilic fraction being extracted into the MeOH/DCM fraction. When water was used for extraction before hexane, a lower concentration of arsenic was obtained in the hexane fraction for all fish meal samples. Due to the very different polarities of water and hexane, this trend cannot be attributed to a partitioning problem of the compounds, but rather to a species transformation of the labile non-polar lipids.[114]

The reduction of arsenic in the hexane fraction following water extraction can result from the hydrolysis of non-polar AsLipids. Hydrolysis of the labile AsLipids short- and medium- chain fatty acids have also been observed in fish oils and was postulated to be as a result of hydrolysis products of their triglycerides.[106] With water as the first extractant results in an underestimation of AsLipids in hexane fraction (non-polar labile lipid fraction). The toxicological implication is that the unstable AsLipids would be in contact with water during digestion and therefore become hydrolyzed when consumed.

On the other hand, with hexane as the first extractant followed by MeOH/DCM, gives a better idea of the amount of non-polar AsLipids (hexane fraction) but gives no information on the amount of AsLipids in the MeOH/DCM fraction (polar lipid fraction). This is due to the partitioning of the hydrophilic arsenic into the relatively polar MeOH/DCM fraction. Therefore, both methods are required to quantify the labile portion of the non-polar AsLipids.

Sequential extractions give an idea of the distribution of arsenic between hydrophilic arsenicals and polar and non-polar AsLipids without using speciation. Sequential extraction indicated that most of the arsenic was present as water-soluble arsenic species and a significant portion of AsLipids was found in the polar MeOH/DCM fraction for fish meal samples.[114] Sequential extraction, and therefore fractionation though they seem helpful in speciation analysis, can however not fully replace identification using HPLC-ICPMS/ESI-MS, since fractionation is somewhat arbitrary.

Although there are many studies identifying organoarsenicals, and new compounds are regularly being reported, their quantification is still proving to be difficult. A further

difficulty in the speciation analysis of arsenic in seafood arises from the ability to reliably identify and quantify the extracted species. The use of orthogonal chromatographic methods is recommended for different groups of arsenicals since it is improbable to achieve efficient chromatographic separation of anionic, cationic and neutral species using a single method.[142] The outcome of inefficient separation was demonstrated in a proficiency study where algal iAs concentration was often overestimated due to co-elution of As^{V} with other cationic organic arsenic species.[143]

Organic arsenic species display great diversity and therefore it becomes almost impossible to routinely identify and determine their relative composition in seafood in the conformity assessment scheme where regulatory limits are established. To simplify speciation analysis, Feldmann et al.[93] developed a routine analytical approach that categorizes arsenic species into three clusters based on the International Agency for Research on Cancer (IARC) classification of the carcinogenicity of arsenic species.

The three categories are 1). toxic inorganic arsenic (iAs) fraction, which is determined as arsenate (As^{V}) after oxidation, 2). AsB, which is established as non-toxic, and 3). the leftover arsenicals, which may contain AsSugars and other non-water extractable, lipophilic arsenicals with potential and unknown acute toxicity (see Fig. 5).

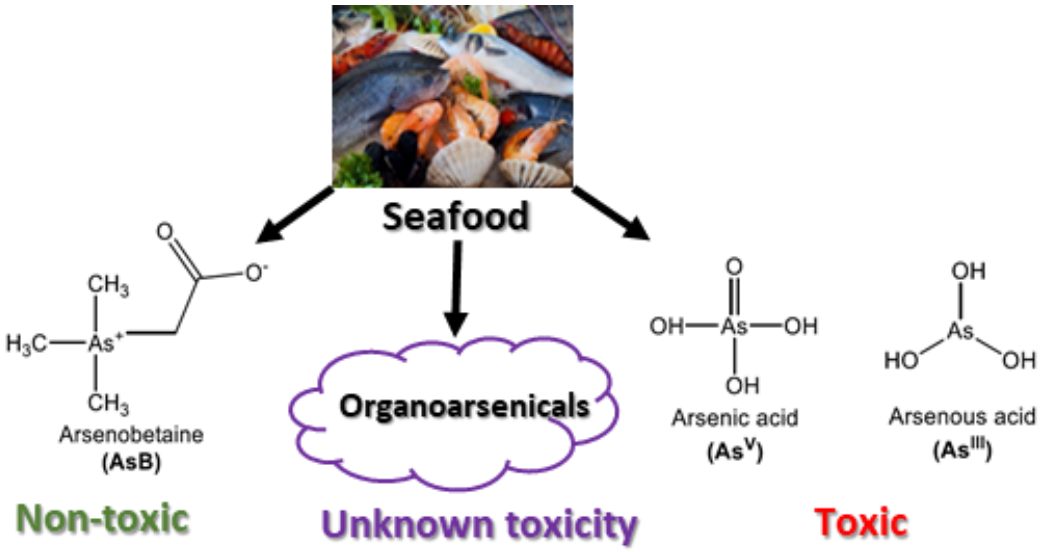


Figure 5: Classification of arsenic species in seafood based on their toxicities.

Arsenic speciation analysis is performed with the objective of understanding the presence and proportions of the various arsenicals from dietary sources.[130] The number of organic arsenic species discovered in seafood continues to increase in tandem with advances in instrumentation and analytical protocols. Significant advances have been made in designing highly sensitive and selective separation and detection techniques for the explicit characterization of known and unknown organoarsenicals.[144]

Characterization and quantification of organoarsenicals in seafood is impeded by lack of widely accepted analytical protocols, well-characterized and commercially available standards to be used as calibrants and certified reference materials (CRMs) for quality assurance and method validation.[145,146] Samples analyzed here are candidate reference materials and the data generated will form part of their certification and property value assignment for interlaboratory intercomparisons respectively.

The selection, acquisition and storage of samples used in this work is discussed in depth in Chapter 5. Briefly, Five classes of seafood, i.e., Spirulina (*Spirulina platensis*), Kelp (*Thallus Laminaria*), Geoduck clam (*Panopea generosa*), Shrimp (Northern brown shrimp, *Farfantepenaeus aztecus* and Pacific white shrimp, *Litopenaeus vannamei*), and Coho Salmon (*Oncorhynchus kisutch*) were selected as proxies for the different human dietary intake to demonstrate the extent of exposure to arsenicals from the same.

Shrimp represented the crustaceans, geoduck clam is a bivalve mollusk, both are filter feeders that have a sedentary lifestyle at the base of the sea. Since geoduck feeds on planktons and macroalgae, it is expected to have high levels of AsSugars and AsB with limited amounts of iAs^{V} and DMA. Kelp is a brown algal seaweed that has been part of human diet for centuries. It is rich in iodine, a vital element for thyroid health and folic acid, thus is used as a dietary supplement. This motivated the selection of kelp as a sample due to its health benefits and as an important exposure route for arsenic, especially AsSugars.

Spirulina a filamentous cyanobacteria represents an important staple diet in humans characterized by high nutritional value with high protein content. Spirulina is also consumed as a dietary supplement with proven and presumed health benefits that will be discussed in Chapter 2. Both the wild-caught and aquacultured shrimps are an important food type because they are the most highly consumed seafood in America. They are a significant contributor to the estimated 90 % of total dietary exposure to arsenic, especially to the US population. Salmon was selected because it is an oily finfish, which is regarded as healthy due to its protein, ω-3 LC-PUFA and vitamin D.

Salmon is a potential source of human exposure to AsLipids. High consumption of spirulina, shrimp and salmon makes them an important route for arsenic exposure.

Studies demonstrated arsenic species in geoduck, spirulina, kelp, and wild-caught shrimp to be hydrophilic, therefore a water extraction method was developed and optimized for their identification and quantification (see Chapter 4). Due to lack of well-characterized and commercially available AsSugars standards and certified reference materials for the determination of this class of organoarsenic species, kelp samples were extracted, fractionated, concentrated, purified, and characterized for AsSugars. Three AsSugars i.e., AsSugar 328, AsSugar 392 and AsSugar 482 were separated and purified and were used for identification and quantification of samples in this work. The process involved in the development of these in-house AsSugars standards is discussed in detail by Yu et al.[147]

The project was divided into three major phases (see Fig 6); Phase I covered the optimization of the extraction process to improve the extraction efficiencies for arsenic species. This involved the study of different factors that affect arsenic extraction from the sample matrix. The parameters studied included, effect of solvent composition, time, temperature, and extraction methods on the extraction efficiency of arsenic in seafood. Geoduck was used as the study material because it was the seafood with the highest diversity of arsenic species based on the selected proxy samples.

Phase II covered the actual determination of total arsenic content and speciation of hydrophilic arsenicals in seafood samples. Total arsenic was performed using ICP-MS after mineralization and quantification by method of standard addition. Arsenic

speciation was performed using several orthogonal methods described in Chapter 4. Further, we studied the effect of oxidizing solvents (HNO_3 and H_2O_2) on the arsenic species stability and extraction efficiency of AsSugars and iAs^V.

Phase III was supposed to be the determination of the lipophilic fraction of the samples, but because of COVID-19 pandemic that adversely affected research work and limited access to the laboratories, modifications to the approach were made. Since there is no known database for the novel AsLipids and because one of the objectives of this project was to identify the lipophilic arsenicals in seafood, the decision to develop an *in silico* database based on extant AsLipids data was reached.

This database is meant to be a cheap, robust, customizable, accessible, and user-friendly tool for identification of all classes of AsLipids using tandem mass spectral data as input. Structural information of extant AsLipids was used to train the model for prediction of the tandem mass spectral data. The combination of the trained model and more than 270,000 predicted AsLipids structures were developed into a searchable molecular library discussed in chapter 6.

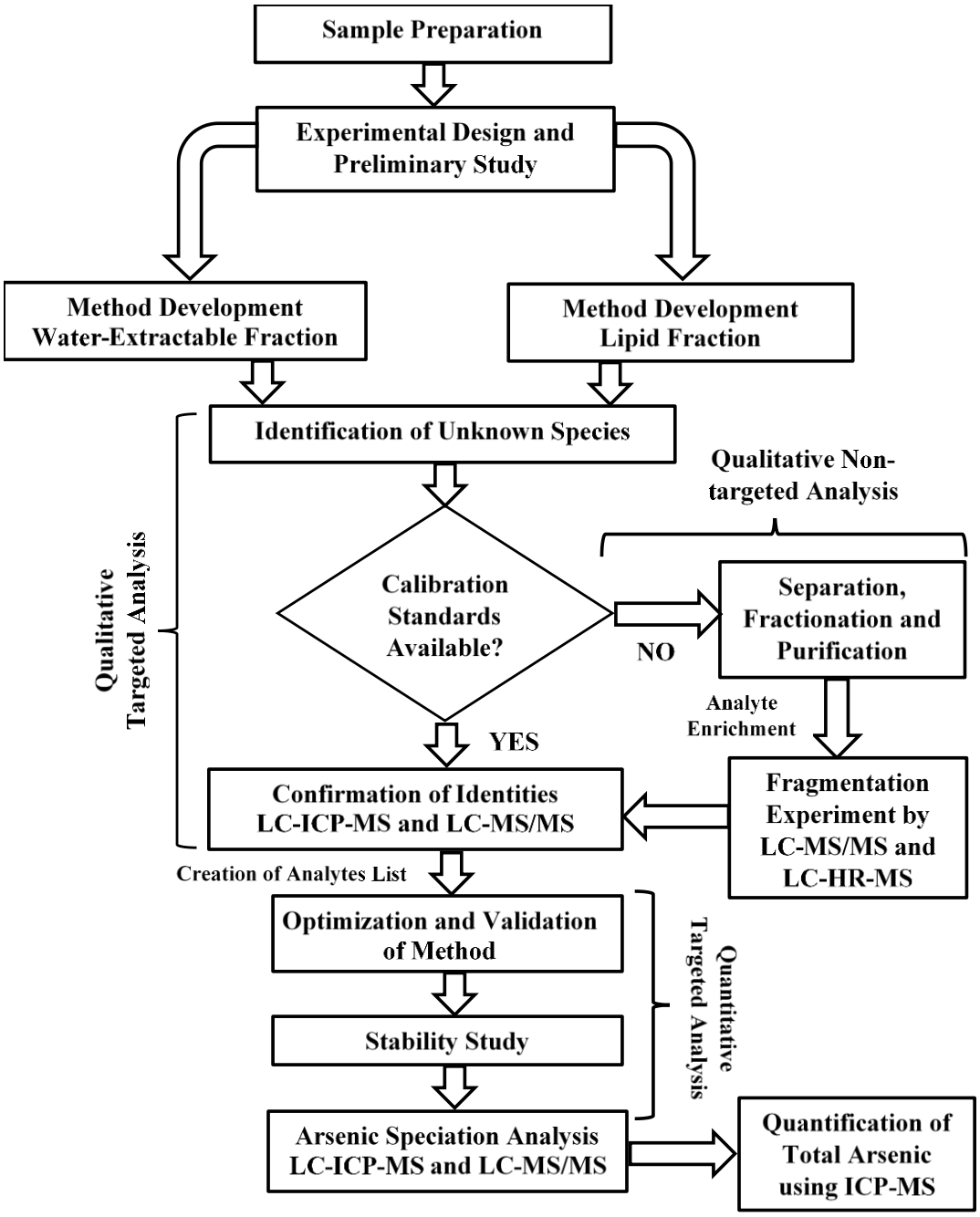


Figure 6: Schematic representation of the project work-flow.

Chapter 2: Organoarsenicals in Seafood: Occurrence, Dietary Exposure, Toxicity and Risk Assessment Considerations.

The work discussed in this chapter has been published in the Journal of Agricultural and Food Chemistry. *J. Agric. Food Chem.* ***2020****, 68, 943-960.*

2.1 Introduction

Seafood encompasses a heterogeneous group of aquatic organisms not only from the marine environment but also freshwater, including shellfish like mollusks and crustaceans, and all types of finfish.[148,149] Shellfish includes mollusks: bivalves (mussel, oyster, and scallop), univalves (abalone, snail, and conch), and cephalopods (squid, cuttlefish, and octopus); crustaceans (crayfish, crab, shrimp, and lobster), and echinoderms.[148] In most cases, seaweeds (macroalgae) and unicellular bacteria (microalgae) are considered as seafood.

Seafood and seaweed are deemed as essential portions of healthy diets as they comprise various nutrients linked with beneficial health effects[150] and they are widely used as dietary supplements. Human consumption of seafood has been increasing steadily mainly because of the reports of health benefits associated with their consumption.[150,151,152] In this section, we are going to consider the different categories of seafood in terms of their nutritional composition, health benefits, consumption patterns and economic importance.

2.1.1. Microalgae (Spirulina)

Spirulina are filamentous blue-green microalga belonging to the *oscillatoriaceae* family that naturally grows in warm alkaline water in tropical and subtropical

regions.[153,154] *Spirulina* are also commercially cultivated in large outdoor or greenhouse ponds under controlled conditions.[155] These microalgae can be harvested and processed easily and have significantly high macro- and micronutrient contents. Cultivated species of spirulina include *S. fusiformis, S. laxissima, S. subsalsa, S. lonar*, *S. labrinthiformis, S. maxima* and *S. platensis*. Among these, *S. maxima* and *S. platensis* are widely exploited for their nutritional and therapeutic aspects.[156]

Spirulina are considered among the most healing and prophylactic food items lacking cellulose cell wall,[154] which makes them easily digestible to yield high protein content (up to 70% by dry weight), with optimal balance of amino acids, particularly essential amino acids, such as leucine (10.9%), valine (7.5%), and isoleucine (6.8%).[157–161] *Spirulina* have up to 10 times more β-carotene than carrots per unit mass.[157] *Spirulina* contains carbohydrates, sterols and some more vital elements like calcium, iron, zinc, magnesium, manganese and selenium.[3,162,163] They have low nucleic acid content and high-value cell components such as vitamin B_{12}, A, C, and E, and a whole spectrum of natural mixed carotenoids and xanthophylls phytopigments.[163–166]

Spirulina is a rich source of essential fatty acids such as palmitic, oleic, lauric, docosahexaenoic acid (DHA, C22:6 ω-3) (up to 9.1% of the total fatty acid content), and it is an interesting source of γ-linolenic acids (GLAs) (20–25% of the total lipid fraction),[167,168] a precursor of leukotrienes, prostaglandins, and thromboxans associated with the regulation of inflammatory, immunological and cardiovascular disorders.[159] *Spirulina* contains clionasterol, which is associated with increasing the formation of the plasminogen-activating factor in vascular endothelial cells.[159,167]

Food and nutrition scientists have progressed from identifying and correcting nutritional deficiencies to designing foods that promote optimal health and reduce the risk of disease.[169] *Spirulina* is thus presented as a "functional food" or "nutraceutical" i.e., food that contain bioactive compounds, or phytochemicals, that may benefit health beyond the role of basic nutrition.[170]

As a functional food, spirulina can promote growth and development, enhance performance, and provide health benefits by reducing the risk of chronic diseases and enhancing the ability to manage the same, thus improving the quality of life. Many people use *spirulina* in their self-care strategy for more energy, nutritional insurance, weight control, and cleansing.[170,171]

Spirulina has positive effects on cholesterol metabolism by increasing high density lipoproteins (HDLs) levels, which can contribute to a healthier functioning of the cardiovascular system.[172] Although the exact biochemical mechanism by which *spirulina* reduces lipid levels is not well understood, some studies have presumed that it's related to the high content of c-phycocyanin (Pc), a biliprotein found in blue-green alga,[173–175] which inhibits pancreatic lipase activity.[176]

Phycocyanin contains an open-chain tetrapyrrole phycocyanobilin, which is responsible for their antioxidant properties.[177] Phycocyanin can activate atheroprotective heme oxygenase-1 (HMOX-1), a key enzyme in the heme catabolic pathway, in endothelial cells improving atherosclerosis.[178] Phycocyanin is also a known apoptosis inducer,[179] wound healing agent,[180–182] and an anti-tumor.[183]

Like other nutraceuticals, *spirulina* is recommended in preventing or managing anemia,[184] viral replication,[163,] hypercholesterolemia,[178,185,186] hyperlipidemia,[187–190] non-alcoholic fatty acid disease,[191] hypertensive effects,[168,186,190,192] inflammation,[162] hyperglycerolemia,[193] obesity,[192] lipid peroxidation,[194–196] and cancer.[197–200] *Spirulina* is also antidiabetic,[201–203] antiarthritic,[204] provides protection against heavy metal chemical-induced toxicity and alteration of antioxidant defense mechanism in the liver, and produces antigen-specific antibodies to help treat depression and attention-deficit hyperactivity disorder (ADHD).[172] Although fewer clinical studies have been performed in humans, *spirulina* does show effective antioxidant activity, making it a potential treatment for chronic obstructive pulmonary disease (COPD) and skeletal muscle damage caused by exercise-induced oxidative stress.[205–207] Most of these effects are linked with phycocyanin activity.[173–175]

These health benefits have caused *spirulina* to receive considerable attention from industry that has led to their large scale production (see Fig. 7).[3,163] The growing demand for *spirulina* in the global market is from nutraceutical and food manufacturers, where it is consumed as a dietary supplement and its added in different food recipes such as salads, breakfast meals, desserts, mixed with drinks such as water, juices, smoothies, and many food products to attract health-conscious consumers.

Estimates by the European Algae Biomass Association (EABA) show that the global market share for *spirulina* which stood at $348 million in 2018, is projected to grow at a compound annual growth rate (CAGR) of 9.4% to reach $629 million by 2025; whereas, in terms of volume, this market is projected to grow at a CAGR of 13.6% from 2019 to reach 68,025.2 tons by 2025.

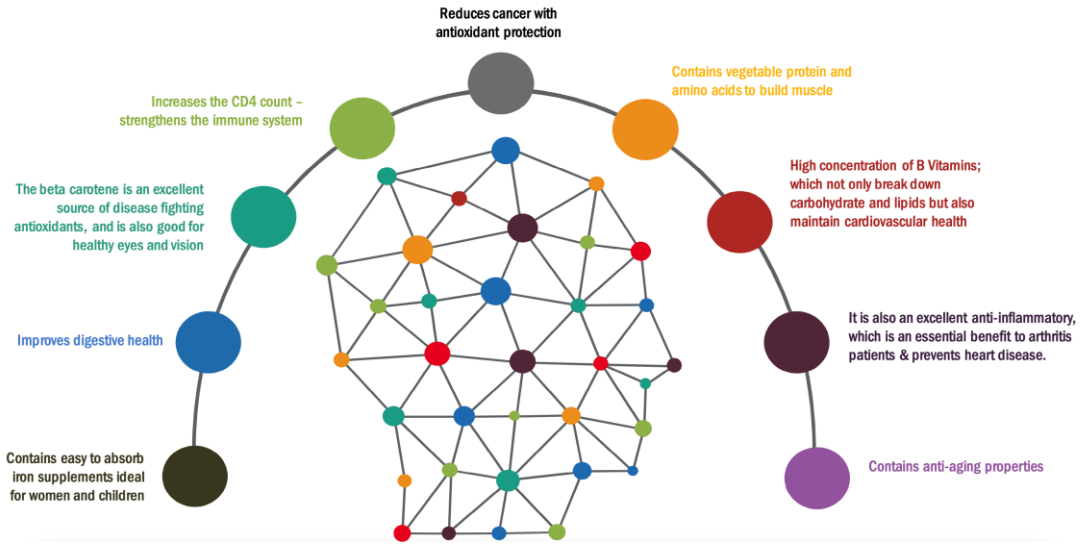


Figure 7: Health benefits for taking spirulina

Spirulina is gaining more attention and worldwide acceptance from health enthusiasts as a food ingredient due to its highly diverse nutritive nature, bioactive and functional properties, and sustainable production, which has been the driving force for the growth of the global *spirulina* market. Moreover, factors such as growing health and wellness trends; stringent regulations regarding the inclusion of synthetic colors and flavors; increasing vegetarianism and preference for *spirulina* sourced products, especially pigments and ω-3 fatty acids; growing use of *spirulina* in aquaculture diets; and increasing consumer awareness for preventative healthcare that has resulted in increasing number of dietary supplements that include *spirulina* as ingredients further support the growth of the global *spirulina* market (Fig. 8).[172]

Japan, USA and European countries are the main importers of *spirulina* powder.[208] The widespread interest in algal foods and/or their functional food potential is evident in numerous recent reviews.[156,167,169,209–216]

The United States Food and Drug Administration (FDA) approved the use of *spirulina* extract as natural blue color additive in different foods and coating formulations in dietary supplement capsules and tablets. *Spirulina* was successfully used by the National Aeronautics and Space Administration (NASA) as a dietary supplement for astronauts on space missions, owing to its ability to modulate immune functions and exhibits anti-inflammatory properties.

Spirulina is "generally recognized as safe" (GRAS) when used as food ingredient in levels ranging from 0.5 to 3.0 g/serving. Though their effects are mild compared to those of pharmaceutical products, they are considered safe, and ingestion through the daily diet as a supplement is responsible for their long-term physiological health benefits.[217] Normally, when *spirulina* is used as a nutritional supplement, it is recommended that no more than 15 g per day on dry weight basis is consumed.[208]

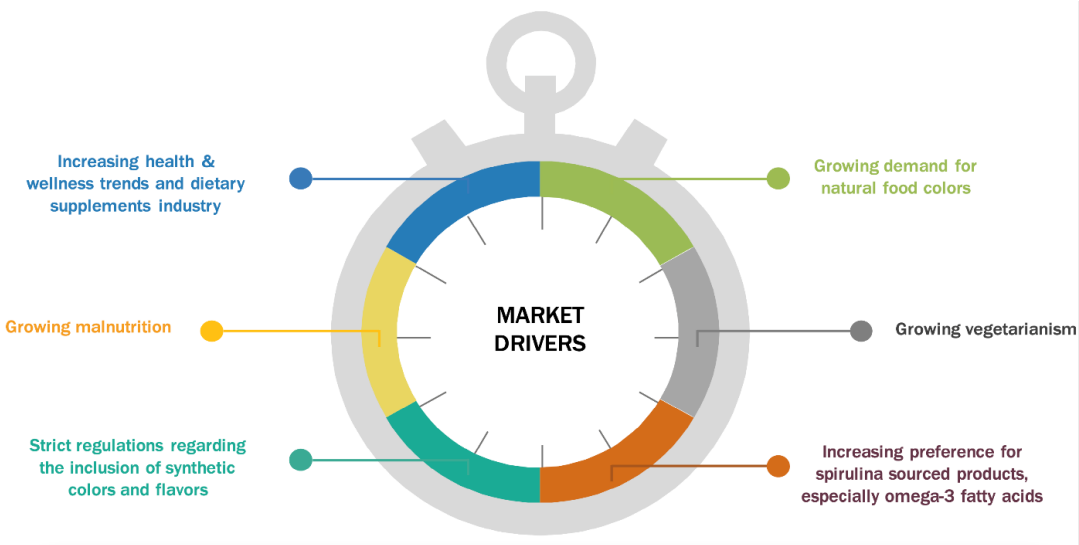


Figure 8: Market drivers for growth of demand for spirulina

2.1.2. Macroalgae (Seaweed)

Multicellular algae that inhabit the sea are called ‘marine macroalgae’ or ‘seaweed’. Seaweed are plant-like organisms that generally live attached to rocks or other hard substrata in coastal areas. Colloquially, they are classified into three groups (see Fig. 9), based on the pigmentation of their thallus, corresponding to phylum *phaeophyta* (brown algae), *rhodophyta* (red algae), and *chlorophyta* (green algae).[218–220]

There are about 10,000 different species of seaweed which are found in all climatic regions across the globe.[221] Red and brown algae are mainly used as human food sources.[222] While many plants and fungi are poisonous and unsuitable for human consumption, all seaweed harvested in clean water are considered safe, except for a few species in the tropics.[221] Therefore, seaweed is a novel food source almost comparable in richness to the addition of the plant kingdom to a hypothetical cuisine based only on animal products.

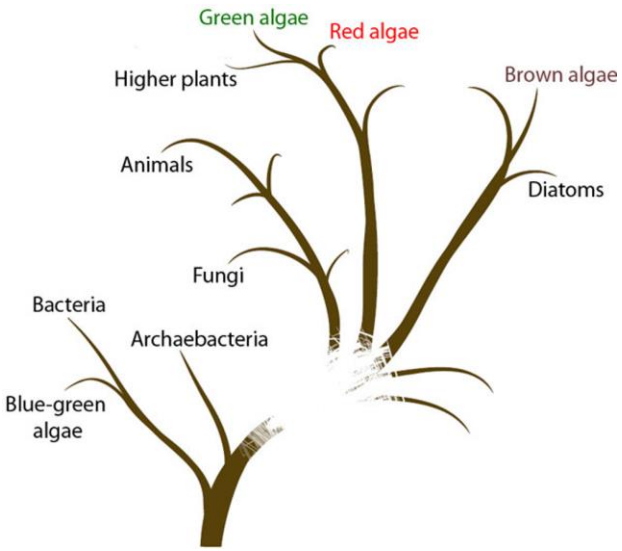


Figure 9: Phylogenetic tree highlighting three classes of marine macroalgae: green, red and brown.

Seaweed are an almost unexploited resource for primary foodstuff in the Western world, whereas they are an essential part of the oriental cuisine,[223] where they have been cultivated in large scale.[224] In the West, especially in Europe, seaweed are exploited mostly as extracts for additives, thickeners, and stabilizers in processed food, specifically alginates, carrageenan, and agar (E400-E407).[221] They are also widely applied in the industrial (pharmaceutical, cosmetic and biogas production) and agricultural (fertilizers and animal feed) sectors.

In the U.S., the use of seaweed has usually been limited to industrial applications,[209] with limited human consumption in a few coastal areas.[225,226] However, demand by the EU and US markets is increasing, owing in part to actual and perceived health benefits of seaweed, as well as the growing popularity of the 'healthy' Asian cuisine.[221]

Seaweed are optimal for human nutrition because they contain a bounty of important minerals, trace elements, vitamins, proteins, iodine and long chain polyunsaturated fatty acids (LC-PUFA).[222,227–231] Seaweed are well known to contain a high proportion of polysaccharides (30-75% on dry weight basis), which are considered dietary fibres for human consumption and hence contain few calories. The levels of dietary fibre are up to 49.8% dw for a red seaweed such as *Porphyra spp* (Nori) and up to 62% for the brown seaweed *H. fusiforme* (Hijiki).[222]

The protein content varies generally from 5% to 47%, and it contains most of the essential amino acids. For example, the red seaweed contain a high level of proteins (47% dw for *Palmaria yezoensis* and 35% for *Palmaria palmata*) while green seaweed,

such as *Ulva lactuca* (sea lettuce), show a lower protein level (10-25% dw) and brown algae, such as *Laminaria japonica*, have the lowest protein content (5-12% dw).

According to these data, the red seaweed seems to be the most suitable source of proteins for human nutrition. These species, especially *Palmaria palmata* (dulse) are described as containing high concentration of methionine, up to 4.5 g amino acid/100 g of protein. However, other species belonging to the brown seaweed (e.g. *Laminaria digitata* and *Undaria pinnatifida*) show a lower concentration in this amino acid, with 1.6 g amino acid/100 g of protein.[232]

The mineral content of seaweeds is generally higher than ten times that of terrestrial plants.[221] Some seaweed possess high levels of mineral elements which have nutritional value such as calcium and magnesium. This is true for green algae (*Ulva spp*), which have been found to contain up to 3.25 g of calcium per kg of dry weight.[231] The brown seaweed, especially the species *Laminaria digitata*, are rich in iodine (up to 10 g per kg dw). However, this high iodine concentration led to a prohibition in France in 1990 against the human consumption of this species. Although it is now permitted, the final iodine content in the *Laminaria* product must be below 500 mg per kg dw.[232] The salty taste of many types of seaweed is due to potassium salts, which generally outbalance the sodium salts. This makes seaweed a good salt substitute in food and maybe an option to combating the increasing global problem of hypertension.[221]

Edible seaweed are characterized by low levels of lipids, generally between 1.5 and 3.3% dw, more than half of the fat consists of unsaturated fatty acids, the majority of which are essential ω-3 and ω-6-LC-PUFA, like arachidonic acid (AA), ω-6

eicosapentaenoic acid (EPA) and ω-3 docosahexaenoic acid (DHA). Microalgae contain all the three fatty acids while seaweed contain only AA and EPA, and very little, if any DHA. The ratio of the amount of ω-3 to ω-6 fatty acids in seaweed is in the range of unity or even greater in certain cases.[222] This is in contrast to a typical European or North American diet, which has ratios of about 0.1 and 0.05, respectively.[233] Their actual nutritional composition depends on the seaweed species, age, geographic origin, season of harvesting, environmental conditions and storage conditions (see Table 1).[221,234]

SNo.	Nutrients in seaweeds	Composition
1.	Proteins, essential amino acids	7% to 35%
2.	Dietary fibre (soluble, insoluble)	45% to 75%
3.	Vitamins	A, B (B_1, B_2, B_3, B_6 and B_{12} of folate), C, E
4.	Iodine	Variable large amounts in some brown species
5.	More K^+ salts than Na^+ salts	
6.	Minerals	Fe, Ca, P, Mg, Cl
	Trace elements	Zn, Cu, Mn, Se, Mo, Cr
	Essential fatty acids	(2% to 5%) ω-3 (EPA, no DHA), ω-6 (AA)

Table 1: Important nutritional elements in seaweeds. Percentages refer to dry weight.

Nutritionists generally recommend that the diet be equally balanced with regard to ω-3 and ω-6 fatty acids.[233] The severe imbalance toward too much ω-6 in the Western diet is believed to be one of the main causes of the burden of ill health, in particular in

relation to heart and coronary diseases, obesity, diabetes and certain mental diseases.[235–237] Seaweed contain high amounts of natural antioxidants such as polyphenols,[227] which might be responsible for protecting LC-PUFA in seaweed when they are used as whole foods.

Seaweed intake has been linked with reduced risk of breast and colorectal cancers, perhaps owing to the extraordinary fiber and vitamin content.[238] Both kelp and laver contain a large amount of iodine, a vital element for thyroid health.[147] Laver is an excellent source of vitamins A and C, while kelp is a good source of folic acid.[147]

Seaweed are believed to have formed the most important component of our ancestors' diet who evolved by foraging in coastal areas.[223] Seaweed may have played a role not only for human nutrition and health but also been pivotal for human brain and neural system evolution since they contain essential elements for brain development, specifically ω-3 & ω-6 LCPUFAs at a proportion close to 1:1,[237] polysaccharides, phlorotannins, taurine, magnesium, zinc, vitamin B_{12}, iodine, and dietary fiber mainly due to indigestible sulfated polysaccharides.[217] Seaweed can therefore be considered to be brain food, and it is their perfect balance of ω-3 and ω-6 LC-PUFA that is particularly beneficial to human health.

Further, seaweed is rarely implicated in allergy risks compared to other seafood, like fish. Only one incident of anaphylactic shock has been reported after the consumption of Ogo-Nori (*Gracilaria verrucosa*). In this respect, seaweed could thus be used as a substitute for some allergenic dietary sources.[239] Seaweed is also probably the most versatile cooking ingredients, because they can be eaten raw, cooked, baked, toasted,

pureed, dried, granulated or deep-fried. They can be eaten on their own or in countless combinations with other hot or cold ingredients.[221] Seaweed is believed to retain the greater part of its nutritional value if consumed unchanged.

Seaweed can be harvested in the wild or grown in the sea in large quantities in a sustainable fashion. They hold great potential to make a significant contribution to the global food supply in future due to their low-carbon imprint of sustainable food production and great contribution to human health and nutrition. These 'vegetables of the sea' will help to renew and balance our diet and may contribute to combating the increased incidences of Western lifestyle-related diseases, such as hypertension, cancer and obesity, as well as mental disorders.[233,236] The global market for seaweed for human consumption amounts to about US $10 billion annually.[240]

In 2018, farmed seaweed represented 97.1 % by volume of the total of 32.4 MMT of wild-collected and cultivated aquatic algae combined. Seaweed farming is practiced in a relatively smaller numbers of countries, dominated by countries in East and Southeast Asia. The world production of seaweed has more than tripled, up from 10.6 MMT in 2000 to 32.4 MMT in 2018 (see Fig. 10).

Despite the slowdown in growth rates in recent years, the rapid growth in the farming of tropical seaweed species (*Kappaphycus alvarezii* and *Eucheuma spp.*) in Indonesia as raw material for carrageenan extraction has been the major driver in the increase of farmed seaweed production in the past decade. Indonesia increased its seaweed farming output from less than 4 MMT in 2010 to over 11 MMT in 2015 and 2016, and similar production levels in 2017 and 2018.

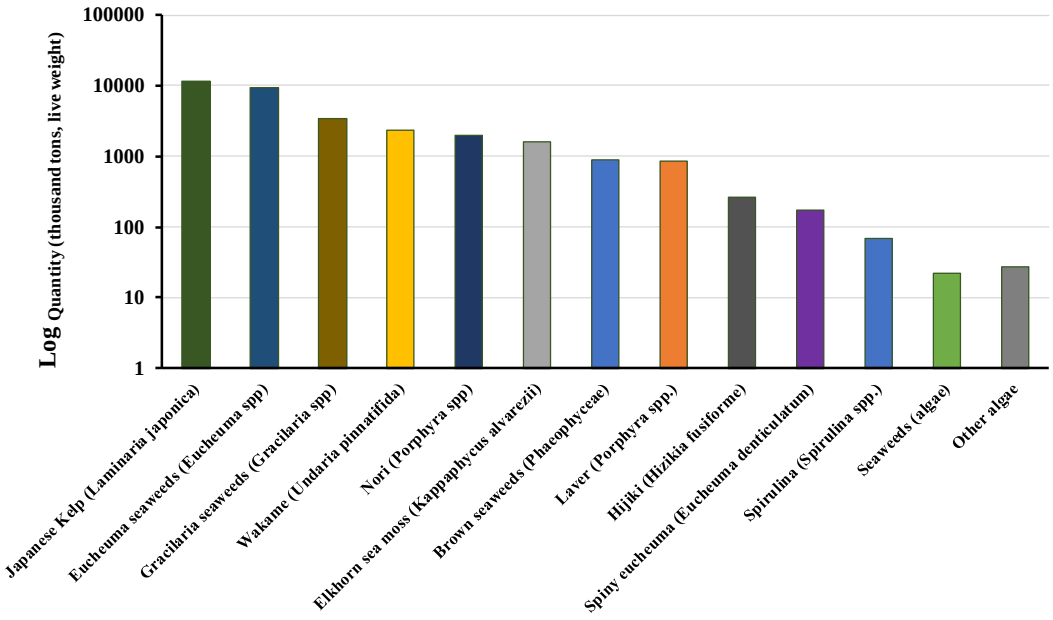


Figure 10: World Aquaculture Production of Aquatic Algae.

Algae are very important in the food chain as they are a source of food to marine and freshwater species. The marine species that are consumed by humans usually form the link between the aquatic environment and humans in the food chain. Seaweed enters the human food chain through direct consumption of edible varieties or is consumed by fish and other marine species that are then consumed by humans.[241] Seaweed have the capacity to accumulate trace metals several thousand times higher than their concentration in seawater.[241]

Due to the role of seaweed in the human diet and potential to accumulate toxic metals, the metal concentrations in seaweed should be monitored. For example, studies on seaweed, which reported high concentrations of iAs in Hijiki (*Hizikia fusiforme)*, resulted in the Canadian Food Inspection Agency warning against its consumption.[65] Other countries that have issued similar consumption advisories on hijiki include Australia and New Zealand,[242] Japan,[243] Ireland,[244] the United Kingdom,[245] Hong

Kong,[246] and Belgium.[247] Conversely, EFSA did not consider hijiki an important source of iAs exposure because of its low consumption rate by the European population.[59]

Although seaweed may not be consumed on a daily basis, health risks may ensue with routine consumption, if toxic elements are at elevated levels.[241] Several countries have set guidance for the level of iAs in edible seaweed; For example, Australia and New Zealand (1 µg/g),[248] Hong Kong (1.4 µg/g),[249] and France (3 µg/g).[250] Furthermore, the EU has set a limit of 2 µg/g iAs in feed derived from seaweed.[251] There are currently no U.S. guidelines for arsenic exposure from seaweed consumption, with the exception of hijiki and oarweeds.

Several factors have been recognized to influence the accumulation of arsenic in seaweed. Bioavailability of the element in the surrounding water, seasonal variation and algal growth-rate play key roles.[252] Environmental conditions including temperature, pH, salinity and nutrient concentration also affect the ability of seaweed to uptake and retain arsenic.[253] Algal taxonomy, especially cellular physiology and biochemistry, contribute significantly to the variation in arsenic buildup.[252]

Studies show that brown algae accumulate arsenic in their cell walls,[254] where polysaccharides such as alginate are abundant.[252,254] Alginate is reported to absorb metals and metalloids in the marine environment,[252] but its effect on the binding of arsenic in brown algae is unclear.[254] While taxonomy is a good indicator, it cannot be taken as a hard and fast rule.

Focusing only on iAs, which is comprised of iAs^{III} and As^{V}, is generally adequate for products that accumulate arsenic in a few forms of defined properties. This approach,

however, disregards species of potential or unknown toxicity when applied to matrices like seaweed [93] where the high concentration of arsenic is due to a great diversity of arsenicals.[255] Lack of detailed toxicological data for some organoarsenic compounds suggest the need for comprehensive speciation analysis for meaningful risk assessment.[93] Wide-ranging determination of arsenic species in seaweed helps to enhance toxicological studies on arsenicals found in these products, and to characterize the chemical identities and properties of unknown arsenic compounds.

2.1.3. Seafood

Seafood here refers to shellfish and finfish. It is estimated that the ocean is inhabited by more than 1,000 species of crustaceans, 50,000 species of mollusks, besides 13,000 species of finfish.[256] Human consumption of seafood has been increasing steadily because of the reports of health benefits associated with their consumption.[150–152]

Like all mammals, humans lack enzymes for the synthesis of omega-3 (ω-3) and omega-6 (ω-6) precursors of DHA and EPA, which are therefore essential fatty acids and need to be provided by dietary sources.[257] Fish is the best dietary source of high-quality proteins and essential amino acids, ω-3 LCPUFAs, and micronutrients, which are linked to a range of health benefits.[258]

ω-3 LCPUFAs, particularly EPA, and DHA, have been demonstrated to have antiatherosclerotic and antithrombotic effects,[151] and are linked to reduced risk of cardiovascular disease (CVD).[259] Benefits on visual acuity and cognitive development have been largely established in term [260,261] and preterm [262,263] infants fed ω-3 LCPUFAs-supplemented formula.[264]

Typically, shellfish contain substantial quantities of digestible proteins, essential amino acids, bioactive peptides, ω-3 LCPUFAs, astaxanthin and other carotenoids, vitamin B_{12} and other vitamins (particularly A, B and D), minerals such as iron, calcium, copper, zinc, inorganic phosphate, sodium, potassium, selenium, iodine, and other nutrients, which offer a variety of health benefits to the consumer.[265,266]

Seafood is low in calories compared with other animal foods. For example, a 100 g serving of shrimp provides approximately 106 kcal, whereas the same amount of fish provides 110 to 150 kcal, lean beef 250 kcal, and roasted chicken 200 kcal.[267] The average daily intake of total fat supplied by fish is fairly low, at about 1.2 g per capita.

This unique nutritional composition means that seafood represents a valuable source for healthy dietary diversification, even in relatively small quantities. This is more important for many low-income food-deficient countries (LIFDCs) and least developed countries (LDCs), where populations may be overly dependent on a relatively narrow selection of staple foods, which cannot provide adequate amounts of essential amino acids, vitamins, micronutrients, and healthy fats.

Fish proteins are essential in the diet of some densely populated countries where the total protein intake is low, particularly in small island developing states (SIDS). For these populations, fish often represents an affordable source of animal protein that may not only be cheaper than other animal protein sources, but preferred and part of local and traditional recipes.[131]

Some malnutrition problems such as anemia in women of reproductive age and prevalence of obesity, including in children, are on the rise at the global level.

Excessive consumption of sugar-rich, high-fat foods and their negative health impacts is a growing problem in both developing and developed world.[268] Therefore, increased seafood consumption, with their diverse and valuable nutritional attributes, can directly reduce the prevalence of malnutrition and correct imbalanced high-calorie and low-micronutrient diets, in line with the world health organization (WHO) and United Nations sustainable development goal (UN SDG 2) - End hunger, achieve food security and improved nutrition and promote sustainable agriculture, by the year 2030.[268]

Full nutritional benefits accrue from the consumption of the entire fish of small species, as their head, bones and skin are rich in micronutrients. This also helps to reduce waste and enhance global food security.

According to the 2020 edition of The State of World Fisheries and Aquaculture published by the UN Food and Agriculture Organization (FAO),[131] the global fish production is estimated to have reached about 179 million metric tons (MMT) in 2018, with a total sale value estimated at US $401 billion, of which 82 MMT, valued at US $250 billion, came from aquaculture production. About 88 % (158 MMT) of world fish production was utilized for direct human consumption. The remaining 12 % (22 MMT) was used for non-food purposes, of which 82 % (18 MMT) was used to produce fishmeal and fish oil.

Aquaculture accounted for 46 % of the total production and 52 % of fish for human consumption. The global fish consumption has increased at an average annual rate of 3.1 % from 1961 to 2017, a rate almost double the annual world population growth (1.6

%) for the same period, and higher than that of all other animal protein foods (meat, dairy, milk, etc.), which increased by 2.1 % per year.[131]

The world aquaculture production attained a record high of 114.5 MMT in live weight in 2018, with a total farmgate sale value of US $263.6 billion. The total production consisted of 82.1 MMT of aquatic animals valued at US $ 250.1 billion, 32.4 MMT of aquatic algae, valued at US $13.3 billion and 26,000 tons of ornamental seashells and pearls valued at US $179,000 (see Table 2). The contribution of world aquaculture to global fish production reached 46 % in 2018, up from 25.7 % in 2000. Inland aquaculture produced most farmed fish (51.3 MMT, or 62.5 % of the world total), mainly in fresh water, compared with 57.7 % in 2000.

SNo.	Type of Seafood	Quantity produced, million metric tons.	Landing Value (US $ Billion)
1.	Finfish	54.3	139.7
2.	Finfish (harvested from inland aquaculture)	47	104.3
3.	Finfish (harvested from marine and coastal aquaculture)	7.3	35.4
4.	Mollusks (bivalves)	17.7	34.6
5.	Crustaceans	9.4	69.3

Table 2: Global Seafood Production from Aquaculture in 2018. Source FAO, 2020.

The global capture fisheries production in 2018 reached a record 96.4 MMT, an increase of 54 % from the average of previous 3 years, which is attributed to marine capture fisheries where production increased from 82 MMT in 2017 to 84.4 MMT in 2018. Catches from inland fisheries peaked in 2018 at 12 MMT. Anchoveta (*Engraulis ringens*) was the top species at more than 7 MMT, followed by Alaska pollock

(*Theragra chalcogramma*) at 3.4 MMT, while shipjack tuna (*Katsuwonus pelamis*) was third at 3.2 MMT. Finfish represented 85 % of total production, with small pelagics as the main group, followed by gadiformes and tuna and tuna-like species. Global catches in inland waters accounted for 12.5 % of total capture fisheries production but accounted for less than 1 % of total captures in the United States.

The share of seafood production destined for human consumption is expected to continue to grow, reaching 89 % by 2030. Total fish production (excluding aquatic plants) is expected to expand from 179 MMT in 2018 to 204 MMT in 2030. Aquaculture will continue to be the driving force behind the growth in global seafood production (see Fig. 11). Aquaculture production is projected to reach 109 MMT in 2030, an increase of 32 % (26 MMT) over 2018. Yet, the average annual growth rate of aquaculture should slow from 4.6 % in 2007–2018 to 2.3 % in 2019–2030.

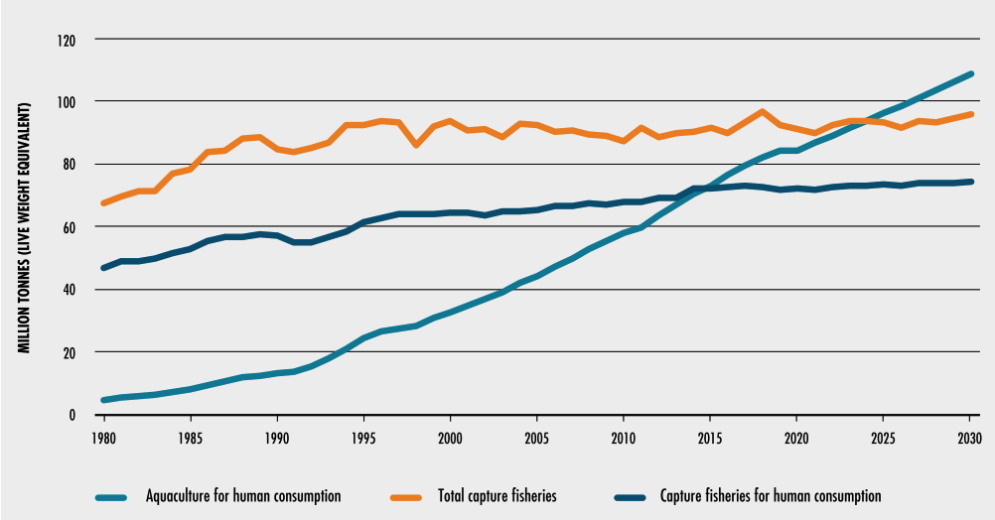


Figure 11: Global Capture Fisheries and Aquaculture Production, 1980 – 2030. Source: FAO, 2020

Exports of seafood and fisheries products are essential to the economies of many countries and regions. In 2018, 67 MMT of fish (live weight equivalent) were traded

internationally, equating to almost 38 % of all fish caught or farmed worldwide. In the same year, 221 States and territories reported some fish trading activity. The total export value of US $164 billion recorded in 2018 represented almost 11 % of the export value of agricultural products (excluding forest products) and about 1 % of the value of total merchandise trade. This does not include an additional US $ 2 billion from trade in seaweed and other aquatic plants (63%), and inedible fish by-products (2%).

Salmonids have been the most important commodity traded in value terms since 2013 and accounted for about 19 % of the total value of internationally traded seafood products in 2018 (see Fig. 12). In the same year, the other main groups of exported species were shrimps and prawn with about 15 % of the total, followed by groundfish (i.e. hake, cod, haddock, Alaska pollock, etc.) at 10 % and tuna (9 %).

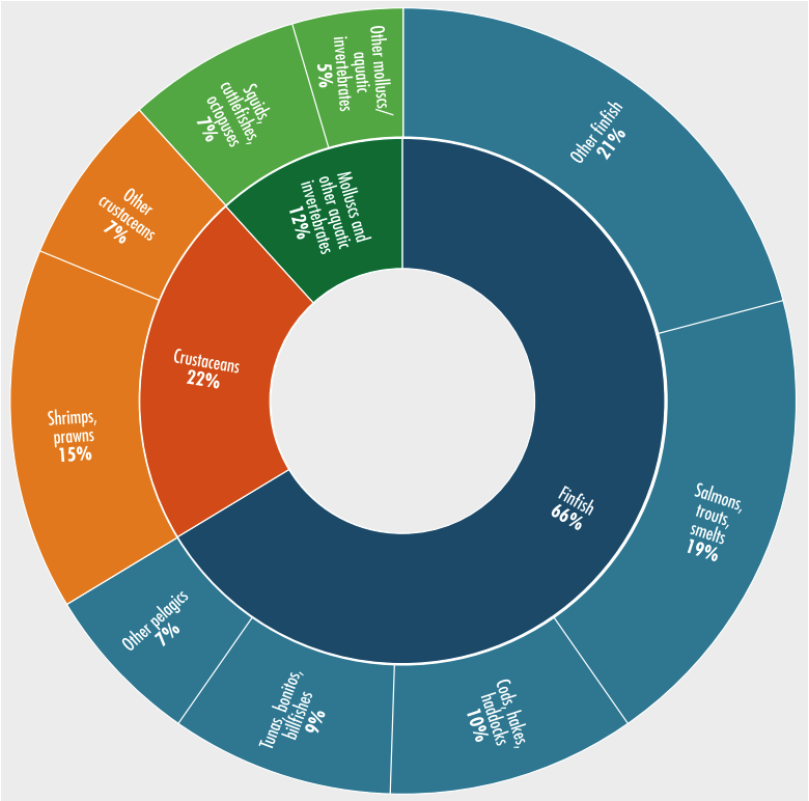


Figure 12: Main groups of species in seafood trade in terms of value, 2018. Source, FAO 2020.

In 2017, fish accounted for 17 % of the global population's intake of animal proteins, and 7 % of all proteins consumed. Globally, fish provided more than 3.3 billion people with 20 % of their average per capita intake of animal proteins, reaching 50 % or more in several countries.

Urbanization has also shaped the nature and extent of seafood consumption in many countries. According to the World Urbanization Prospects: 2018 Revision published by the UN, Department of Economic and Social Affairs, Population Division (UN DESA),[269] the urban population has accounted for more than half of the world's population, since 2007 and it continues to grow. The number of megacities (cities with more than 10 million inhabitants) reached 33 in 2018, of which more than 15 are in developing countries.

Urban inhabitants typically have more disposable income to spend on animal proteins such as seafood, and they eat out more often. In addition, the infrastructure available in urban areas allows for more efficient storage, distribution and marketing of fish and fish products. Hypermarkets and supermarkets are developing rapidly throughout Africa, Asia and Latin America, and fish products are increasingly sold through these channels as opposed to traditional fishmongers and fish markets.

At the same time, the ease of food preparation represents an increasingly important consideration for urban dwellers with fast-paced lifestyles and higher demands on their time. As a result, the demand for seafood products prepared and marketed for convenience, through both retail and fast-food services, is rapidly increasing. The dietary preferences of modern urban consumers are characterized by an emphasis on

healthy living and a relatively high interest in the origin of the foods they eat, trends likely to continue to influence seafood consumption patterns in both traditional and emerging markets.

The EU was the largest fish importing market (34 % in terms of value) in 2018, followed by the USA (14 %) and Japan (9 %). While the markets of developed countries remain dominant in seafood imports, the importance of developing countries as consumers has been steadily on the rise (see Fig. 13).

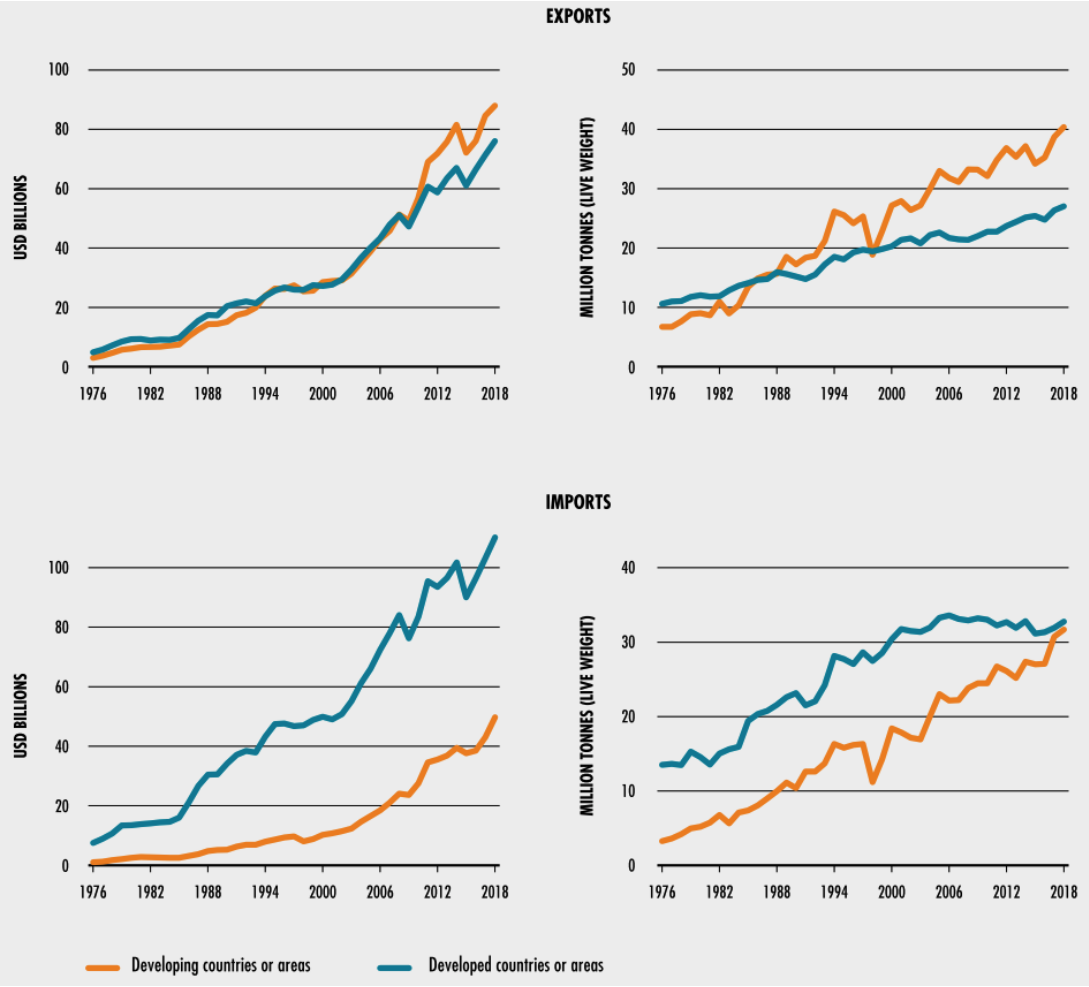


Figure 13: Trade of Seafood and Fisheries Products. Source: FAO 2020.

In 2018, fish exports of developing countries were valued at US $88 billion, and their net fish export revenues (exports minus imports) reached US $38 billion, higher than those of other agricultural commodities (meat, tobacco, rice, and sugar) combined. Both as a source of export revenue and as a provider of employment, trade in seafood and fisheries products represents an important contributor to economic growth in developing countries.

An estimated 59.51 million people were engaged in the primary sector of capture fisheries and aquaculture, in 2018, 14 % of them being women. In total, 20.53 million people were employed in aquaculture and 38.98 million in fisheries (see Fig. 14). Of all those involved in primary production, most are in developing countries and most are small-scale, artisanal fishers and aquaculture workers. Overall, the highest numbers of fishers and aquaculture workers are in Asia (85 % of the world total), followed by Africa (9 %), the Americas (4 %) and Europe and Oceania (1 % each).

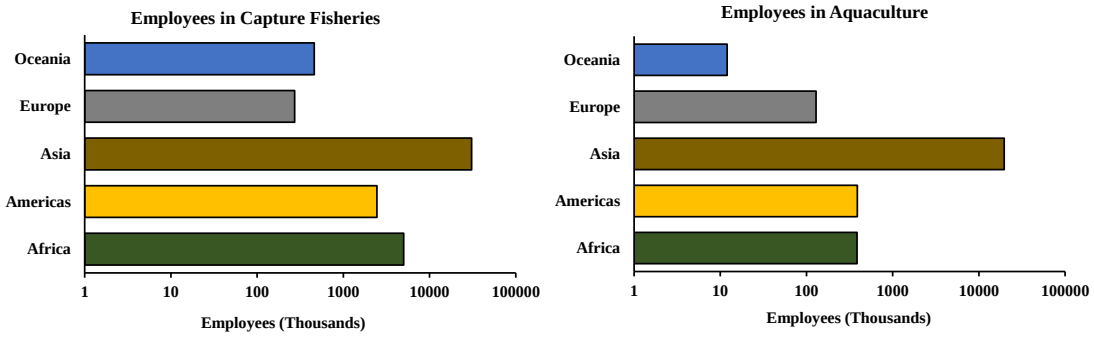


Figure 14: World Employment for Fishers and Fish Farmers by Region in 2018

Globally, the proportion of women in the total work force in aquaculture (19 %) is larger than that in capture fisheries (12 %). In most regions, women are less involved in offshore and long-distance capture fishing. For example, in the USA, women in the

Alaskan fisheries are mainly engaged in the near-shore salmon fisheries.[270] Aquaculture is being promoted as a significant growth sector, and as an activity that can empower women and young people, notably by facilitating women's decision-making on the consumption and provision of nutritious food.[271]

Evidently, seafood is a highly consumed and traded commodity therefore intentional or unintentional contamination with toxic elements like arsenic may become technical barriers to trade. For example, in December 2013 China imposed a ban on all U.S. imports of geoduck clams (*Panopea generosa*), a large edible saltwater clam found along the Pacific Northwest extending from northern California to southeastern Alaska, citing high levels of arsenic contamination. Revenue from U.S. exports of geoduck are upwards of US $80 million annually with about 90 % of all exports going to China.[272]

2.2. Arsenic speciation in Seafood

Seafood is the major dietary source of total arsenic in humans,[6] excluding regions with widespread elevated drinking water contamination.[5,7–9] Organic arsenic predominates in seafood, however, this is not always the case as there are reported cases of elevated inorganic arsenic levels in seafood such as edible seaweed *Hijiki* (60 µg g^{-1} to 150 µg g^{-1}, iAs),[273] fish from Thailand,[274] and mussel from Norway.[55]

Marine algae and shellfish are the seafood exposure sources with the greatest diversity of arsenicals.[31] Among these arsenicals the potential for biotransformation upon ingestion varies considerably.[275] Arsenic speciation (Fig. 15) is complicated and diverse arsenic species display great difference in toxicities.[3]

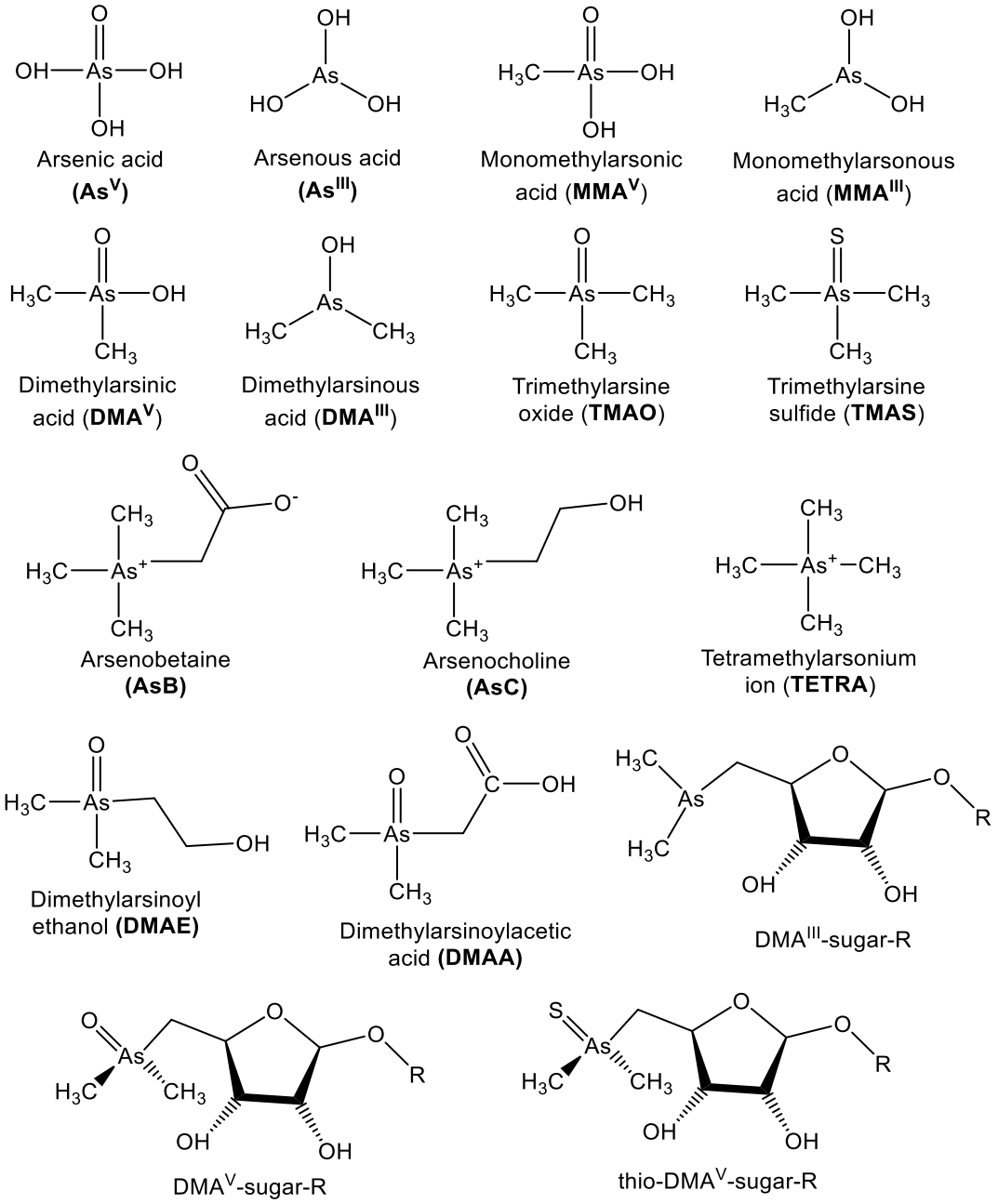


Figure 15: Structures of common arsenic compounds.

Arsenic exists in four oxidation states in the inorganic form as trivalent arsenite (iAs^{III}), and thermodynamically stable pentavalent arsenate (iAs^{V}).[276] Elemental arsenic (As^{0}), and arsine, H_3As (As^{-III}), which exist in strongly reducing conditions.[277–279] Arsenic

exists in organic form where arsenic is bonded to carbon as; low molecular weight compounds like MMA, DMA, AsB, AsC, TMAO, and TETRA; high molecular weight AsSugar and AsLipids; and in complexed form as arsenopeptides glutathione (As-GSH), and phytochelatins.[2,12,280]

AsB is the main arsenical in seafood, commonly comprising in excess of 90% of the total arsenic in fish.[11,93,130,281] AsB is the major polar arsenical, which together with AsSugars and a range of other lipophilic arsenicals account for over 200 naturally-occurring organoarsenicals found in seafood.[7,21]

There is great diversity in the level of arsenic in seafood, but arsenic in most samples falls within the mass fraction range of about 5 μg g^{-1} to 100 μg g^{-1} dry mass.[21] Sirot et al. reported the levels of inorganic arsenic (iAs) in 30 fish species collected in France as 0.005 μg g^{-1} to 0.073 μg g^{-1} on wet mass basis. This clearly demonstrated that the proportion of iAs to total arsenic content, mostly AsB, was 100-fold lower.[282]

2.3. Metabolic transformation and toxicity of Arsenic

A large number of biologically relevant arsenic species has been characterized in samples of dietary sources,[21,61,130,141,144,283] which is an essential step in the toxicity assessment. Arsenic toxicity, bioaccumulation and mobility is greatly dependent on the chemical state in which the element appears and the extent of methylation through the metabolism process.[8,31,39,42] iAs^{V} and iAs^{III} are categorized as non-threshold Class I carcinogens,[10] with acute toxicity of [LD_{50}: (15 to 42) mg/kg body mass], while simple methylated arsenicals are deemed to pose intermediary toxicity (LD_{50}: 890 mg/kg body weight to 10,600 mg/kg body weight), and the tetraalkylated compound AsB, present

in fish and the principal dietary source of arsenic exposure for humans, is non-toxic with LD_{50}: >10,000 mg/kg bw, and is primarily eliminated by humans in the urine.[11,20]

Typically the lower the oxidation number the higher the toxicity, and the higher the methylation the lower the toxicity, thus producing the following order of toxicity for arsenic species in human cell lines: $MMA^{III} > DMA^{III} > As^{III} > As^{V} > TMA^{+} > DMA^{V} = MMA^{V} > TMAO > AsC > AsB$.[284–287] Moreover, AsB, AsC, TMAO, AsSugars, and AsLipids are usually mildly toxic compared to iAs species.[3,21,288]

Due to the extreme toxicity of iAs, microalgae and other living organisms may undergo dissimilar processes to reduce the toxic effects, including cell surface adsorption, arsenite oxidation and arsenate reduction,[289,290] methylation,[291] conversion to AsSugars or AsLipids,[292] chelation of iAs^{III} with glutathione and phytochelatins,[293,294] and elimination from cells.[3] As detoxification occurs through a series of biotransformation in biotic systems producing a wide range of organoarsenicals, whereby arsenic is covalently bonded with one or more carbon atoms containing functional groups.[294,295] Toxicity is initiated once liver methylation ability is impeded or surpassed.[296–298]

It is important to note that discussions on arsenic toxicity are mainly in reference to iAs and methylated arsenicals (MMA and DMA) since their toxicity mechanism is well established and understood, unlike for AsSugars and AsLipids, which are yet to be fully elucidated. Organoarsenicals have not demonstrated acute toxicity therefore their toxicity may arise from metabolic transformations that lead to formation of highly toxic metabolites.[20,141,280] For example, the toxicity of AsSugar and AsLipids may arise from their metabolic breakdown to other arsenicals such as DMA^{V}, which is also the

metabolite of iAs found in urine and is a known tumor promoter and confirmed carcinogen in experimental animals.[299,300]

Liver, kidneys, heart and lungs are main repository organs of arsenic, with slight buildup in brain and muscle tissues.[39] This buildup is linked with a variety of ailments including cancer, diabetes, hepatotoxicity and nephrotoxicity. Arsenic can cause thiamine deficiency by lowering its accessibility leading to lactic acidosis by enhancing lactic acid concentration.[39] In addition, arsenic may cause genotoxicity by impeding DNA repair mechanism, and further stimulates oxidative stress by producing reactive nitrogen species (RNS) and reactive oxygen species (ROS).[39,301,302]

In biological systems, arsenate can replace phosphate and form esters that resemble phosphate esters, which are abundant in biomolecules, from the sugar phosphates of intermediary metabolism, to membrane phospholipids, to the phosphate back-bone of genetic materials like deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).[303] However, these compounds are less stable because they are more delicate than the subsequent phosphate esters, which is partly attributed to the bond lengths and bond angles.[304] The P–O bond length in phosphates is almost 1.5 Å, whereas the bond length of As–O in arsenates is about 1.6 Å to 2.0 Å.

Arsenic usually form lengthier bonds than phosphorus (Fig. 16).[304] In addition, arsenic angles are considerably less obtuse than the phosphorus angles,[303] i.e. the O-P-O bond angle is about 117°, whereas O-As-O bond angle is roughly 100°, with minor variabilities in diverse compounds. Therefore, since longer bonds are fragile, arsenate esters disintegrate more easily than phosphate esters.

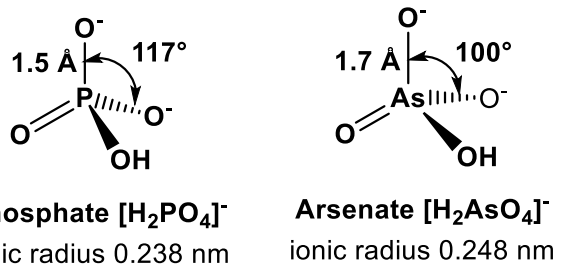


Figure 16: Structures of deprotonated oxo-anions of arsenate and phosphate

Within the cell, metabolic enzymes that utilize phosphate may also incorporate arsenate in alkylation, acylation, or phosphorylation reactions because of their isosteric and isoelectronic nature.[305] For example, arsenate inhibits the formation of ATP as a result of generation of delicate anhydrides and also uncouples ATP synthesis during oxidative phosphorylation by coupling with adenosine diphosphate (ADP) in the presence of succinate in the mitochondria forming ADP-arsenate rather than ATP.[306–308]

This disrupts phosphorus metabolism since ADP-arsenate may be utilized as a substrate for hexokinase, which under normal conditions produces glucose-6-phosphate, the first intermediary in the glycolytic pathway.[306] Therefore, hexokinase is inhibited by negative feedback mechanism. The glucose-6-arsenate produced by hexokinase is then transformed to glucose-1-arsenate by phosphoglucomutase, proving that intermediary metabolism can produce arsenicals.[305]

The rate constant for spontaneous breakdown of glucose-6-arsenate has been shown to be 4×10^{-4} s^{-1}, compared to 4×10^{-9} s^{-1} for glucose-6-phosphate. Therefore, the short half-life of arsenate ester causes it to spontaneously hydrolyze 10^5 faster than the phosphate ester, which is the major cause of decoupling action of arsenate in oxidative phosphorylation.[306] Cells starve for scarcity of metabolic intermediates.[3,294,303,305,309]

The other factor that potentially contributes to the instability of arsenicals is the fact that iAs^{V} is swiftly reduced to iAs^{III} within the cells.[303] The biological half-life of As is roughly four days, which is dependent on the manner of exposure. iAs^{III} is considered to have a shorter half-life in comparison to iAs^{V}.[310] iAs^{III} is the most hazardous and toxic form of inorganic arsenic, which block sulfhydryl (-SH) groups by creating robust bonds of metallic nature with thiols in proteins and small molecules.[303]

Toxicity of iAs^{III} is due to its high affinity for thiol groups causing allosteric inhibition of respiration by binding to vicinal thiols in pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase,[309] with resultant membrane destruction and cell death by generating reactive oxygen species (ROS).[3,311] The allosteric inhibition of PDH, a vital precursor of acetyl-CoA, not only restricts the generation of ATP in the electron transport chain, but also impedes the formation of gluconeogenesis intermediaries.[312–315] Moreover, research findings indicate that arsenite is able to traverse across the blood-brain barrier.[316–318]

Due to scarcity of toxicity and long-term exposure data for organoarsenicals in humans or other mammals, health hazards from exposure to organoarsenicals are challenging to evaluate. Most of the injurious effects of iAs have been documented, but the uncertainty concerning the threat to the people exposed to organic arsenic, especially from seafood, and the dosage needed to trigger these effects still linger.

Numerous investigations have contemplated the latent carcinogenicity from the formation of the metabolite DMA^{V},[40,41,140,319,320] centered on elevated dose exposure studies in rats to DMA in water [300] or diet.[321] There is, however, compelling indication

that the rat model is invalid for human exposure to DMA, because of the difference in the metabolic pathways of rats and humans and also because these studies are not capable of evaluating the pathway to DMA (from iAs or organic arsenic compounds), and the intermediary effects.[322]

On the basis of projected exposure level and expected metabolism, it seems unlikely that arsenic in seafood can significantly promote arsenic-associated carcinogenic effects.[140] The bulk of arsenic in seafood exist as AsB, which is benign and is rapidly excreted from the body intact. Amounts of TETRA resulting from dry cooking or AsB-containing fish are unlikely to reach toxic levels. In addition, levels of iAs and methylarsenicals in seafood are relatively low to allay suspicions of their potential detrimental effects in seafood consumers.[140,323]

Whereas no deductions can be made regarding the effects of organic arsenic species, proof of toxicity from AsLipids and organoarsenic metabolic intermediates from *in vitro* assessment confirms the necessity for animal and human research to assess probable health impact of arsenic in seafood.[324,101] Majority of research on natural AsLipids has focused on the polar lipids, leading to the characterization of AsFAs and AsHCs. Profound discernment of arsenic biochemistry may perhaps be garnered from characterization of the additional lipid fractions.[122]

2.3.1. Methylated Arsenicals

Methylated arsenic species are present in marine food as MMA and DMA at low levels. Other methylated arsenicals include TMAO, AsB, AsC and TETRA. Methylarsenicals in marine ecosystem are generated by phytoplankton, bacteria, and microbial

breakdown of botanical matter from iAs and are introduced into the seafood chain.[289,325] Phytoplankton absorb iAs^{V} in the euphotic surface waters and successively transform it to DMA, and MMA. [326–330] Anaerobic members of archaea and bacteria are known to biotransform iAs into both volatile (methylarsines) and nonvolatile (MMA and DMA) species.[4,331–333]

Three metabolic pathways have been proposed for arsenic biotransformation.[70,75,326,334] iAs undergoes enzymatically biotransformation into several methylated metabolites following the classical Challenger's metabolic pathway as follows: $[iAs^{V}] \rightarrow [iAs^{III}] \rightarrow [MMA^{V}] \rightarrow [MMA^{III}] \rightarrow [DMA^{V}]$.[70–72,75,335,] DMA^{V} can be reduced to DMA^{III} and further methylated to TMA^{III}, via TMAO intermediate.[336] Mammalian systems do not subsequently produce arsines except under extraordinary conditions.[337,338]

The Challenger pathway illustrates the reduction of pentavalent iAs^{V} and MMA^{V} to their trivalent species, iAs^{III} and MMA^{III}, followed by an oxidative methylation phase where S-adenosylmethionine (SAM) acts as the methyl donor, producing MMA^{V} and DMA^{V} as major metabolites. Although the precise pathway in humans has never been entirely understood,[39,339] it was for long deemed as a detoxification process.[340–342]

Biotransformation on iAs results in the production of MMA^{III} and DMA^{III},[343,344] which are more toxic than iAs, therefore, biotransformation of iAs should not be generalized as a detoxification process in microorganisms.[312] The Challenger pathway is coherent with the distribution of arsenicals in urine and can be entirely verified using $(CH_3)_3S^{+}$ as a CH_3^{+} donor and SO_2 as the reducing agent in likeness with the Meyer reaction,

which is an uncatalyzed oxidative addition reaction employed in the preparation of MMA^{V} from iAs^{III} and methyl halide.[310,70,71,336]

Other pathways proposing glutathione- or protein-conjugated intermediaries have been advanced by Hayakawa et al.[71] in 2005 and Naranmandura et al.[72] in 2006 (see Fig. 3), respectively, though their chemical basis is questionable because they involve accepting CH_3^- group.[345] They, however, appear to be in agreement with the belief that trivalent arsenicals were confirmed to be readily absorbed by the organs/tissue and linked to cellular proteins, as a substitute to elimination.[346]

The two pathways propose that DMA^{V} and MMA^{V} ought to be final products (instead of intermediaries) of arsenic biotransformation,[347] because trivalent arsenic, whether in glutathione or protein complex states, is subjected to reductive methylation without being oxidized.[310] MMA^{III} that has long been considered as a transitory intermediate in the methylation pathway is rather a stable metabolite of iAs that has been detected in appreciable level in hamster liver, rat bile, and human urine upon exposure to iAs.[336,348]

Production of methylarsenicals is linked to the growth phase or phytoplankton nutritional state.[326] Production of DMA^{V} increases gradually, while DMA^{III} and MMA^{III} remain fairly constant during the lag phase of phytoplankton growth. Formation of DMA^{V} is elevated when the proportion of phosphate to arsenate declines implying enhancement of production at phosphate-replete conditions.[326,349]

MMA and DMA are generally available in trace amounts or not present in seafood.[140] Measurable amounts have mainly been detected in fatty types of fish.[350] Extremely low amounts (i.e. µg As/kg) of DMA have been detected in mackerel and herring and in

prawns but not measurable in cod, dab, haddock, sole, plaice, tuna or whiting.[140,350–352] DMA has been detected in seaweed like Kelp. *In vivo* studies have shown that methylated arsenic metabolites can traverse the placental barrier, although methylation capacity is enhanced during gestation, as protective measure for the developing fetus.[343]

It has been suggested that MMA^{III} is more hazardous and toxic than iAs to the liver, skin and lung cells.[353] In addition, DMA^{III} is more toxic than DMA^{V} and iAs[354] because DMA^{III} has neutral charge and can readily permeate cells (up to 16%); but DMA^{V} having a negative charge can scarcely enter cells (0% to 2%).[355]

The methylated trivalent arsenicals, MMA^{III} and DMA^{III}, have higher cytotoxicity than As^{III} and As^{V}, which are more cytotoxic than the methylated pentavalent arsenicals, MMA^{V} and DMA^{V}.[310] The adverse effects of arsenic are therefore intimately connected to its metabolism and is significantly reliant on the methylation level and the valence state of the metabolites (see Table 3 below).[356]

2.3.2. Trimethylarsine oxide (TMAO)

TMAO has been isolated in various species of aquatic organisms as a minor arsenic species, seldom found except in miniscule amounts.[2,357] Quantities are much lower in stored, frozen fish than in fresh fish, likely due to post-mortem degradation, but dietary ingestion of TMAO is most likely small.[2,140,358] TMAO is fundamentally benign, with an acute oral LD_{50} for arsenic in mice of 5,500 mg/kg (see Table 3 below).[140,359]

2.3.3. Arsenocholine (AsC)

Arsenocholine is a metabolic predecessor for AsB in aquatic animals.[140,357,360] After inoculation of labelled AsC, it is swiftly taken up and converted to AsB with minimum breakdown to iAs, MMA or DMA.[140,360,361] Even though findings on AsC toxicity are scanty, it is deemed to be benign.[140] The acute oral LD_{50} for AsC in mice was 6,500 mg/kg, whereas the acute intravenous LD_{50} was 187 mg/kg (see Table 3 below).[140,361]

Table 3: Experimental biological activities of different arsenicals

SNo.	Arsenic Species	LD_{50} (mg kg^{-1})	Animal	Reference
1.	MMA^{III}	2	Mice	Petrick et al.[362]
2.	MMA^{V}	916	Mice	Kaise et al.[359]
3.	DMA^{V}	648	Mice	Kaise et al.[359]
4.	AsC	6,500	Mice	Kaise et al.[361]
5.	AsB	>10,000	Mice	Kaise et al.[20]
6.	TMAO	5,500	Mice	Kaise et al.[359]
7.	TETRA as TMA-chloride	890	Mice	Shiomi et al.[363]
8.	TETRA as TMA-hydroxide	No Toxicity	Mice	Sakurai et al.[364]
9.	$DMAs^{III}$-Sugar-Glycerol	6.56×10^{-2} †	Human UROtsa	Andrewes et al.[238]
10.	$DMAs^{V}$-Sugar-Glycerol	1.968 †	Human UROtsa	Andrewes et al.[238]
11.	AsHC 332	3.25×10^{-3} †	Human UROtsa	Meyer et al.[42]
12.	AsHC 360	1.73×10^{-3} †	Human UROtsa	Meyer et al.[42]
13.	AsHC 444	2.31×10^{-3} †	Human UROtsa	Meyer et al.[42]

LD_{50}: Concentration at which 50% of a population dies.

† IC_{50}: Concentration at which the cell viability is reduced by 50%

2.3.4. Arsenobetaine (AsB)

Seawater contains traces of arsenic (~2 µg/L), which is bioaccumulated by aquatic organisms by up to five orders of magnitude.[2] The bulk of arsenic in aquatic organisms exist as AsB, mainly found in fish and shrimp, and AsSugars, mainly found in marine algae.[238] AsB is an arsenic analogue of the amino acid derivative, glycine betaine, and it is extremely stable to hydrolysis or metabolism.[31]

The source of AsB in the food web is vague, though various speculations concerning its biosynthetic pathway have been proposed.[46,365] The arsenic atom in AsB is oxidized with four stable carbon bonds, which are enzymatically or thermally recalcitrant. Even though AsB can be degraded by microflora existing in human gut, their incubation time (7 days)[366] is longer than practical gut retention, and this metabolic pathway has not been witnessed *in vivo*, thus AsB is not converted in humans and other mammals.[2,31,140]

This makes AsB biochemically quasi-inert, which may explain why this species, though readily accessible, does not convert into other metabolites when consumed by humans, and is swiftly eliminated intact from the mammalian body.[93] The postulation that the four stable As-C bonds account for the innocuous nature of AsB earns credence from the fact that tetramethylarsonium ion (TETRA) and arsenocholine (AsC), both of which are structurally analogous to AsB, also display no indication of toxicity.[93,288]

AsB is non-mutagenic and does not show cytotoxicity, immunotoxicity or biotransformation in mammalian cells, which is aptly reflected by its acute oral LD_{50} in mice being more than 10,000 mg As/kg (see Table 3 above).[20]

There is adequate proof that higher aquatic animals do not produce AsB, but the complete description of its formation remain unclear.[357] Experimentally, AsB has been proven to be efficiently assimilated from seawater by mussels, whereas shrimp and fish accumulate AsB efficiently only from food, which includes phytoplankton among others.[26,28] In mussels, retention of AsB is dependent on the salt content of their ambient water, which supports the notion that AsB can mimic an osmolyte.[31]

The tendency to increase total arsenic with salinity was witnessed among three species of pelagic fish, where AsB is presumed to be the main arsenic species, also implying AsB absorption and retention is linked to salinity.[31,28] This experiment gains credence from data that show a positive correlation between arsenic content and salinity of mussels kept in aquatic environment of changing salinities.[26]

2.3.5. Tetramethyl arsonium ion (TETRA)

TETRA is a minor arsenical in finfish but the main species in various mollusks.[2,69,357] Amounts ranging from 0.2 µg/g to 16 µg/g were reported in different organs of a few clams.[357] Concentrations of TETRA can be enriched by freezing or dry cooking (grilling, roasting, and baking) at temperatures above 160°C, particularly in burnt meat, possibly owing to thermal decarboxylation of AsB.[367]

Consequently, TETRA concentrations above 1.0 µg/g dry mass have been documented in cooked fish where TETRA was not present before cooking.[140] The halogenated TETRA has substantial acute toxicity; in mice, the acute oral LD_{50} of TETRA-chloride was 890 µg/g. Conversely, such toxicity may arise from the halogen anion and not TETRA as analyses of synthetic TETRA-hydroxide has revealed non-toxicity.[364]

The highest documented amounts in grilled or roasted fish are 0.571 µg/g fw and 1.79 µg/g dw.[140,367,368] Due to the low contents of TETRA in dietary fish, its acute toxicity is improbable.

2.3.6. Thioarsenicals

Thio-arsenicals and oxo-arsenicals are structurally similar with sulfur replacing oxygen and are produced when oxo-arsenicals are exposed to hydrogen sulfide (H_2S).[369] Knowledge of thio-organoarsenicals is fairly recent and few studies have examined their production processes and detection techniques.[370] The main focus of arsenic speciation has been on oxo-arsenicals, owing to their abundance in nature.[371]

Speciation analysis of thio-arsenicals is a challenging task and there is need to be cautious during sample storage, pretreatment, extraction, separation, and detection.[310] Despite the latest advances in characterization and detection of thioarsenicals, there are lingering intricacies in their analysis in biological matrices, especially seafood and seaweed, owing to the complexity of arsenic metabolism, the homophyly of oxo-arsenicals and thio-arsenicals,[371] and lack of standards for thio-arsenicals.[310]

Many thio-arsenical standards must be produced in specific laboratories and, in certain instances, the production is grueling owing to the instability of species, like $DMMTA^{III}$.[310] The names, abbreviations, and chemical structures of the main thiolated arsenicals involved in arsenic metabolism are presented (see Fig. 17) below.[310]

Presence of dimethylmonothioarsinic acid ($DMMTA^{V}$) and dimethyldithioarsinic acid ($DMDTA^{V}$) in human and animal urine, which may cause interferences with metabolic

processes, after ingestion of AsSugars has led to the recent upsurge in the research on thio-arsenic species.[370,371] Methylated thio-arsenicals have been identified in urine after long-term ingestion of iAs-contaminated drinking water or intake of AsSugars.[342]

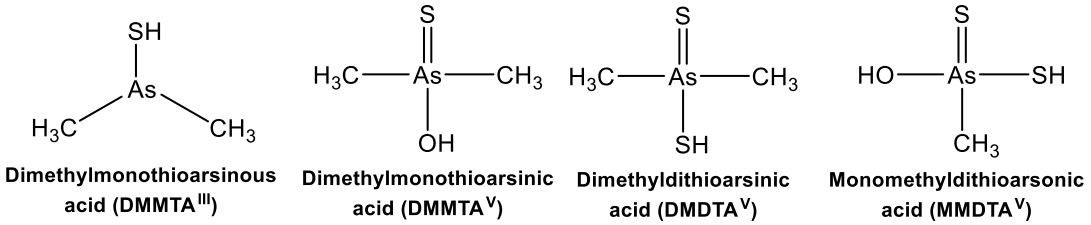


Figure 17: Structures, names, and abbreviations of methylated thioarsenical compounds.

$DMMTA^V$ appeared at trace levels in the urine of Japanese males after intake of AsSugars.[372,373] Elevated levels of 2-dimethylarsinothioyl acetic acid (thio-DMAA) were detected in the urine of sheep consuming algae.[371] Trace amounts of thio-DMAA and thio-DMAE were detected in the serum following intake of an oxo-AsSugar.[374] Wang et al.[375] reported the presence of thio-DMAA in human saliva following ingestion of Chinese seaweed, with the usual excretion profile observed in urine.

Toxicological significance of thio-arsenicals to organisms is still uncertain, but there are signs that methylated thio-arsenicals have appreciable toxicity compared to their oxo-anion counterparts.[376] As regards human epidermoid carcinoma, $DMMTA^V$ has higher cytotoxicity than DMA^V (LD_{50} 10.7 µmol/L and 843 µmol/L, respectively).[377]

$DMDTA^V$ has injurious consequences in culture cells that result in DNA impairment.[378] Thio-DMA^V has been observed as a product of both iAs[342] and AsSugar metabolism.[373,374] Considerable toxicity from thio-DMA^V has been witnessed in skin, bladder, liver, and lung cells, which is partly linked to its extreme cellular

bioavailability.[379–384] Thio-DMA^V has been proven to generate ROS in healthy cells,[379–381] and to disturb cellular stress response, even at picomolar levels, in oxidatively stressed cells.[40,41,319]

Thio-DMA^V exhibited no genotoxic mode of action in lung cells,[382] but DNA injury and alterations in gene expression were witnessed in bladder cells exposed to such species.[381] Epigenetic effects from long-term exposure to thio-DMA^V have also been detected at low picomolar levels.[385]

2.3.7. Arsenosugars (AsSugars)

AsSugar is a general expression that denotes ribofuranosides containing arsenic.[238] There are at least 20 known AsSugars in different molecular forms, of which four (AsSugars-OH, $-PO_4$, $-SO_3$ and $-SO_4$) are commonly occurring seafood.[31,93,140,238] AsSugars are the predominant arsenicals present in macroalgae,[97,111,363,386–396] and have been reported in clam,[397,398] gastropods,[399] shrimp,[77] mollusks,[67] and oyster tissue.[400]

Most AsSugars contain a dimethylarsinoyl group, where arsenic is pentavalent and connects to two methyl moieties, at C5 of the ribose ring and to oxygen, with a variety of substituents at the C1 position of the sugar backbone (see Fig. 18). The dimethylarsinoyl moiety of the oxo-AsSugars is prone to be protonated at low pH (below 3), thus bestowing a cationic and polar nature to the molecule. However, this property is countered by the aglycone moiety if it holds an acidic moiety because the acid-base characteristics witnessed are essentially regulated by the aglycone.[144]

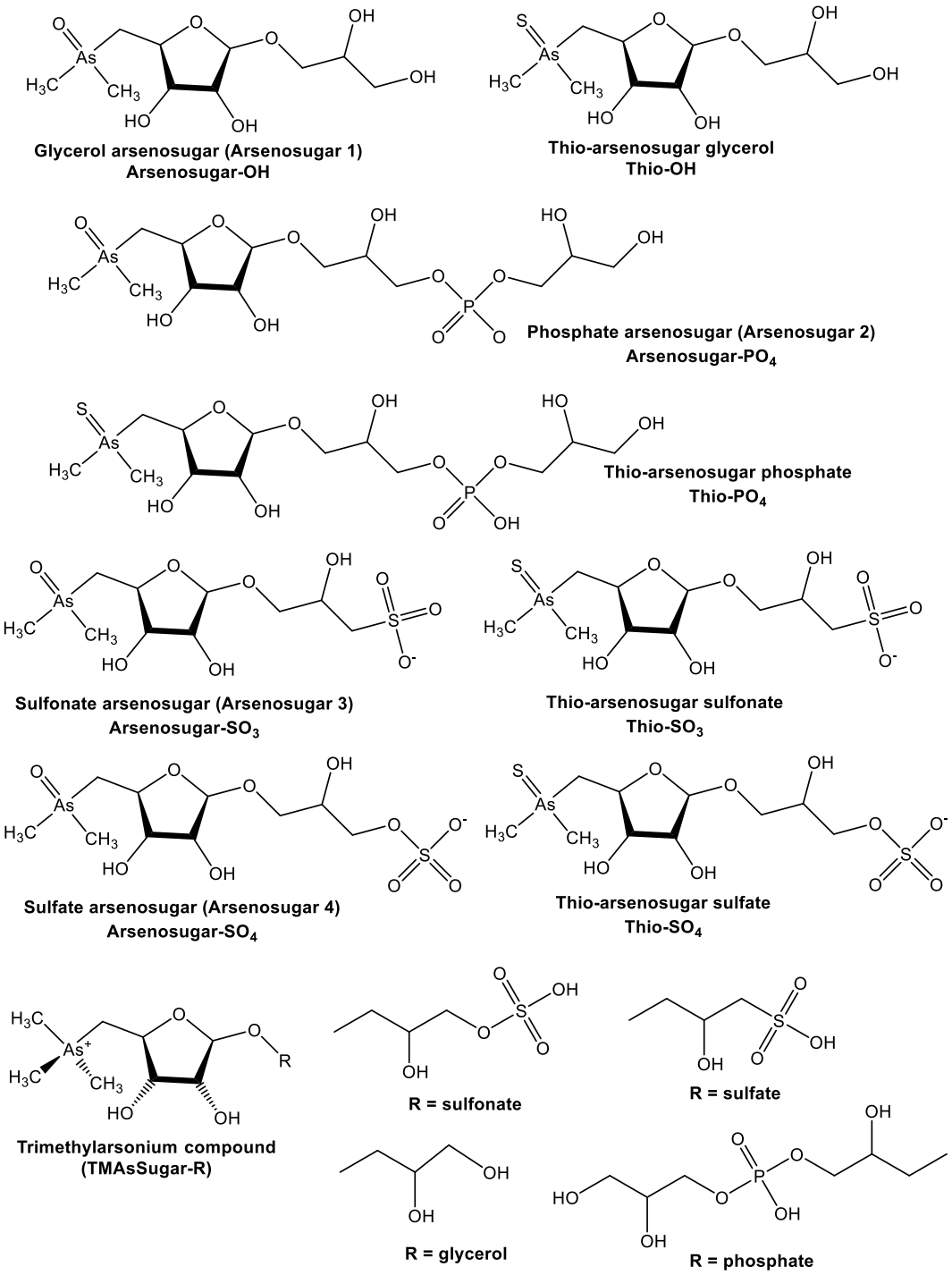


Figure 18: Structures, names, and abbreviations of Arsenosugars.

Apart from AsSugar-Glycerol, the other oxo-AsSugars with widespread occurrence possess a strongly acidic group. The acidic potency intensifies from the phosphoric acid ester, followed by the sulfuric acid ester through to the sulfonic acid ester.[401,127]

AsSugars may occur as thio-AsSugar where the oxygen is replaced by sulfur and as trimethylarsonium compounds (see Fig. 18).[93,238] Naturally, corresponding oxo and thio analogues are present, though the pentavalent dimethylated oxo species predominate.[144] Most AsSugars are polar. There are several other lipophilic arsenic-containing oxo-ribofuranoside. The molecular structure of the first AsSugar-PL 958 identified by Morita et al. in 1988 (see Fig. 19).[97] García-Salgado et al. identified additional AsSugar-PLs from two species of brown macroalgae in 2012.[111]

The polarity of AsSugars, which is based on their behavior in reversed-phase chromatography, shows that oxo-AsSugars are more polar than their thio analogues, with the AsSugar-OH being the species with the highest polarity.[144,373] The presence of diastereomeric sulfonate and carboxylate oxo-AsSugar species in natural samples was revealed following NMR studies.[144,402]

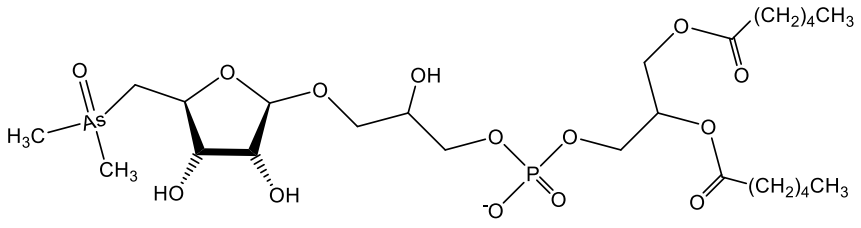
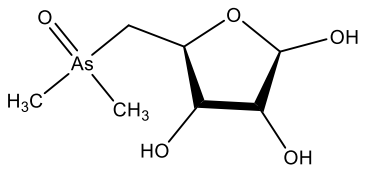


Figure 19: Chemical structure of AsSugar-PL 958 extracted from brown algae.

AsSugars are substantially more chemically labile than AsB, and biodegradation is probable when they are exposed to an acid or base hydrolysis or modeled gastric-type environment.[82,403,404] However, degradation of AsSugars is not entirely stimulated by the chemical conditions, and may be initiated by enzymatic and/or microbial activity.[405] Only limited information is available with regards to temperature stability of AsSugars

which are neither decomposed by cooking of seaweed nor by stomach acid digestion, which suggests that the occurrence of DMA^V after AsSugars ingestion is attributable to either enzymatic or microbial activity in the human body.[403,406]

Oxo-AsSugars stability persisted during transitory heating to 100°C for 10 min even though this does not demonstrate normal cooking conditions. At elevated temperatures and under acidic conditions, some AsSugars undergo acid hydrolysis to yield the disintegration product AsSugar 254 (DMAsSugarHydroxy), see figure 20 below.[405]



AsSugar 254
(DMAsSugarHydroxy)

Figure 20: Structure of AsSugar 254, DMAsSugarHydroxy

Not much is known concerning the source or potential function of AsSugars in biological systems, though it is assumed to be produced in organisms because of detoxification and excretion after ingestion of iAs^V naturally occurring in seawater.[24,407] Probable pathways for their formation and conversion have been illustrated in the literature.[238,407,408]

AsSugars are not just the major arsenicals in seaweed, but also exist in large quantities in filter feeding herbivorous mollusks and gastropods from consuming algae or phytoplankton.[31,140,144,409] Many research activities focused on the characterization and quantitation of AsSugars present in diverse aquatic macroalgae, regularly referred to as seaweed or kelp, and commercially accessible algae products, whose intake is

promoted for their health benefits, but may be a cause of exposure to arsenic that is intrinsically present in them.[144,410,411] Aquatic organisms have higher levels of AsSugars compared to the freshwater and terrestrial organisms where the topmost detected amounts in sea algae and commercial kelp powders were 10 µg As/g to 40 µg As/g (dry weight).[412]

In cells of mammals, generated AsSugar was not cytotoxic at micromolar levels.[5] Of the four AsSugars with widespread occurrence in seaweed, only two (AsSugar-OH [40,41,238,413] and AsSugar-SO_3 [40,41,319]) have been assessed and demonstrated significantly reduced cytotoxicity in comparison to iAs. However, a trivalent derivative of AsSugar-OH (DMA^{III}-AsSugar-OH) displayed substantial toxicity to the cell, but this arsenic species has at no time been witnessed in biological systems.[238]

DMA^{V}-AsSugar-OH has been assessed with cell cultures and presented no cytotoxicity at micromolar level, indicating that the AsSugars in their native state in dietary sources have extremely decreased toxicity and are likened to AsB, though this conclusion was made upon the evaluation of a single AsSugar.[93] AsSugars that were previously reported in literature as not exhibiting cytotoxic or mutagenic activities,[394] have demonstrated bioaccessibility following metabolism within the human body.[403]

The high intake of AsSugar and the resemblance they share with iAs with regards to metabolism and accumulation suggest that AsSugar may display more toxicity than earlier assumed.[238] For example, trivalent AsSugars are more cytotoxic (IC_{50} = 200 µmol/L, 48h exposure) than the corresponding pentavalent species (IC_{50} > 6,000 µmol/L, 48 h exposure) in typical human epidermal keratinocytes.

AsSugar metabolites may also occur in trivalent forms, which have thus far not been identified in biota, likely as a result of their reactivity, but have demonstrated elevated cytotoxicity while directly linked to plasmid DNA at the μmolar level.[238] Reduction of DMA^V-AsSugars is envisaged to happen promptly *in vivo* via reaction with thiol compounds, thus making these AsSugars hazardous to human health.[238,414,415]

AsSugars are metabolized and biodegraded to various minor compounds after retention in the body.[403] Assimilation and elimination of AsSugars [372,403,409] is much slower than AsB or AsLipids,[416,417] and highly variable between individuals.[403] Volunteers from single consumption studies of seaweed showed either no buildup or just a slight elevation in urinary arsenic content, while others eliminated up to 95% of the consumed arsenic.[372,374,403,404,409,418] The same consumption experiment was repeated with volunteers who showed the least (4%) and the most (95%) recovery of the consumed AsSugars, and consistent outcomes were reported.[374] Difference in metabolism by gut microflora, permeation past intestinal barriers, and conversion within the liver may provide explanations for the retention variabilities between individuals.[31,419]

DMA^V is the main metabolite for AsSugar in human urine, although the transformation sites are yet to be confirmed.[238] Feldmann et al. studied sheep, which perennially fed on seaweed and therefore were chronically exposed to AsSugars, to provide further insight into the metabolism of AsSugars because of their metabolic similarity with humans.[414] Elevated urinary arsenic concentration peaked about 20 h after ingestion were reported in all twelve sheep in the study.[415] However, tissue arsenic concentrations were not significantly elevated,[414] and only 4% to 20% of consumed arsenic was detected in feces, indicating that most arsenic was eliminated via urine.[415]

The results could not be verified due to challenges in acquiring 24 h urine samples from the sheep.[415] Unfortunately, sheep that were used as proxies to study human susceptibility to cancer risks have a limited lifespan of 4 to 6 years, which is not sufficient to assess the long-term exposure effects. However, the sheep study underscores the need for thorough investigation of foodstuff that contains arsenic in a state that is metabolized, but whose toxicological latency is unknown.[93]

2.3.8. Arsenolipids (AsLipids)

AsLipids occur as derivatives of fatty acids (AsFAs),[7,17,80,102–104,107,113,122,420–423] hydrocarbons (AsHC),[7,80,102–104,107,109,113,122,420–423] phosphatidylethanolamine (AsPE),[115,424] phosphatidylcholine (AsPC),[115,424,425] fatty alcohols,[103] and AsSugar-PLs.[107,111] Human exposure to AsLipids arises from consumption of seafood for example fatty fish,[11] algae,[107,111] and crustaceans.[1,2] However, there is a scarcity of knowledge with regard to abundance, identity and toxicity of these compounds.[11,99]

AsLipids are emerging species of interest.[96] In seafood, AsLipids comprises up to 70% of the total arsenic content.[18] The greatest quantities are found in fatty fish like herring and mackerels.[11] AsLipids are believed to propagate in the food chain starting with algae to higher organisms such as fish, with the possibility of endogenous production in the organism since the detected AsFAs show similarities to common fatty acids found in aquatic organisms.[11,324] AsHCs are present in various aquatic systems, such as herring,[80] tuna,[81] and cod,[108] fish oils,[18,102,113] and edible brown algae.[96,107,142]

Several molecular structures of AsLipids have been elucidated based on their exact mass and their product ion mass spectra, demonstrating the chemical complexity of

these compounds in seafood.[7,17,107,111,421] Subsequent biochemical studies showed that AsLipids in unicellular algae involved three main lipid types,[426] and similar species were also detected in clam tissues resulting from algal-clam symbiosis.[420,427]

Following Morita et al. successful isolation and structural elucidation of AsSugar-PL 958 in brown macroalga, Wakame (*Undaria pinnatifida*),[97] Garcia-Salgado et al.[111] and Raab et al.[107] were able to extract other AsSugar-PLs from brown macroalgae. AsLipids were first detected and identified in fish oils in 2008, where arsenic was directly linked to either a long chain fatty acid [17] or a hydrocarbon (see Fig. 21).[18] Characterization of AsLipids is work in progress with more than 50 known AsLipids.[31]

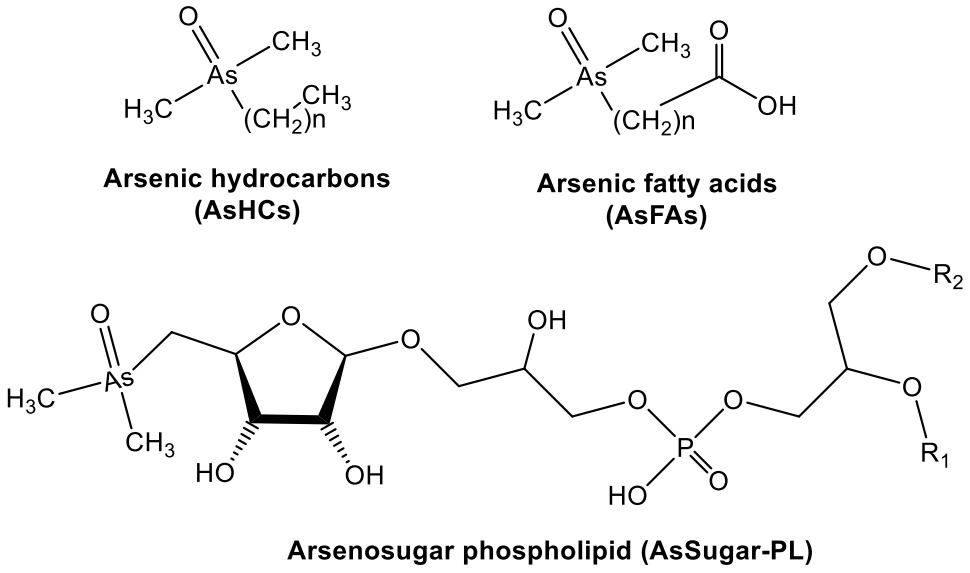


Figure 21: Structures, names, and abbreviations of arsenolipids

The identification and quantification of AsLipids has been made possible by concurrent analysis using LC-ICP-MS for element specific detection and ESI-MS/MS with mass analyzers with high resolving power and mass accuracy for structural determination.[104,109,420–423] Several AsHCs and AsFAs have been synthesized for

confirmation of identity.[116,420,428,429] However, there are still no standards or reference materials available for AsLipids and AsSugar-PLs. Standards for unsaturated AsLipids are difficult to synthesize and therefore must be isolated from fish muscle.[80]

Lack of standards is a progressive challenge because as more compounds get identified the higher is the demand for new standards. Pure compounds are necessary for accurate quantification and to aid in the investigation of their metabolic transformation and potential toxicities.[12] Improved knowledge with regards to chemical structures, amounts, bioavailability, and toxicity of the specific AsLipids is necessary for a more comprehensive risk assessment of arsenicals found in food and feed.[31]

AsLipids are of toxicological concern because their metabolites are similar to iAs^{III} a well-characterized carcinogen.[42–45,98,99,324,430,431] However, the molecular modes of action regarding their toxicity as well as their metabolism in liver remain unclear.[98] AsHC 332, AsHC 360 and AsHC 444 have recently been found to exhibit substantial toxicity in various *in vitro* and *in vivo* systems.[42] In an *in vitro* blood–brain barrier model, it was shown that AsHC 360 [1-(dimethylarsinyl)heptadecane] was up to five-fold more cytotoxic than iAs^{III}, followed by AsHC 332 [1-(dimethylarsinyl)pentadecane)], and AsHC 444 [1-(dimethylarsinyl)tricosane], which were 3.7-fold and 1.8-fold more cytotoxic than iAs^{III}, respectively.[99] AsHC 332 and AsHC 360 are effective permeability enhancers that would allow other food borne toxicants easy access to the brain.[99] Cytotoxic latency of AsFAs and their water-soluble metabolites were much lower in comparison to iAs^{III} and AsHCs. No substantial cytotoxicities were detected for AsFA 362 [15-(dimethylarsinyl)pentadecanoic acid], and AsFA 388 [17-dimethylarsinyl-9-heptadecenoic acid].[99,45]

Elevated cellular bioavailability in human differentiated neurons, liver and bladder cells [42,43] together with the detected increased intestinal bioavailability in the Caco-2 intestinal barrier model[45] implies that AsHCs appear to effortlessly permeate cell membranes, along with other physiological membranes. This explains how AsHC 332 is able to transport into the brain of *Drosophila melanogaster*,[101] suggesting these compounds are bioaccessible to the brain.[31,44,116] In the same way iAs^{III} and DMA^{V} have been demonstrated to traverse the blood-brain barrier in mice and rats.[316–318]

Due to their amphiphilic structure, intact AsLipids are seemingly able to substantially traverse across physiological barriers like intestinal barriers[45] and the blood-brain barrier.[44] Interestingly, AsHCs were neither metabolized in the *in vitro* blood–brain barrier model [99] nor in the *in vitro* intestinal barrier model.[45] In contrast, AsLipids present in cod liver oil ingested by humans were biotransformed to small AsFAs and DMA, the same metabolite for iAs^{III}.[417] AsHCs have been shown to disturb mitochondrial function leading to reduced cellular ATP levels in fruit flies,[42] while in humans AsHCs not only reduced the mitochondrial membrane potential, but also induced apoptosis. Such effects were not observed with iAs^{III}.[324,430]

A small number of studies have looked into bioaccessibility and metabolism of AsLipids, and data suggests that AsLipids are swiftly taken in via the gut, and as opposed to AsB, are metabolized before excretion in urine within 6 h to 15 h of intake.[31] After ingestion of cod liver oil mainly consisting of AsLipids by two human subjects, more than 85% of the ingested arsenic was eliminated after two days. DMA^{V} constituted up to 70% of the arsenic excreted in urine with no intact AsLipids detected.[416,417]

Other metabolites included AsFAs derivatives like dimethylarsenopropanoic acid (DMAP), dimethylarsenobutanoic acid (DMAB) and their thio-analogues (thio-DMAP and DMAB).[324,416,417] DMAP and thio-DMAP did not trigger any adverse effects in human liver cells (HepG2), human bladder cells (UROtsa), or differentiated neurons.[43,45,324] The fact that AsLipids can occur at high levels in food, have been shown to be bioavailable to humans, and extensively degrades to small arsenic species justifies the great interest on the possible toxicities of these compounds.[420]

The latest study performed by Al Amin et al.[432] on 17 food composites projected the daily intake of AsLipids in the Japanese population. It showed that the population is exposed to AsHCs, AsFAs, and AsSugar-PLs. AsLipids were identified mainly in algae, fish, and shellfish of the 17 dietary composites with amounts between 4.4 ng As/g and 233 ng As/g fresh weight (fw).[432]

Of concern was that two AsLipids, AsHC 332 and AsHC 360, with known cytotoxic effects were identified in algae, fish, and shellfish. Amounts ranging from 33 ng As/g fw to 40 ng As/g fw, with an approximated mean daily intake of about 3,000 ng As/person/day and 360 ng As/person/day, or 50 ng As/kg body weight (bw)/day and 6.0 ng As/kg bw/day, respectively were reported.

From these findings, it is apparent that diet contributes to substantial amounts of the daily intake of toxic AsHCs, fortunately the margin of exposure does not seem to present a health risk with respect to IC_{50} values of 3.05 μg As/g and 1.73 μg As/g for human liver and bladder cells exposure to AsHC 332 or AsHC 360, respectively. [42,432]

2.4. Considerations for Risk assessment

There is need for regulatory limits based on speciated data, especially for seafood because data based on total arsenic only as an indicator for risk assessment is inadequate.[93,137] Detection of arsenic species that exceed regulatory limits can become a technical barrier to trade as was witnessed in 2013 when China imposed a ban on all U.S. imports of geoduck (*Panopea generosa*) with an annual value of $80 million.[272]

2.4.1. Risk assessment

EFSA provided a risk profile for arsenicals in diet that highlighted the need to legislate in relation to toxic arsenic in food and to generate more speciated arsenic data.[14] Currently, there is no tolerable intake level set for As, since the latest EFSA scientific opinion on As concluded that the previous provisional tolerable weekly intake (PTWI) of 15 μg kg^{-1} bw was no longer appropriate.[14]

The EFSA review was an important instrument that stimulated appropriate allocation of resources to more detailed scientific assessments that led to the generation of relevant information. However, this information needs to be systematically collated and evaluated with the objective of establishing regulatory limits, especially for organoarsenicals in seafood, which do not currently have set limits.

The review article by Feldmann et al.[93] highlighted lack of structural and toxicity data on organic arsenic species, and the authors suggested the categorization of food samples into three clusters based on IARC [10] classification that categorizes all organoarsenicals as potentially toxic. New information has emerged since then with the

identification and characterization of some novel organoarsenicals having toxicities like iAs^{III}, a known carcinogen.[42–45,98,99,324,430,431]

The molecular structures of more than 200 lipophilic organoarsenicals have been assigned and new analytical methods have been developed.[17,181,217,219,221–226,228,229,237,238,256–259,] This information has changed the landscape of risk assessment with respect to organoarsenicals in food, especially seafood.

Risk assessment is a scientifically based process consisting of four main stages: Hazard identification, hazard characterization, exposure assessment and risk characterization. Hazard identification entails the screening process with the purpose of ascertaining the presence of a hazard, defined as a biological or chemical agent capable of causing an adverse health effect and that may be present in a food or group of foods.

Based on this definition, iAs^{III}, a well-characterized carcinogen, is hazardous. Other hazardous arsenicals include arsenic-containing hydrocarbons like AsHC 332, AsHC 360 and AsHC 444,[42,44,45,98,99,324,430,431] arsenic-containing fatty acids like AsFA 362 and AsFA 388,[43,45] and methylated trivalent arsenicals like MMA^{III} and DMA^{III}.[310,343,354,362] DMA^{V} is also a known tumor promotor in rat liver.[299,436]

There is need for more toxicity studies on the new organoarsenicals to identify all the potential sources of hazards. Toxicity studies aim at establishing the severity and frequency of the associated adverse health effect (response). Toxicity studies should not only be limited to the organoarsenicals but should also be extended to their metabolites, because it has been established that most arsenicals are not acutely toxic, but toxicity usually emanates from metabolic transformations.[3,8,40,144,238,367,391,437–441]

There are still many organoarsenicals that haven't been tested for toxicity and are assumed to be non-toxic because of the benign nature of some of the known organoarsenicals such as AsB. However, these compounds are not known to be non-toxic. It is therefore imperative that toxicity studies are performed for these recently identified arsenicals.

Most arsenic toxicity information was garnered from studies using laboratory animals.[20,343,344,347,359,361–363,413,442,443] This information is important but not necessarily sufficient in its current form for establishment and/or accurate simulations of regulatory limits. It is therefore imperative that the LD_{50} values obtained from laboratory animals are converted to enforceable limits for human subjects. This call for additional data and information that has been systematically obtained to enable the setting of exposure metrics that can practically be implemented in regulatory practices.

To appreciate the exposure level to organoarsenicals, there is need to conduct exposure assessment studies, which entail dietary studies considering the potential sources of exposure with regards to seafood. Exposure assessment requires data from the number of servings of potentially dangerous food ingested (provided from dietary studies), and the level of contamination (provided by information from arsenic speciation analysis), which determines the magnitude of exposure (dose).

A few dietary studies have been performed targeting certain foods in different countries.[32,39,54,55,97,132,432,255,268–272] While carrying out dietary studies it is important to consider the vulnerable section of the population who may be ingesting dietary supplements based on seafood, because they may be contaminated with organic arsenic

species. Dose-response assessment links the amount of the hazard ingested (dose) with the chance of developing adverse health effects and the severity of the same. These studies enable the establishment of exposure metrics like PTWI and allowable dietary intake (ADI) that are important in establishing regulatory limits.

2.4.2. Instrumental needs

It would be beneficial to have access to an instrument that can concurrently identify and quantify novel organoarsenicals without the need for standards. Even with standards available there is still the challenge of co-elutions and isobaric interferences that significantly affect quantification by LC-ICP-MS. There is therefore great need for an element-sensitive detector with high resolving power and mass accuracy. This will hopefully enable the concurrent identification and detection of organoarsenicals. Such an instrument should be affordable, robust and with high sensitivity.

The identification of AsHCs, AsFAs, arsenic-containing fatty alcohols (AsFOHs), and AsSugar-PLs has been aided by high resolution mass spectrometers with high resolving power and mass accuracy, such as quadrupole ion trap (QITMS),[390,449] quadrupole time-of-flight (Q-ToFMS),[7,108,124,398,421–423,450,451] OrbitrapMS,[111,80,102,103,113,433] and Fourier transform ion cyclotron mass spectrometers (FT-ICT-MS).[17,450]

These instruments are not affordable and require high level of expertise for operation, which makes them beyond the reach of many laboratories. In addition, the structural information for the identified arsenicals need to be confirmed, which requires the use of techniques like nuclear magnetic resonance (NMR) spectroscopy.[82,392,395–397,437]

Additional information can be provided by X-ray crystallography,[392,407] X-ray absorption near-edge spectroscopy (XANES) or infrared spectroscopy, and mass spectrometry.[452] Unfortunately, these techniques require analytes at high concentrations and yet the amounts of AsLipids present in the seafood samples are usually very low. Therefore, the arsenicals must be synthesized and characterized in the laboratory for confirmation, which also require in-depth knowledge of synthetic protocols and characterization of the synthetic products.

2.4.3. Standards and Reference Materials needs

It is almost impossible to synthesize standards for all known arsenicals. A pragmatic approach would be to synthesize the standards for arsenicals with known toxicities and to also synthesize their labeled analogues. Due to the monoisotopic nature of arsenic, it is impossible to find labeled arsenic standards. However, for organoarsenicals, the heteroatoms of the synthetic standards can be labeled with ^{13}C or ^{2}H, and thus enable their use as internal standards for identification and quantification of analytes of interest. Concurrent use of these synthetic standards can also be useful in overcoming co-elutions, and isobaric and polyatomic interferences associated with quantification of organoarsenicals using ICP-MS.

There is currently no agreement on a method that is internationally acceptable for arsenic speciation analysis. There is an urgent need for higher order analytical protocols for the detection and structural assignment, especially for the novel organoarsenicals. There are currently no certified reference materials for the new organoarsenicals.

Access to CRMs can be helpful in the validation of analytical methods. Interlaboratory comparisons like proficiency testing (PT) schemes can also provide an additional level of confidence in the measurement results as a tool for assessing the robustness of the validated analytical protocols and evaluating the equivalence of measurement results.

The concurrent use of the synthetic standards and labeled standards as internal standard in combination with reliable and robust analytical method for exact quantification of selected arsenicals will play an important role in the establishment of regulatory limits for the toxic organoarsenicals. With reliable analytical methods and availability of standards, development of matrix-matched CRMs will become a reality.

As an initial step, further effort should be expended in the characterization of lipophilic organoarsenicals to obtain a more exhaustive list that can then be tested for toxicity. There is still a lot more to be learnt from the hexane extracts of fatty and oily fish that is mostly discarded because of high matrix effect. Improvement in the sample cleanup techniques may also allow access to more information from the non-polar portions of the samples.[18,81,102,103,108,113,122,124,420–423,433]

Another area that might require attention is analysis of samples with high organic content because analysis of intact AsLipids require them to be dissolved in organic solvents that are not amenable with ICP-MS. The current approaches for simultaneous identification and quantification will play a critical role in arsenic speciation.

Analytical instruments have enabled species identification and structural elucidation, however, there is still need for standards and CRMs.[12] These materials will be used for the identification and quantification of arsenolipids in food samples.

Once the hazard identification, hazard characterization and exposure assessment have been concluded, then the information thereof is used as input for risk characterization, which is simply the estimation of risk that informs the setting of regulatory limits. Some of the implementation considerations include availability of standards, certified reference materials, validated analytical methods, and established regulatory limits.

Chapter 3: Analytical Methodologies for Determination of Organic Arsenic Species in Edible Marine Species.

Work discussed in this chapter has been published in the Journal of Agricultural and Food Chemistry. *J. Agric. Food Chem.* ***2020****, 68, 1910-1934*.

3.1. Introduction

Arsenic occurs naturally in seafood in a variety of organic chemical forms. There are hydrophilic arsenicals such as AsB, AsSugars, and lipophilic arsenicals like AsLipids. Inorganic arsenic (iAs) is known to be toxic. Total arsenic as an indicator for dietary risk is inadequate,[93] and accurate account for the myriads of arsenic species in the seafood present considerable challenge for food safety regulatory authorities.[61,136,137]

Current regulations for arsenic exposure focus mainly on iAs, a well characterized Class A carcinogen.[31] Setting of standards for arsenic in food is complicated owing to the enormous metabolic diversity of organic arsenic species in humans and lack of reliable speciation data on dietary sources.[93] Furthermore, the regulatory limits for iAs are derived from studies of high exposure from regions with endemic contamination of drinking water.[138,139] iAs is 100% bioavailable in drinking water. Therefore, the mode of action and exposure levels invalidates the significance of these risk assessments with regard to seafood as a source of arsenic exposure.[140]

Additionally, lack of data on arsenic toxicity in humans and other mammals from intake of significant amounts of seafood [407] provides supporting evidence against arsenic acute toxicity.[140] However, since consumption of seafood may result in production of metabolites that are important in arsenic-induced carcinogenicity, it may be prudent to

assess the effects of chronic exposure to arsenic in seafood and their contribution to long term cancer risk.[140]

The intricate distribution of arsenic in marine organisms imply that evaluation of risk focusing primarily on iAs may provide a warped outlook.[93] In addition, a myriad of compounds where arsenic is attached to an organic group have been detected in seafood, besides the toxic iAs. These organoarsenic compounds constitute more than 85% of the total arsenic content in most seafood, especially fish.[140,453,283]

Considering only the iAs fraction in determining toxicity might therefore underestimate the risk, since the major fraction containing arsenic may be present in a form with potential and unknown toxicity.[137] This would contravene the precautionary principle of risk assessment that errs on the side of caution, since focusing only on iAs, especially for seaweed, where AsSugars with unknown toxicities predominate, would misrepresent the level of potential toxicity.[93]

Seafood is considered safe owing to the benign nature of AsB that predominates and the low levels of iAs. Knowledge of arsenic speciation is the key as the chemical form of arsenic controls its bioavailability, mobility and toxicity.[12] The need for speciation data to fully assess the environmental, biological, or toxicological role of elements has been embraced by the scientific community.[137]

The challenge remains how to practically implement these ideas into the food safety regulatory framework; the case of arsenic being particularly complex owing to its presence in foods in a myriad of chemical forms, and due to the scarcity of information on the toxicity and metabolism of such arsenicals.[137]

Speciation analysis of seafood samples require analytical methods that can quantitatively characterize diverse forms of arsenic from dietary sources, however, matrix complexity and the general dominance of AsB is an impediment.[144,454–456] It is also imperative that the arsenic species in the sample are maintained in the form that they naturally occur in food.[457] This requires analytical methods that prevent species interconversions. Unfortunately, there is currently no robust, simple and affordable method available.[21,93]

A systematic assessment of methods is essential for reliable arsenic speciation in seafood.[130] The practical approach in arsenic speciation analysis comprises four main aspects: extraction, separation, detection and characterization (identification).[31] Every analytical step must be optimized with special attention to interferants including doubly charged species, isobaric polyatomic species and organic signal enhancers.[12]

AsSugars and AsLipids have attracted a lot of interest because little is known about their toxicity to humans. AsSugars occur in high concentrations (10-40 μg g^{-1}, dw) in marine organisms,[412] including those used as human food, there is considerable interest regarding their toxicological behavior.[451]

Studies suggest that AsSugars exhibit no acute cytotoxicity or mutagenicity, however, these compounds may be metabolized within humans to form toxic metabolites.[394] DMA a known tumor promotor and metabolite of iAs,[300,322] is also a metabolite of AsSugars and studies have revealed elevated levels of DMA in human urine after consumption of seafood containing high levels of AsSugars.[458,403]

The cytotoxicity of three AsHC, namely AsHC 332, AsHC 360 and AsHC 444 was investigated in cultured human bladder (UROtsa) and liver (HepG2) cells.[42] The three AsHC showed toxicity comparable to that of arsenite.[42–44] Similar studies were performed on two AsFA, namely AsFA 362 and AsFA 388 [43] which were less toxic than AsHC and As^{III}, though they demonstrated significant effects at µM level.[42–44]

These latest findings underscore the urgent and dire need for more speciation and toxicological information of organoarsenic compounds, and drive the development of robust methods for routine analysis that will support the establishment and implementation of regulatory requirements in food, especially seafood.[61,93]

A risk-based approach should be adapted in the development of new regulatory framework targeting the known toxic arsenicals, i.e., iAs, AsHC 332, AsHC 360, AsHC 444, AsFA 362 and AsFA 388. This new approach should also pay attention to the neglected area of arsenic speciation research; enhancement of extraction efficiency.[31,438,455] The proportion of un-extractable arsenic is considerable, especially in seafoods and this portion may contain potentially toxic forms of arsenic with unknown identities.[93]

Arsenic speciation analysis intent should be to understand the presence and proportions of the various arsenicals in dietary sources.[130] However, the major challenge, especially with respect to organoarsenicals is the availability of standards and CRMs. Therefore, owing to the general variabilities in toxicities based on the chemical forms present, significant risk assessment related to arsenic must exploit speciation data, which entails the deployment of reliable, robust and widely accepted analytical methods.[459]

This chapter aims to provide an overview of the current state of practice in arsenic speciation analysis of edible marine species, which include seafood and seaweed from extraction to detection, quantitation, and characterization (Fig. 22), while highlighting the general analytical considerations. Discussions will focus on hydrophilic organic arsenic species, including methylated arsenicals and AsSugars, and lipophilic organic arsenic species, including As-HCs, As-FAs and AsSugar-PLs also known as AsLipids.

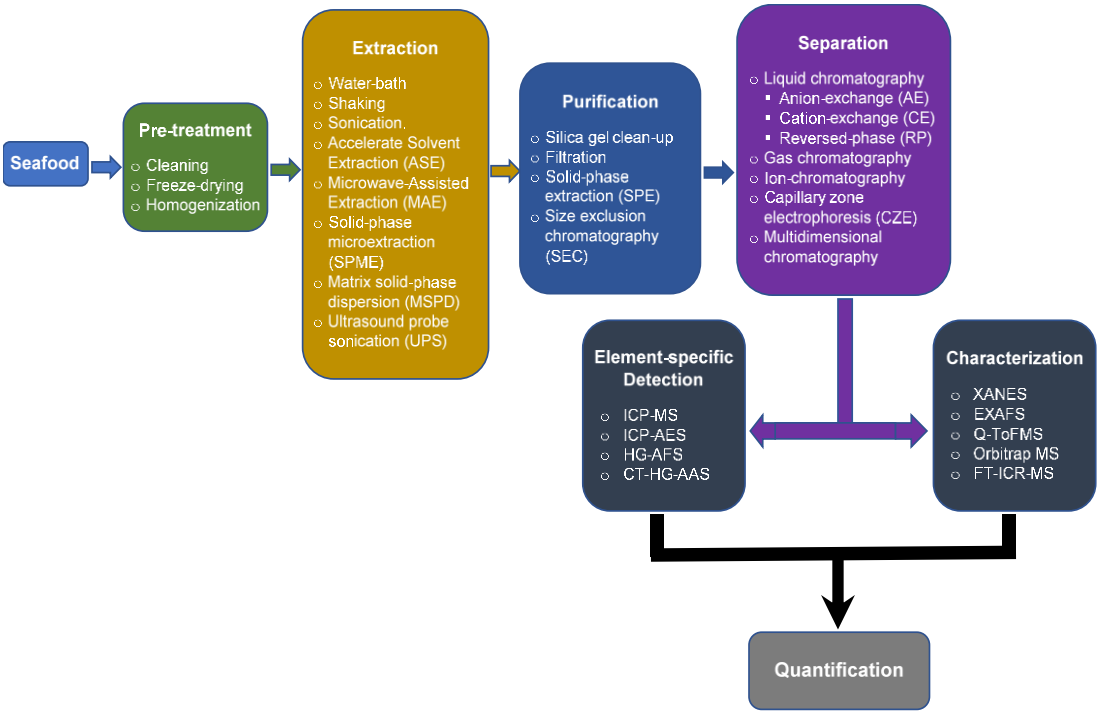


Figure 22: Analytical procedure for arsenic speciation in seafood.

3.2. Sample handling and pretreatment

Sample pre-treatment is a crucial step in speciation analysis because of the complex nature of seafood matrices and the low limits of detection required. Sample handling and pretreatment is critically important in arsenic speciation and need to be carefully optimized in order to guarantee species integrity.[144] Factors such as pH, temperature,

light, microbial activity and the container material are critical for sample handling and have direct effect on species stability.[441] Procedures such as freezing, cooling, acidification, sterilization, deaeration and storage in the dark have been recommended for preservation of species integrity.[460]

3.2.1. Cleaning

Macroalgae often need to be cleaned to remove surface material and epiphytes before analysis, as they may contain arsenic species that can bias speciation results.[12] Cleaning of seaweeds may be achieved using a dilute saline solution that does not disrupt cells due to osmotic pressure differences.[461]

It is almost impossible to completely eliminate contaminating organisms in some samples. For example, seaweed contain symbiotic fungi that are part of the plant matrix and cannot be removed by washing or scrapping.[462] AsB is an arsenic species not known to be produced by seaweed, but likely to be produced by associated epiphytes, yet several studies have reported its presence in seaweed.[78,461]

Seafood need to be depurated before analysis as their digestive systems may contain sediment particles or undigested plant and animal tissues, which can contain arsenic species that can bias speciation results. For example, many fish that fed on seagrass epiphytes contain undigested seagrass blades which contain mostly iAs,[462] and if the seagrass material is not depurated, elevated levels of iAs will be reported in seafood.

3.2.2. Freeze drying

For marine samples where bacteria naturally exist, storage at low temperatures, or even lyophilization, may be required to prevent biological activity that could modify the nature of the sample.[463] Other key issues that must be considered and which have implications on the speciation pattern observed include storage conditions and storage time.[144] Total arsenic and oxo-methylated arsenic species have been shown to be stable for long periods in frozen and freeze dried tissues of seafood and seaweed.[464–466] However, Dahl et al. [465] demonstrated that a decrease in arsenic concentration may occur in blue mussels that are frozen for periods greater than 1 to 3 months.

Some degradation of arsenic species in unfrozen samples may occur due to microbial action. For example, the production of $(CH_3)_3As$ as a degradation product has been reported in fish.[467] Freeze dried samples are unlikely to result in arsenic speciation alteration as microbial or chemical conversion cannot occur in freeze dried samples as long as they are desiccated.[12] However, although most arsenic species are stable in frozen samples, freezing and thawing of seaweed samples before drying and extraction may result in the loss of organic arsenic species. Therefore, seaweed samples should not be frozen and thawed before freeze drying.[12]

3.2.3. Homogenization

Most seafood samples are often cleaned, freeze dried and homogenized by cryogenic grinding. However, the particle size is rarely specified or characterized.[12] Particle size is critical because it determines the extraction efficiency based on sample wetting, which is directly proportional to the surface area of the sample that comes into contact

with the extractant. Narukawa et al.[468] who showed that the extraction efficiency of arsenic from rice with deionized water decreased by 10% to 30% when particle size increased from <150 mm to 500 mm. Similarly, Alava et al.[469] showed that extractable arsenic from rice increased from 70% for whole grain, to about 80% for particle size below 1 mm, to 90% for particle size below 0.5 mm to 100% when particle size was reduced to a powder. Even though the studies were performed on rice, which is not a seafood, the importance of particle size on arsenic extraction was demonstrated.

3.3. Extraction

Extraction is the key analytical step in arsenic speciation analysis because it releases the target analytes from the matrix into a solvent that must be amenable with the detection method of choice.[127] Regardless of the effectiveness of separation techniques or the sensitivity of the detectors, the limiting step that determines the quality of the analytical results is sample preparation, in particular extraction and sample cleanup.[470]

Extraction methods have been developed focusing on enhanced efficiency with reduced extraction times and volume of extraction solvents.[463] Arsenicals are difficult to extract from solid tissues making optimization of methods for each matrix type a necessity. High extraction efficiencies are desirable and are not only dependent on the species and type of tissues examined, but may show variability for different species of the same family.[471,472] For example, fish tissues gave 90 to100% extraction,[473–476] while oyster and red and brown algae allowed 85 to 100% extraction with water/methanol mixture.[394,477] Quantitative extraction is possible in a few cases but complete extraction of arsenic in seaweed or fatty seafood is mostly challenging.[463]

Extraction techniques should be designed with the aim of achieving the highest recovery, maintaining the integrity of analyte species, and ensuring the composition of the extractants are compatible with intended separation and detection methods.[31,478,479]

3.3.1. Extraction Techniques

There are many approaches and techniques employed in extraction of arsenic species from marine dietary sources. Environmental considerations such as low toxicity of the extractants and low waste generation have been the driving force towards improving the classical extraction techniques in order to obtain faster, more reliable, and environmentally friendly methods.[480] Different procedures are utilized including optimization of solvent polarity, sample acidification [437,454] to enhance recoveries of the species, enzymatic hydrolysis (EH),[68,481] and use of chelating agents.[482–485]

The classic sample extraction technique is solvent extraction using different solvents and/or solvent mixtures with microwave,[486] magnetic stirring,[487] sonication,[488,489] heating,[490] or physical shaking [437,454] applied to aid in solvent extraction.[491] Solvent extraction as a stand-alone technique is less used because it is characterized with long extraction times, use of voluminous toxic solvents, and low preconcentration factors.[492]

Modern extraction techniques tend to reduce solvent consumption, e.g. by supercritical fluid extraction (SFE),[493,494] accelerated solvent extraction (ASE) or pressurized liquid extraction (PLE),[68,495–497] microwave-assisted extraction (MAE),[66,478,498–500] make use of solvent free methods such as solid-phase microextraction (SPME),[501] or sorbent extraction phases, which is the case for matrix solid-phase dispersion (MSPD),[480] and physical treatments as in ultrasound probe sonication (UPS).[502]

Most of the modern extraction techniques have demonstrated higher capabilities for organic analyte extraction from complex matrices, like seafood, by simultaneously performing both the extraction and cleanup stages.[480]

3.3.1.1. Solvent extraction

Solvent extraction by mechanical shaking or magnetic stirring,[487,503,504] or assisted procedures by ultrasound water-baths [505,506] have widely been used in the quantitative extraction of polar arsenicals in seafood and marine-based products. Unfortunately, the procedure is laborious, time consuming and less efficient for lipophilic samples.[492,507]

Marine samples are complex and there is no single method or extractant that exists with the capability to extract all the arsenic species.[66,508] Common extraction solvents for marine samples include ultrapure water,[352,438,474,509,510] hexane,[17,102,103,113,433] methanol-water, [352,387,405,410,450,474,511–513] dilute acids,[474,514] and chloroform.[352,515]

A number of approaches, which involve a combination of several polar and non-polar organic solvents as extractants have been reported to achieve successful extraction of arsenic species from seafood.[96,102,103,105,352] For example, a mixture of Methanol/DCM [102,103,273] and Methanol/chloroform mixtures,[81,142,352,516] which have been separately employed to extract AsLipids from fish. Other commonly used solvents include:

3.3.1.1.1. Methanol-Water mixture

This is the most used solvent mixture in arsenic speciation. Ultrapure water is environmentally friendly and by far the best extractant for speciation analysis due to the polar nature of most arsenic species. As a soft extractant water cannot extract all

arsenic due to the presence of lipophilic arsenicals in seafood.[514] Methanol is also extensively employed as an extractant for seafood owing to its limited co-extraction of non-arsenicals and ease of removal by evaporation.[517]

The combination of the individual superior qualities of water and methanol as extractants has motivated their wide application in arsenic speciation analysis.[352,387,405,410,450,474,511–513] The methanol-water mixture therefore affords a fitting balance between arsenic solubility and simplicity of solvent elimination, since the majority of naturally occurring arsenicals in seafood are polar and water soluble.[144]

Aqueous extraction is suitable for polar arsenicals and more importantly preserves them in their innate chemical form.[93] Subsequent analysis of the aqueous extract is therefore less challenging as it does not involve any complex sample manipulation other than filtration and dilution. This strategy is convenient and gives a clearer outlook of the distribution of marine arsenicals rather than applying harsh conditions in an attempt to extract all arsenicals simultaneously.[130]

However, when extracting a lipophilic matrix, common in seafood samples, sequential extraction procedure is recommended because it aids in fractionating arsenicals on the basis of their polarities to realize adequate extraction efficiencies.[459,518]

Owing to the great diversity of arsenic species in marine samples, each arsenical should be extracted using customized extraction methods.[514,517] For example, physical extraction techniques like mechanical agitation and sonication have been coupled with methanol as an extractant to enhance the extraction efficiency. Unfortunately, low recoveries of arsenicals have been reported for seaweed, even with repeated (3 or 4)

extractions,[66,410,401,519] and for oily seafood having high proportions of non-polar arsenicals.[365,447]

3.3.1.1.2. Acidic extraction conditions.

Acidic extraction conditions are reported to improve extraction efficiencies of AsSugars [520,521] due to acid hydrolysis that releases arsenic degradation products in the lipid and protein fractions.[31] However, these harsh conditions are also believed to be responsible for the production of a single riboside species observed from the degradation of different AsSugars.[405,520] Severe degradation is usually experienced when high temperatures and high acid contents are employed over lengthy periods.[522]

3.3.1.1.3. Basic extraction conditions.

Tetramethylammonium hydroxide (TMAH) has been used by Gamble et al., [434] for extraction of AsSugars, which are difficult to extract in oysters and shellfish. They reported improved extraction efficiencies. However, at high concentration, AsSugars degradation based on an S_N2 mechanism was observed.[434] Ackley et al. also reported highest recoveries using 5% TMAH on spiny dogfish muscle (DORM-2).[478]

Neutralized TMAH extracts shifted peak retention times when injected on a C_{18} column.[93] Therefore, regardless of the extraction technique or choice of extractant combinations, especially for AsSugars, species integrity is usually compromised in pursuit of higher extraction efficiencies.

3.3.1.1.4. Enzymatic hydrolysis (EH)

Extraction of proteins, lipids and sugars present in marine samples may require more aggressive leaching or solubilization methods with the potential to cause species interconversion.[492] Enzymes have been used in speciation analysis because of their ability to break down specific bonds in the substrate under mild pH and temperature conditions thus allowing selective release of the analytes from sample matrix without species transformation.[68,523,524] For example, enzymes such as trypsin, pancreatin and phospholipase D have been used for arsenic speciation extraction.[516,525,526] Trypsin, a proteolytic enzyme, has been used in arsenic speciation studies on fish species such as cod, dab, haddock, mackerel, plaice and whiting.[352,405]

Enzymes can be used to determine bioavailable fraction of species by mimicking living environments, e.g. gastric digestion processes.[492] Artificial gastric juice has been demonstrated to have higher extraction efficiency for arsenic species as compared to commonly used extractants such as ultrapure water, methanol-water and 0.15 M HNO_3.[527] Therefore, the artificial gastric juice extraction could be used to simulate the physiological conditions for the dissolution of arsenic species in the human body. Many factors could influence the efficiency of enzyme-assisted extraction, including the enzyme dosage, the pH value, the extraction temperature and incubation time.[524]

A general drawback of conventional enzymatic hydrolysis is the long incubation times, typically from 12 to 24 h, the need for incubation in a bath at 37°C and relatively high cost of the reagents, which limits its applicability in speciation analysis.[481] However,

combination of MAE, PLE, or UPS to EH significantly reduces the extraction time from several hours to 30 min,[83,528] 10 min,[23] and 30 s to 2 min,[529,530] respectively.

Ultrasonication provide effective disruption of the cell walls, thus facilitating enzyme interaction with liberated cytosolic components.[531] Improvements on the enzymatic hydrolysis under microwaves is attributed to pressure effects on the enzyme and/or the substrate-enzyme interaction and conformational changes in the protein that permit exposure of the new cleavage sites to enzymatic hydrolysis,[531,532] which leads to efficient contact between the solvent molecules and the solid particles.

3.3.1.2. Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) uses CO_2 as extractant virtually exclusively, so its scope is restricted to non-polar analytes.[533] Therefore, the application of this technique in speciation analysis is rare owing to its low extraction efficiency for highly polar or ionic compounds, which form the bulk of organoarsenicals. Wenclawiak et al. were able to extract DMA, MMA As^{III} and As^{V} from spiked samples with CO_2 in the presence of thioglycolic acid methyl ester.[534]

The derivatization reaction was carried out in supercritical CO_2, leading to the formation of derivatives that were determined reproducibly by gas chromatography, reporting recoveries from 90 to 103% for MMA and DMA under the optimum extraction conditions compared to the liquid-solvent extraction technique. See Table 4 below for comparison of extraction techniques, along with their advantages and disadvantages.

Table 4: Comparison of sample extraction techniques

Solvent Extraction	
Advantages	**Disadvantages**
1. Relatively robust extraction procedure that allows efficient transfer of analytes into the extraction solvent.	1. Time consuming. Long procedure for sample extraction with extraction times between 8 h to 48 h.
2. Applicable to complex sample matrices like seafood.	2. Uses a lot of solvent most of which is toxic and thus generates a lot of hazardous waste.
3. Can be directly applied to unfiltered samples.	3. Has low preconcentration factor.
Supercritical Fluid Extraction (SFE)	
Advantages	**Disadvantages**
1. Simple, faster and high precision extraction as compared to conventional solvent extraction methods.	1. Since it uses CO_2 as an extractant, the scope of application is limited to non-polar analytes.
2. Uses CO_2, an environmentally friendly extractant that reduces the need for consumption of and exposure to toxic organic solvents. CO_2 is non-toxic, nonflammable and relatively cheap.	2. Application in arsenic speciation is rare due to low extraction efficiency for highly polar or ionic compounds which forms the majority of the organoarsenicals.
3. Low viscosity and diffusion co-efficient contributing to rapid mass transfer of solutions and enhanced interactions at the molecular level which favors the solubilization process.	3. Poor selectivity which requires advanced optimization.
4. Capable of extracting thermolabile species owing to the use of CO_2 as an extractant, which has low critical temperature that allows extractions under mild conditions, thus suitable for speciation analysis.	
Accelerated solvent extraction (ASE)	
Advantages	**Disadvantages**
1. Analyte and matrix independent technique	1. Extraction efficiency asymptotically reaches a maximum at which point the quantitative nature of extraction becomes matrix dependent.
2. Provides cleaner extracts than conventional extraction procedures.	2. No exact volume control of solvent used for extraction is provided.
3. Applicable for extraction of analytes in complex matrices.	3. Only fixed and relatively high-volume extraction cells are commercially available.
4. Relatively short sample extraction time.	4. Limited application in speciation analysis.
Microwave-assisted extraction (MAE)	
Advantages	**Disadvantages**

1. Highly efficient extraction method for a wide range of sample matrices. Solubility and not solvent diffusion is the only critical parameter to obtain good recovery. 2. Capable of hyphenation to chromatographic and spectroanalytical techniques. 3. Suitable for extraction of thermolabile species 4. Supports derivatization reactions. 5. Fast and effective extraction method. 6. Environmentally friendly because of reduced solvent waste.	1. Extraction medium, microwave power and exposure time must be carefully optimized to avoid species losses or transformation. 2. Requires polar solvents.

Ultrasound probe sonication (UPS)

Advantages	Disadvantages
1. Simple extraction procedure with fewer operations thus less prone to contamination. 2. Cavitation increases the polarity of the system, including extractants, analytes and matric, which increases the extraction efficiency. 3. Allows addition of a co-extractant to increase further the polarity of the liquid phase. 4. Allows the extraction of thermolabile analytes, which are altered when using conventional extraction techniques. 5. Allows extraction of a wide variety of compounds with various polarities therefore can be used with any solvent. 6. Generally expeditious, inexpensive and effective alternative to other extraction techniques with possibility of full automation. 7. Safer for acid digestion because it doesn't require high pressure or temperature	1. Being a batch system, the solvent cannot be renewed during the process therefore its efficiency is a function of the partitioning coefficient. 2. The need for filtration and rinsing after extraction lengthens the overall duration of the process and increases solvent consumption and the risk of losses or contamination. 3. Particle size is a critical factor 4. Less robust since the extraction efficiency can be altered as the surface of the ultrasonic probe ages. 5. Lower precision resulting from the use of ultrasonic bath in which energy distribution is not uniform and ultrasound energy is wasted. 6. Not reproducible.

Matrix solid-phase dispersion (MSPD)

Advantages	Disadvantages
1. Mild extraction technique that maintains species integrity 2. Suitable for speciation analysis. 3. Allows simultaneous extraction and cleanup of samples. 4. High capability for organic analyte extraction from complex matrices.	

3.3.1.3. Accelerated solvent extraction (ASE)

ASE, also known as pressurized liquid extraction (PLE), is an analyte and matrix independent technique which provides cleaner extracts than the time-consuming classical procedures used for extraction of compounds from complex matrices.[68,495–497] The process is based on applying increased temperatures, accelerating the extraction kinetics, and elevated pressure, keeping the solvent below its boiling point, thus enabling safe and rapid extractions.

Solvent composition and solvent temperature are the parameters that produce the most dramatic increase in extraction efficiency and hence they must be optimized.[495] Since the first instruments became commercially available in the mid 90's, this technique gained widespread acceptance for extraction of organics.[495] Unfortunately, applications in speciation analysis are rare. ASE has been used in the extraction of organic arsenic species from ribbon kelp.

3.3.1.4. Microwave-assisted extraction (MAE)

Microwave-assisted extraction is an alternative to conventional solvent extraction, where microwave energy is used to heat solvents that are in contact with solid samples, this enhancing their penetration into the sample to facilitate the partitioning of the analytes of interest from the sample into the solvent.[535] A low-power focused-microwave field, typically 20–90 W can be employed to accelerate leaching of arsenic species without affecting carbon–arsenic bonds at atmospheric pressure.[492]

Microwave heating is currently extensively used in the extraction of arsenicals from seafood and seaweed with significant improvements shown in the extraction efficiencies when compared to shaking and sonication.[66,127,478,486] Low-power microwaves, employed to decrease extraction times while maintaining efficiency, have been applied to edible marine algae,[142] oysters,[536] mussels [537,538] and fish tissues,[476] allowing 98%, 97%, 85% and 95% extraction efficiency, respectively.

On-line procedures can easily be implemented by using flow-injection for hyphenation to chromatographic and spectroanalytical techniques.[492] It is suitable for extraction of labile species and also supports derivatization reactions.[539]

3.3.1.5. Ultrasound-assisted extraction and ultrasound probe sonication.

Ultrasound-assisted extraction (UAE) has been demonstrated to significantly speed up the extraction procedure and increase the extraction efficiency.[502,540,541] UAE can accelerate the permeability of the solvent and increase the dissolution rate of extracted components by sonoporation.[524] The thermal and mechanical effects of ultrasound accelerate the diffusion of the extracted components and facilitate their extraction. Compared to MAE, UAE can achieve high extraction efficiency within minutes without damaging the components by high temperature and pressure.[524]

Ultrasound probe sonication (UPS) is a fast, relatively cheap, and effective alternative to other extraction techniques. The driving force of sonochemical action in UPS is the acoustic cavitation, provoked by bubbles formed by the soundwave in a liquid that continuously compresses and decompresses.[542] This results in extreme local temperatures and pressures generated in the liquid as well as solute thermolysis and

formation of hydroxyl radicals and hydrogen peroxide,[542] the latter in case of aqueous solvents. Consequently, when a solid is present in a solvent, compounds present in the solid may be partially or totally extracted into the liquid medium faster than when using other classical methods.[481] These features have made the use of focused UPS one of the upcoming approaches in sample treatment.[502]

3.3.1.6. Solid-phase microextraction (SPME)

SPME is based on the partition equilibrium of target analytes between a polymeric stationary phase attached onto a fibre and the sample matrix, combining analyte extraction and preconcentration into a single step.[492] The analyte is then desorbed from the fibre at high temperature into an appropriate separation and detection system, usually GC. Since the extracting phase is non-volatile, only extracted analytes are introduced into the instrument. In the great majority of the cases, extraction of metal species has been carried out using the commercial 100 μm polydimethylsiloxane (PDMS) coated fibre.[492] Volatile organometallic compounds can be collected by SPME from the sample headspace or liquid phase, directly or after derivatization.

The suitability of this technique for speciation purposes is fairly limited by the range of characteristics of commercially available stationary phases, although an increasing number of tailor-made coatings is presented in a review by Diezt et al.[543] Non-volatile analyte species can be collected from the sample liquid phase and separated by LC or HPLC, this has been done for arsenic speciation,[501] using ICP–MS detection. The application of SPME in the field of trace metal speciation is discussed in the review by Mester et al.[544]

3.3.1.7. Matrix solid-phase dispersion (MSPD)

Matrix solid-phase dispersion (MSPD) technique was introduced by Barker et al.[545] in 1989 as an extraction method for organic compounds. MSDP disrupts the sample by mechanical blending with a solid-support bonded phase to provide a material suitable for extraction.[546] The shearing forces generated by the blending process disrupt the sample architecture and provide a more finely divided material suitable for extraction by sequential elution using different solvents.[547] Therefore, due to the disruption of the sample as a consequence of dispersion, analytes are weakly bonded to the newly formed solid-support/sample matrix, and analyte extraction is possible using dilute and less toxic reagents.[548] In this way, MSDP reduces solvent consumption, amount of sample, and time required for analysis.[545,549,550]

Detailed theoretical aspects about MSDP technique is available in literature.[546,549,550] MSPD is therefore a mild extraction technique that is suitable for arsenic speciation analysis in various matrices including seafood.[480,548,551] This fact together with the possibility of performing a cleanup step simultaneously or just before extraction makes MSPD technique a potential frontrunner in modern arsenic speciation analysis.

3.3.2. Extraction efficiency and arsenic species transformation

A neglected area of arsenic speciation research is that dealing with the "non-extractable" fraction, which is thought to comprise "protein-bound" arsenic and/or "lipid arsenic".[31,438,455] Quantitative extraction must therefore overcome interactions between analytes and matrix, which partly depend on matrix composition.[542] Lack of quantitative extraction is a common challenge associated with inadequate release of

analyte from insoluble constituents of the sample matrix (e.g. protein, lipids and cells)[127] due to entrapment or strong physicochemical binding.[552]

Research in these areas is currently hindered by lack of suitable extraction techniques capable of quantitative extraction without arsenic species interconversion, especially for arsenolipids and arsenopeptides. Aggressive methods are therefore employed to improve the extraction efficiency of organoarsenicals from seafood, which may affect the integrity of the arsenic species.[553,67] For example, the use of severe extraction conditions [553] and the application of high temperature [500] could extract all the arsenic species and accelerate the extraction process, however, this may lead to species interconversion and at times degradation of species like AsSugars.[514]

There is no universal procedure that ensures species integrity during extraction and analysis since arsenic species stability depends on the sample matrix, the concentration level and the sample extraction procedure.[554] Therefore a delicate balance must be maintained between achieving higher extraction efficiencies and preserving the integrity.[31,405] Some of the modern approaches to overcome this challenge is by combining enzymatic treatment with ultrasound probe sonication (UPS).[555]

This approach was developed for various sample matrices where optimum extraction efficiencies achieved for both total arsenic and arsenic species was 70 to 109% and 86 to 91% respectively.[529] Stability studies are therefore necessary to ensure there is no species transformation during sample extraction and analysis.[441] Simulated gastric juice has been used as an extractant to aid in understanding ‘bioavailability’ and the balance between quantitative extraction and arsenic species-specific integrity.[405]

3.4. Sample cleanup procedures

Extraction is rarely selective. The raw extract may contain the analytes of interest and also co-extracted compounds, most of which interfere with the analytical process.[542] Therefore adequate removal of matrix is necessary to improve the sensitivity and reliability of instrumental analysis and to decrease interferences in chromatographic separation related to matrix as well as in analyte detection.[144] For example, presence of arsenic-free carbohydrates may hinder purification.[556] However, the choice for further purification is strongly dependent on the nature of the sample and the separation and/or detection methods to be employed.[144,495]

3.4.1. Silica gel cleanup

In the analysis of AsLipids, the lipid extracts are characterized with high normal lipid matrix interference.[122] Silica gel fractionation has been used to remove normal lipid matrix interferences from hexane extracts of fish oil, thus simplifying the analysis.[103,113,122] AsLipids are separated from normal lipid in the sample matrix because of their profound affinity for silica, since most of them contain the dimethylarsinoyl, $(CH_3)_2OAs$,[122] moiety which interacts with the acidic silica causing them to be strongly adsorbed on the column whilst normal lipids are eluted with low to moderate polarity solvents.[96]

AsLipids have such a high affinity for silica that copious amounts of highly polar solvents, like methanol, are required to elute them.[122] Caution must therefore be taken to avoid quantitative transesterification of the less-polar arsenic-containing fatty acids (AsFA) that may form fatty acid methyl esters (FAMEs) in the column.

Silica gel has also been used for the cleanup of MeOH/DCM extracts of marine algae to improve chromatographic separation.[31,96] Studies of silica chromatography clean-up procedure by Glabonjat et al.[96] demonstrate that the technique is effective for AsHCs and AsSugar-PLs with apparent negligible loses. However, when the procedure was applied on lipids in fish oil containing high amounts of AsFA conjugates, most of the initial compounds were altered by the procedure.[420,457] Therefore, washing the column with H_2S/Acetone mixture that convert the oxo-AsLipids to their thio analogues, which are less polar and readily elute from the column may address this challenge.[122]

3.4.2. Solid-phase extraction (SPE)

SPE and solvent extraction share the same principle involving a partitioning of solutes between two phases. However, instead of two immiscible liquid phases, partitioning between a liquid (sample matrix) and a solid (sorbent) phase is exploited.[557] The basic approach involves passing the liquid sample through a column, cartridge, tube or disk containing an adsorbent that retains the analytes and subsequent recovery upon elution with an appropriate solvent.

The mechanism of retention depends on the nature of the sorbent, and may include simple adsorption, chelation, ion-exchange or ion pair solid phase extraction.[557] Main advantages of the SPE approach are the possible integration of columns and cartridges in on-line flow injection systems, less solvent consumption, ease of use and possible application as species storage device for field sampling.[492]

Ionic compounds may selectively be preconcentrated using anionic or cationic cartridges, besides avoiding possible signal overlapping with related species in

complex matrices when using atomic detectors. In that way the iAs^{III} interference on AsB in arsenic speciation could be avoided by placing an anionic cartridge before the separation column, leading to retention of iAs^{III}, iAs^{V}, MMA and DMA.[558,559]

3.4.3. Size exclusion chromatography (SEC)

SEC is a separation technique based on molecular-weight. It is not used as an analytical technique, but as a cleanup technique.[386] SEC is important in purifying complex seafood matrices characterized with complex biopolymers like sugars, lipids and proteins [389,560] that may bind to the stationary phase or co-elute with analytes of interest.[144] Rigorous cleanup of extracts is necessary to prolong the lifetime of LC columns, minimize matrix interferences during detection, reduce deterioration of chromatographic resolution and fractionate highly concentrated extracts before separation of AsLipids in lipid fractions, which will ensure achievement of good separation of arsenic by RP-LC on C_8 or C_{18} columns.[81,103,389,433]

Secondary mechanisms, such as adsorption and ion-exchange effects, may impact the retention of analytes.[144] Therefore, arsenic charge state should be considered when determining SEC conditions in order to avoid retention based on factors other than size and molecular weight.[144] Strong electrostatic attractions are induced when charged groups of the stationary phase material interact with the quaternary TMAsSugars in the presence of water as an eluent, which may result in inhibition of their elution, especially in the absence of an acidic group in the aglycone moiety in the C1 position.[357]

While investigating the effects of mobile phase composition and flow rate on SEC for AsSugars cleanup, McSheehy et al. chose not to use a buffer to enhance the non-

specific interactions of AsSugars with the column and to minimize the salts in the collected and lyophilized fractions.[387] Under the same conditions the dimethylarsinoyl moiety of the AsSugars was preferentially protonated which resulted in less retention of the AsSugars with acidic aglycone.[144]

Electrostatic repulsion between the anionic stationary phase and the AsSugars' anionic functional groups may explain why AsSugars act as though they were ejected from the pores.[387] Based on these conditions McSheehy et al. succeeded in fractionating the acidic AsSugars from AsSugar-OH that lacks an acidic group in the aglycone in C1 position. AsSugar-OH was therefore voided in the dead volume with other cationic species thus eliminating the need for additional purification steps.[144,387]

3.5. Separation

There are numerous separation techniques available for arsenic speciation analysis, which includes capillary zone electrophoresis (CZE), gas chromatography (GC), and liquid chromatography (LC). The choice of separation technique depends on among others, sample matrix, extraction techniques, analytes stability and detection systems.

The advantages of CZE are low running costs, low sample volumes for analysis, fast analysis, and simultaneous separation of anionic and cationic species with high resolution.[524] However, CZE application is mainly limited to pure standard solutions or simple matrices, has poor sensitivity,[561,562] interfacing with various detection systems is difficult [524] and the analytes that are to be separated by CZE must carry an ionic charge.[563] Not all arsenicals, especially AsLipids are ionic in nature, which greatly limits the application of CZE in arsenic speciation analysis.

GC can provide excellent separation of volatile arsenic species and can easily be interfaced with various element-specific detection systems like electrothermal atomic absorption spectrometry (ET-AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), hydride-generation atomic fluorescence spectrometry (HG-AFS), and inductively coupled plasma mass spectrometry (ICP-MS). However, it is not widely used in arsenic speciation analysis because most organoarsenicals are non-volatile and thermolabile.[517] Derivatization (hydride-generation) is therefore required to form arsines, however, most of the organic arsenic species do not form arsines, which further limits the scope of application of GC in arsenic speciation analysis.

LC is often preferred over GC or CZE because it is generally more versatile, capable of being applied to a wide range of sample matrices and can analyze non-volatile polar and lipophilic organoarsenicals.[564]

Ion-chromatography (IC) is predominantly used for arsenic speciation in one of the three separation modes: ion-pairing,[488,489] ion-exclusion, or ion-exchange chromatography.[564–566] For neutral arsenic species, especially for AsLipids analysis reversed-phase liquid chromatography (RPLC) is employed.[487]

LC can also be interfaced with numerous detection systems including ICP-MS, HG-AFS and tandem mass spectrometry, making it the most suitable separation technique for arsenic speciation. The major drawback for LC is post column dispersion, coelution of species with similar physicochemical properties and the need for standards necessary for retention time matching and species identification.[452,524,567]

3.5.1. Capillary Zone Electrophoresis (CZE)

CZE separations arise as a result of differences in electrophoretic mobilities of arsenic species in an electrolyte buffer under the influence of an electric field based on their charge-to-size ratio, which can be carefully controlled by appropriate choice of buffer constituents and pH adjustments.[563] CZE offers high separation efficiency, rapid analysis, and chemical simplicity.[533]

CZE boasts of better resolution than chromatographic separations and has been applied to the separation of organoarsenic species,[563,568] but, because of matrix interference, the analysis of real samples has proved to be challenging.[561,562] Moreover, its sensitivity is relatively poor and its connection to various detection systems is difficult because of buffer incompatibilities with the ionization process.[563]

Currently the majority of CZE separations of arsenic have been limited to pure standard solution or simple matrices. Sample-stacking, an on-column pre-concentration technique with a column-switching facility was introduced to improve CZE detection sensitivity with respect to arsenic species.[463] The coupling of CZE to mass spectrometry (MS) combines the advantages of CZE and MS so that information on both high separation efficiency and molecular masses and/or fragmentation can be obtained in one analysis, which has great potential for arsenic speciation in marine samples.[562]

CZE separations occur in the liquid phase while MS detection is a gas-phase process, therefore, electrospray ionization (ESI) is the suitable interfacing technique. ESI is an atmospheric pressure ionization (API) technique that produces gas-phase analyte ions directly from solution.[569,570] In this regard, the ESI interface with a coaxial sheath liquid

arrangement is considered highly effective.[571,572] However, challenges encountered when combining CZE and MS online include: (i) CZE background electrolytes are limited to volatile compounds;[573] (ii) migration times of analytes in a CZE-ESI-MS separation can be affected by the sheath liquid composition;[574] and (iii) CZE-ESI-MS has limited absolute concentration sensitivity.[561,562]

3.5.2. Gas chromatography (GC)

GC has successfully been utilized for the analysis of AsHCs in capelin oil,[109] using GC-MS and in canned cod liver [108] and commercial fish oils,[110] using GC-ICP-MS. GC has the advantage of overcoming the challenges associated with the introduction of organic solvents to the ICP-MS associated with RP-HPLC.[110] Organic solvents destabilize the argon plasma in ICP-MS requiring several modifications of the ICP-MS, including low flow and oxygen addition,[575] for analysis of lipophilic samples using LC-ICP-MS.

Fortunately, the ICP-MS plasma will not be affected by organic solvents when using GC. Additionally, GC is commonly used for the separation of fatty acids and other lipids that can be volatilized be derivatization.[110] Despite this, only few studies have, so far, focused on the use of GC for analysis of arsenicals in lipophilic marine samples.

3.5.3. Liquid chromatography (LC)

Hydrophilic arsenic species appear in diverse ionic forms that are pH dependent, which makes it difficult to find a single scheme capable of separating all the common water-soluble arsenicals.[12] Lipophilic species are mostly neutral with hydrophobic properties owing to the alkyl and acyl groups.[487] Chromatographic approaches like ion-pairing

reversed-phase, ion-exchange, ion-exclusion, and reversed phase are reported to facilitate speciation of arsenicals in marine sample extracts owing to variabilities in their physicochemical properties.[12,524] For example, at pH 7 MMA at (pK_a = 2.6) and DMA at (pK_a = 6.1) are anions; AsC, TETRA and TMAO at (pK_a = 3.6) are cations, while AsB at (pK_a = 2.18) is zwitterionic.[576,577]

Methylated arsenicals and AsSugars have successfully been isolated on anion-exchange (AE) columns,[488,489] while cation-exchange (CE) provides effective isolations for AsB, AsC, DMA, TMAO, TETRA and DMAA.[401,578] AsLipids, however, require additional separation, using reversed-phase liquid chromatography (RP-LC) typically with a C_8 or C_{18} column.[102,96,7]

3.5.3.1. Ion-exchange chromatography

Ion-exchange chromatography has been used for the separation of ionic and ionizable arsenic species by employing the mechanism of exchange equilibria between a stationary phase, which contain surface ions, and oppositely charged ions in the mobile phase.[579,580] Ion-exchange chromatography may be used in either of the two separation modes: anion-exchange (AE) or cation-exchange (CE).

AE is used to determine anionic arsenic species, where the arsenic species are initially retained on the column by anion exchange and subsequently eluted by a competitive anion included in the mobile phases.[564–566] Ion-exchange chromatography enjoys significant application for arsenic speciation analysis.[144] Over fifty hydrophilic arsenic species have been identified in marine biota extracts using AE and CE chromatography.[12]

The factors that influence the separation and retention of analytes in ion-exchange chromatography include: the ionic strength of the solute, the pH of the mobile phase, the ionic strength and concentration of the buffer, temperature, flow rate, and the introduction of organic modifiers into the mobile phase.[564]

3.5.3.1.1. Eluent systems

In ion-exchange chromatography, the retention time of the analyte is dependent on eluent composition, including the nature of the competing ions, eluent concentration, and pH.[483–485]

3.5.3.1.1.1. Competing ions

The nature of competing ion is the main parameter that determines whether target ions are eluted from the column by competitive ion exchange with the eluent anion.[581] To obtain arsenic speciation within a reasonable retention time, it is necessary to optimize the eluting system. Generally, phosphate is a weak competing ion.[582] However, changes in the eluent strength and pH can significantly improve both the resolution and retention.[581] For example, when an eluent of 5 mM $NH_4H_2PO_4$ at pH 4.6 was used, there was poor resolution between As^{III} and DMA and it took more than 20 min for the target ions to be eluted. However, when the pH was increased to 7.9, there was a significant improvement in the resolution and decrease in the retention time.[581]

In general, an increase in eluent pH reduces the retention time. Unfortunately, at higher eluent pH, metal ions present in the matrix may begin to precipitate.[581] In addition, the higher the concentration of the competing ions in the eluent, the more effectively the

eluent displaces target ions from the stationary phase, the faster the elution of target ions from the column, and therefore the shorter the retention time. Sadly, a loss of resolution occurs with increasing eluent concentration.

3.5.3.1.1.2. Effect of pH on apparent charge (Q_{app}) of arsenic species

The retention of organic arsenic species by ion-exchangers relies mainly on their electrostatic interactions with the cationic or anionic sites of the stationary phase, and therefore on their apparent charges (Q_{app}), which are dependent on the pH.[583] Other factors such as hydrophobic interactions of the various species with the polymeric stationary phase may also influence the retention, especially for hydrophilic arsenicals.

Methylated arsenicals and AsSugars contain acidic moieties in their chemical structure, so their apparent charge (Q_{app}) depends on the pH of the mobile phase.[584] Elution of organic arsenic species electrostatically retained by the mobile phase at a given pH depends mainly on the apparent charge of the mobile phase (ACMP) ions.[583] Buffer concentration also has a significant influence.

Guerin et al.[583] established the pH domains in which a good separation of arsenic species may be achievable (i.e., domains in which Q_{app} values differ sufficiently) by plotting Q_{app} as a function of pH for each species. They found that in the pH ranges 4-6 and 9-10, the differences between Q_{app} values for some arsenic species were quite low, and the optimal overall conditions were established to be in the reduced pH domain 6-9. The aglycone functionality of AsSugars results in different retention characteristics that are dependent on the pH of the mobile phase that affects the Q_{app}.[144]

For example, at pH below 5, the apparent negative charge of AsSugar-PO_4, AsSugar-SO_4 and AsSugar-SO_3 may decline resulting in decreased retention.[577]

At pH ranges 3.8 – 9, AsSugar-OH doesn't dissociate and cannot be retained in the AE column and therefore is eluted with or close to the solvent front because it has no charge.[577] Identification and verification of AsSugar-OH is therefore performed using CE column.[144] Tukai et al.[66] observed that AsSugar-OH was eluted after the void volume if the pH was adjusted from 5.6 to 9.2, which also helped reduce peak broadening for AsSugar-SO_3.

3.5.3.1.1.3. Effect of pH on apparent charges of mobile phase (ACMP) ions

In aqueous solution, the arsenical compounds protonate to an extent determined by the pH of the mobile phase and their dissociation constants.[585] The mobile phase acidity will determine the Q_{app} of the arsenicals and consequently the composition of the mobile phase. For example, changes in the pH of 5 mM $NH_4H_2PO_4$ eluent from 4.6 to 7.9 directly influenced the Q_{app} of competing and solute ions, in this case the Q_{app} of phosphate ion changed from negative one ($H_2PO_4^-$) to negative two ($H_2PO_4^{2-}$), resulting in an increase in eluting power.[586] Similarly, the Q_{app} of DMA (pK_a 6.1) and MMA (pK_{a1} 2.6; pK_{a2} 8.2) were influenced by the eluent pH, however, it was only DMA that was influenced by the pH change because its pK_a is 6.1 which resulted in dissociation to its ionic form causing it to elute after MMA.

ACMP should be carefully optimized in order to obtain good separation of the various arsenic species, because high ACMP will lead to a reduced resolution while low ACMP will considerably increase the retention times and eventually lead to poor elution of the

most strongly retained species.[583] The retention time of a given analyte depends mainly on both the Q_{app} value at a given pH and the ACMP value at the same pH.

Secondary effects, such as hydrophobic interactions, may alter this prediction to a certain extent,[27] but major information can nevertheless be obtained from Q_{app}/ACMP plots as a function of pH. A plot of Q_{app}/ACMP as a function of pH revealed the optimum separation conditions for arsenic species using acetate buffers covered the pH range 4-6, phosphate buffers 6-9 and carbonate buffers 8-10.[583]

3.5.3.1.2. Gradient elution

Ordinarily, AE is performed in isocratic mode, mainly utilizing aqueous mobile phases consisting of buffer salts with pH ranges covering neutral, basic, and acidic conditions.[144] Gradient elution can shorten the analysis time without compromising resolutions of target analytes, allows a broad range of retention, has high peak capacity capable of handling complex matrix like seafood, and can overcome the general issues associated with elution.[576,587] Gradient elution protocols are rarely used,[588] because they cause baseline instabilities that could lead to inaccurate quantification and they generally require extra instrument maintenance in comparison to isocratic elution.[410,589]

3.5.3.1.3. Chloride interferences

In AE, where mass detection is used, separation of the arsenic species often suffers from chloride interference in the matrix due to the formation of $^{40}Ar^{35}Cl^{+}$ and $^{38}Ar^{37}Cl^{+}$ polyatomic species both of nominal m/z = 75, which can interfere with detection of monoisotopic $^{75}As^{+}$ using ICP-MS.[564,565] This problem can be chromatographically

solved by using an eluent system that can separate the chloride from the arsenic species and therefore avoid the chloride interference.[581,590] Such eluent systems include ammonium phosphate,[582,591–593] sodium phosphate,[594,595] ammonium carbonate,[147,478,596–598] tartaric acid,[599] nitric acid,[600] TMAH,[601] sodium hydroxide,[602] phthalic acid,[603] and formate.[604] Cations are eliminated in the void volume from the anion column which aid in reducing matrix interference.[12] Unretained cations, like sodium and potassium, elute in the solvent front in AE chromatography with potential interference in AsSugar-OH determination.[365]

3.5.3.2. Reversed-Phase Liquid Chromatography (RP-LC)

RP-LC is based primarily on partitioning and is used mostly for analysis of intact AsLipids. RP-LC coupled to ICP-MS has been employed to identify different AsLipids in cod liver oil,[17,433] capelin oil,[18] fish,[81,103,122,421,422] macroalgae,[97] fish meal from capelin,[102,113] cod liver.[108,124,423] In contrast to ion-exchange chromatography, RP chromatography is more prone to matrix and pH effects.[579,605,606]

Sodium and potassium phosphate buffer mobile phase systems are often used in AE and RP-LC analysis when UV-vis detectors are used because these phosphate buffers are UV transparent.[597] However, these buffer mobile phase are not amenable with MS detectors because they leave non-volatile buffer salts on the lenses and skimmer cones resulting in signal drift and high level of maintenance for cleaning the inner surfaces of the MS detector. In addition, high concentration of sodium decreases plasma stability since sodium is readily ionizable.[597] Ammonium salts of organic acids, as well as ammonium carbonate are amenable to the ICP-MS because at 5000K to 10000K

plasma leaves little other than NH_3, CO_2 and H_2O.[564] Phthalate, formate and TMAH buffers have the same advantages with ICP-MS detector.[564]

3.5.3.3. Ion-Pair Chromatography

RP-LC uses aqueous solutions as mobile phase, which may contain a portion of organic modifiers that aids in the separation of analytes on the stationary phase that is less polar than the mobile phase. In reversed phase ion-pair chromatography, a counterion is added to the mobile phase, and a secondary chemical equilibrium of the ion-pair formation is used to control retention and selectivity.[564,579,580]

Ion-pair chromatography has the advantage of separating both ionic species as well as uncharged molecular species. Therefore, it has great utility in arsenic speciation analysis. Common ion-pair reagents are long-chain alkyl ions such as heptane sulfonate anions and tetraalkylammonium salts.[12,458] Ion-pair reagents are usually maintained at low concentrations in the mobile phase, typically 20 mM or less.[564] The main challenge of using counterions is that they are non-selective and can pair also with matrix components, hence altering the retention times.[398]

Aqueous solutions with organic modifiers, usually methanol, are used to achieve elution and separation when using ICP-MS detection for arsenic speciation analysis. The selectivity of chromatographic separation of analytes in ion-pair chromatography is influenced by several factors including the hydrophobicity of the counter ion, the concentration of the ion-pair reagent, buffer concentration, the pH and ionic strength of the mobile phase, and the properties of the stationary phase.[12] For example, switching from tetramethylammonium hydroxide (TMAH) to tetrabutylammonium

hydroxide (TBAH) lengthens the retention times of arsenicals due to reduced polarity.[579] When using the less polar TBAH, the separation mechanism changes from counterion formation to dynamic ion exchange. In essence, the solute ions are bound to counterions which are now attached to the stationary phase, as a result of their increased hydrophobicity.[12]

3.5.3.4. Micellar Liquid Chromatography (MLC)

Micellar liquid chromatography (MLC) is a variant of RP-LC that has been used in arsenic speciation analysis.[607] In MLC, a relatively high concentration of surface-active agents (surfactants) is used as counter-ions and the formation of 'micelles' occurs. ICP-MS is usually used for detection. MLC offers advantages over RP-LC, such as concurrent separation of both ionic and non-ionic analytes, faster analysis times and improved detection sensitivity and selectivity,[608] which arise from its unique three-way equilibrium mechanism where micelles acts as a pseudo phase in addition to the mobile and stationary phases.[607]

3.5.4. Multidimensional Chromatographic Techniques

Chromatographic separations based on a single interaction mechanism show limited selectivity in the presence of a variety of species,[609] especially in complex matrices like seafood.[144] Therefore, orthogonal chromatographic approaches using AE, CE, RP, and ion-pairing may be used individually or in combination to separate arsenic species.[524] Simultaneous use of multiple complementary separation techniques, like AE, CE, and RP chromatography facilitates the complete separation of arsenic species while

reducing chances of co-elution and thus enhances the reliability of analytical results.[144,365,610]

The general approach involves the use two or more orthogonal chromatographic separations to isolate arsenic species with varying chemical properties resulting in efficient resolution and high arsenic species retention capacity.[611] The sequential application of orthogonal isolation techniques may enhance chromatographic separation efficiency and particularly helps in obtaining baseline resolution.[386,449]

Reversed phase ion-pairing chromatography is an ideal alternative for simultaneous separation of neutral and ionic species.[524] TMAH, TBAH, and tetraethylammonium hydroxide (TEAH) are commonly used cationic pair reagents for separation of arsenic species.[612] Various alkyl sulfonates have been used as anion pair reagents, while sodium 1-butanesulfonate, malonic acid and TMAH have been used as chelating agents to increase the retention capacity of arsenic species on C_{18} column.[524]

3.6. Detection Techniques

Atomic spectroscopy and molecular mass spectrometry are the main detection techniques used for speciation analysis.[144] While techniques such as hydride generation (HG) atomic absorption spectrometry (HG-AAS), HG atomic fluorescence spectrometry (HG-AFS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) are element-specific with high sensitivity, mass spectrometry (MS) provides additional information on the structure of the analyte based on fragmentation patterns.[613,614]

3.6.1. Atomic Absorption and Atomic Fluorescence Spectrometry

Atomic absorption spectrometry (AAS) and atomic fluorescence spectrometry (AFS) have traditionally been the most widely used detection techniques in arsenic speciation because of their sensitivity, simplicity and precision at low ppb levels.[479] While using AAS and AFS, often hydrides have to be generated to enable analysis of arsenic.

HG, as a means of sample introduction can provide unique benefits for arsenic speciation analysis, including separation and enrichment of analytes from the matrix, high sample introduction efficiency and significant elimination of spectroscopic or matrix interferences from samples with high salt and acid concentration.[524]

LC coupled with HG-AAS or HG-AFS is a simple and convenient method for simultaneous separation and determination of arsenic species in marine products. The method combines the high separation efficiency of LC, the unique gas-liquid separation techniques of chemical vapor generation, and the efficient post column online derivatization.[524] However, the efficiency of HG is affected by the chemical forms and valence states of the analytes, with trivalent arsenic species readily undergoing HG compared to their pentavalent counterparts resulting in lower detection sensitivity.

In addition, there are limitations for the number of organoarsenic species capable of generating hydrides with chemical reagents,[487,615] therefore chromatographic eluent is often irradiated with ultraviolet (UV) rays [460,536,615] or microwave digested [476] to change inactive species into active species prior to analysis by post-column derivatization.[463]

3.6.2. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is the most widely used analytical technique for detection of arsenic species since its introduction in the 1980s, due to its amenability to front-end separations and sample-introduction strategies, high element selective limit of detection, high sensitivity, accurate isotope-ratio determination, wide linear dynamic range and multi-elemental detection at low concentration levels (1 ng/L) with minimal sample preparation constraints.[11,31,509,616,617]

ICP-MS is quite robust and less susceptible to matrix effects.[144] High sampling and data acquisition rates of the ICP-MS enable baseline separation of neighboring peaks and quantification without loss of peak resolution.[12] Depending on the physics of mass analysis, analyzers could be a quadrupole, magnetic sector, ion trap, time-of-flight (TOF), or Fourier transform (FT).[618]

3.6.2.1. ICP-MS signal interferences

Since the resolution of single quadrupole mass spectrometer is not high enough (about 0.75 amu), it cannot eliminate spectroscopic interferences.[524] ICP-MS is therefore affected by polyatomic interference from marine samples in form of molecular isobars $^{40}Ar^{35}Cl^{+}$ and $^{38}Ar^{37}Cl^{+}$, which have the same mass to charge ratio (m/z) as $^{75}As^{+}$. In addition, high concentrations of rare earth elements, like Samarium (Sm) with *m/z* 150, in extracts are an important source of doubly charged interferences since they have low ionization potential (11-12 eV) and readily form doubly charged ions $^{75}Sm^{2+}$. Several approaches can effectively overcome these interferences caused by polyatomic ions.

Interference by chloride forming polyatomic species are seldom a challenge in practice since they can be chromatographically isolated from arsenic species.[12] These interferences can also be alleviated using ICP triple quadrupole mass spectrometry (ICP-MS/MS) using H_2 or He collision cell and O_2 reaction cell technologies to detect arsenic at *m/z* 75 and *m/z* 91, respectively via chemical reaction.[488,489]

In oxygen reaction mode, the quadrupole mass filters can be set to only allow ions with *m/z* = 75 ($^{75}As^+$, $^{40}Ar^{35}Cl^+$, and $^{75}Sm^{2+}$) to pass through the first quadrupole (Q1) to the reaction cell, $^{75}As^+$ is easily oxidized to form $^{75}As^{16}O^+$ with *m/z* = 91 in the reaction cell, but $^{40}Ar^{35}Cl^+$ and $^{75}Sm^{2+}$ do not react with oxygen gas to form ionic species with *m/z* = 91 and are therefore rejected and filtered out in the third quadrupole (Q3).

Alternatively, arsenic can be detected in high resolution mode ($m/\Delta m$ >10000) by resolving $^{40}Ar^{35}Cl^+$ interferences spectroscopically.[31,619] The mass-to-charge (*m/z*) ratio of 77 must be monitored during method development, to establish whether the presence of $ArCl^+$ would be a source of interference for the detection of arsenic, since about 25% of all the $ArCl^+$ is expected to have *m/z* 77, owing to $^{40}Ar^{37}Cl^+$ contribution.[478]

For ion chromatographic separations, sodium ions arising from high concentration of NaOH in the mobile phase must be removed as they suppress the arsenic signal [522,620] and cause severe baseline drift.[12] Self-regenerating suppressors may be installed to remove Na^+ while OH^- ions are electrochemically converted to water before sample introduction to ICP-MS.[12,621]

The main shortcoming in testing AsLipids is associated with incompatibility between ICP-MS and organic solvents.[488,489,575] Organic solvents are necessary for analysis of

lipophilic compounds, which has hampered advances of AsLipids research until recently.[11] Mobile phases with high organic content may trigger arsenic signal enhancement, or extinguish the plasma,[622] which may require addition of oxygen to the plasma to help in the removal of carbon that builds up on the sampling cones of the interface due to incomplete combustion.[31,102,463] This may impact analytical performance resulting in loss of the analyte or reduction in signal intensity.[144,411]

These issues are overcome by employing a specifically designed interface, e.g., cooled spray-chamber, membrane desolvator,[623] or post-column dilution, by utilizing micro-bore LC columns,[411] by allowing low solvent flow, adding oxygen to the plasma gas, or by including a post-column flow split.[463,624,625]

Grotti et al. used small-bore columns in conjunction with low dead volume interfaces to overcome challenges associated with conventional LC-ICP-MS, because these conditions provided faster separation and a lower flow rate of mobile phase, which lead to the reduction of matrix plasma load, reagents consumption and waste generated.[626]

3.6.2.2. Sensitivity improvement by Hydride Generation

The sensitivity of LC-ICP-MS can be considerably amplified by the integration of a hydride generation (HG) system post chromatographic separation of arsenic species.[12] HG systems are typical for operation of AFS. Many arsenic species form volatile hydrides that allow approximately 70-80% of the arsenic that is converted to hydride gas to reach the ICP-MS plasma, as opposed to only about 1% of the arsenic species that reach the ICP-MS plasma via the spray chamber.[12]

$NaBH_4$ is a typical reductant for As-hydride generation,[627] which is dependent on the arsenic species and sample matrix.[12] For example AsB and AsC don't produce volatile hydride species, while AsSugars form hydride species but with a very low efficiency (~5%) or 21-28% when using an optimized HG system.[628]

The use of HG eliminates the overestimation of iAs, especially when the levels are used to satisfy food regulation requirements, by eliminating the signal of the other arsenic species that do not generate hydrides.[629] However, if the organoarsenical content is obligatory, a post column reactor is essential to convert these compounds to hydride forming species in a process that involves UV-photolysis in the presence of an oxidant, which converts benign arsenic species, such as AsC and AsB, to As^V before HG.[460,630] Cysteine is used to reduce As^V to As^{III} [631] before the formation of arsine (AsH_3).[12]

Kumar et al. proposed that the As-cysteine complexes where oxygen is replaced by a thioalkyl (SR) group, readily reacts with less sterically hindered BH^- as compared with As^{III}.[632] Cysteine permits lower acid contents to be used [633] to obtain the same response for As^{III}, As^V, MMA and DMA. Pohl et al. reported that the use of charged surfactants enhances the generation of As-hydride complexes,[634] while Karadjova reported the suppression of hydride generation by some organic solvents like ethanol.[635]

3.6.3. Hydride Generation- Atomic Fluorescence Spectrometry (HG-AFS)

LC-ICP-MS is the analytical technique of choice for arsenic speciation.[66,253,552,577,578] HG-AFS coupled to LC offers an alternative technique.[142,320,461,491,504,615,630] HG–AFS has been reported to be similar to ICP-MS with regards to sensitivity and linear

calibration range, although it has other beneficial qualities like simplicity of use, and lower acquisition and running costs for arsenic speciation analysis.[460,636,637]

Due to the low efficiency in generating volatile hydrides, the destruction of the organic part of organoarsenicals is necessary for their determination by HG-AFS.[638] Organic arsenic species are usually converted to inorganic species subsequent to chromatographic separation by photo-oxidation using a strong oxidant in basic media and UV radiation before HG-AFS detection.[142]

The utility of HG-AFS has been successfully demonstrated in diverse seafood samples. For example, ˇSlejkovec et al. analyzed six CRMs of marine origin (dogfish muscle and liver, lobster hepatopancreas, oyster tissue, brown algae, and scallop) by LC–(UV)–HG–AFS, using both AE and CE chromatography. They identified AsB, DMA, TETRA, AsC, TMAO, As^{V}, MMA and two AsSugars, together with four unidentifiable compounds.[615] Sánchez-Rodas et al. applied a similar analytical technique for the analysis of aqueous extracts of oysters.[630] These two research groups highlighted the possibility of using LC-(UV)-HG-AFS as an alternative technique to LC-ESI-MS for the detection of AsSugars in crude extracts, since the signal response in HG-AFS is less susceptible to matrix effect as compared to LC-ESI-MS analysis that require additional sample cleanup steps.

In a separate experiment, ˇSlejkovec et al. applied LC-(UV)-HG-AFS technique in the determination of arsenicals in ten different marine algae (red, green, and brown) from the littoral zone along the Adriatic Sea coast of Slovenia. They were able to identify

AsSugars as the predominant arsenicals in most of the analyzed algae samples together with AsB, As^{III}, As^{V} and DMA.[461]

Schaeffer et al. were able to determine twelve arsenicals in mussels, anchovies, seabreams, sea bass and sardines by LC–(UV)–HG–AFS, using both AE and CE chromatography. AsB was predominantly detected in all the samples, with trace levels of As^{III}, DMA and AsC, whereas AsSugars were detected only in mussel samples.[504] Geng et al. analyzed six seafood samples and four seaweed samples using cryogenic trap-hydride generation-atomic absorption spectrometry (CT-HG-AAS) following alkaline digestion for arsenic speciation and found that the results were comparable with those obtained using LC-(UV)-HG-AFS.[491]

3.7. Characterization

The monoisotopic nature of arsenic means that it lacks a distinctive isotope pattern, therefore, it is challenging to find authentic isotopically labelled standards and CRMs for identification, quantitation and method validation.[450] Standards are mandatory for accurate and reliable identification and quantification of arsenicals using LC-ICP-MS, however, in the absence of standards, as is the case with organoarsenicals in seafood, indirect or complementary approaches may be employed.[102]

Identification of organoarsenicals in seafood require either fractionation and cleanup of analytes followed by fragmentation experiment using high resolution tandem mass spectrometry for identification and structural assignment of analytes;[147] indirect confirmation of structures by comparison with synthesized arsenicals that have been fully characterized by molecular mass spectrometry.[420] However, the analytical workup

schemes in these methods are extensive and identification of potentially co-eluting lipophilic arsenicals in seafood, which are in low concentrations is very challenging.

The main techniques capable of addressing the analytical limitations of LC-ICP-MS are X-ray absorption spectroscopy (XAS) for *in situ* identification,[452] and molecular mass spectrometry with a soft ionization technique and mass analyzers with high resolving power for structural elucidation.[113,423,611]

3.7.1. Identification using LC-ICP-MS coupled to X-ray Absorption Spectroscopy (XAS)

X-ray absorption spectroscopy (XAS), which includes X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) is an element specific spectroscopic analytical synchrotron technique that uses the principle of X-ray fluorescence to probe the changes in the chemical environment of metal centers by means of X-rays.[639,640] XAS enables the elemental characterization in terms of oxidation states, site ligation, and coordination.[640] Analysis of the absorption spectra, their features and underlying principles are detailed in the review by Nearing et al.[452]

XAS presents unique capabilities over other arsenic speciation techniques by allowing *in situ* arsenic speciation analysis in nearly all types of sample matrices including crude extracts, frozen hydrated samples, freeze-dried samples and in subcellular compartments independent of their actual physical state (whether solid, liquid, or gaseous), which is not possible with conventional techniques,[452] and thus reducing sample preparation steps that might modify the elemental species present [640] while giving detection limits of about 1 to 10 µg/g, depending on experimental conditions.[452]

XAS is compatible with LC-ICP-MS, which it can be coupled with for structural elucidation of novel compounds like AsSugars and arsenolipids in their native state in seafood, some of which the reported structures have been postulated from those of known fatty acids or hydrocarbons owing to lack of identification methods and standards.[102,17] The unique speciation analysis capability offered by XAS is also key to understanding the cellular mechanisms of As biotransformation in toxicity studies.[640]

Some of the disadvantages of XAS are that XAS uses hard X-ray beams with high energy that can potentially damage the sample; the absorption edges for some arsenic and selenium compounds may be very close or even identical, which makes the use of standards mandatory. However, most standards are not available for organoarsenicals of interest like arsenosugars and arsenolipids. XAS is less sensitive to metals bound to the lighter elements like O, P, N and S functional groups.

Arsenic compounds with similar nearest-neighbor environments have similar white line energies and may be misidentified in XAS without comparison with the LC-ICP-MS. For example, arsenobetaine (AsB), arsenocholine (AsC), and tetramethylarsonium ion (TETRA) have the same white line energy (11872.6 eV).[452] Finally, XAS requires high level of skill to operate and interpret since the resulting spectrum is the weighted sum of all species present in the analyzed volume, which may be very difficult to interpret in complex matrix like seafood.

3.7.2. Simultaneous identification and quantification using LC-ICPMS/HRMS

ICP-MS is a widely accepted analytical technique in elemental analysis at trace levels, because of its high sensitivity and element selective detection,[11] however, it has a

number of limitations: its reliance on extraction step usually from solid matrix that may be incomplete or result in species interconversion, risk of co-elution of arsenic species, impossibility of species identification in the absence of well-characterized standards, lack of molecular information, and the risk of species misidentification based on the retention time matching with standards.[524,567] These challenges highlight the need to adapt complementary analytical techniques to gain a better understanding of the arsenic chemistry in seafood samples.

Marine samples have numerous arsenic species with similar physicochemical properties and the insufficient chromatographic separation efficiency of a single separation technique makes co-elution of similar species practically inevitable.[577] This challenge can partly be addressed by careful optimization of separation conditions,[641] or by running the same sample using orthogonal separation techniques, sequentially [387,386] or concurrently [102,113,389,423] for simultaneous quantification and confirmation.

Earlier arsenic speciation analysis were performed by first acquiring the "sample profile", which required element-specific detection, the most commonly used being LC-ICP-MS.[104,499,642,643] Other element-specific detection techniques used included LC-(UV)-HG-AFS,[12,142,504,615,630] CT-HG-AAS,[491] and graphite furnace atomic absorption spectrometry (GF-AAS).[644] This was followed by determination of the molecular mass profile of the intact molecules that required soft ionization and high-resolution mass analyzers.[17]

This opened the possibility for structural assignment of unknown arsenicals by either fraction collection from natural sources,[147] off-line identification by matrix-assisted

laser desorption ionization time-of-flight mass spectrometry (MALDI-ToFMS),[17] ESI-MS,[17,102,113,420,423] or indirectly by comparison with synthesized AsLipids standards that were well characterized by molecular mass spectrometry.[420]

Structural assignment for unknown species has been realized by high resolution mass spectrometry, an approach that was successfully applied by Taleshi et al. while characterizing AsLipids in multiple matrices [420] and Nischwitz et al. in characterization of AsSugars.[411] Miguens-Rodriguez et al. demonstrated the utility of electrospray ion trap multi-stage mass spectrometry (ESI-IT-MS^n) [449,390] in generating distinctive product ions that enabled the rapid screening and sensitive characterization of four AsSugars in unrefined seaweed extracts with minimal sample preparation.[390]

The recent trend is towards concurrent sample analysis using LC-ICP-MS with another orthogonal detection technique, which is not necessarily element-specific, like tandem mass spectrometry from the same chromatographic run by splitting the effluent flow from the HPLC column between the two mass spectrometers.[645] The high-resolving power of various mass analyzers (see Table 5 for figures of merit) provide accurate mass data that is essential for distinguishing the different isobaric and isomeric arsenicals with high precision and thus aid in the elucidation of their molecular formulas and provide structural information.

Concurrent use of LC-ICP-MS and molecular mass spectrometry with high mass accuracy offered by the high resolving power of mass analyzers like Q-ToF, Orbitrap™, magnetic sector, and FT-ICR mass spectrometry provide an additional separation dimension for isobaric and co-eluting analytes.[102,113]

Mass Analyzer	Resolving Power (FWHM)	Mass Accuracy (ppm)	Sensitivity (g)	Advantages	Disadvantages
Quadrupole	Unit-mass resolution Modern instruments can reach up to 5000	50	10^{-15} (SRM)	1. Good choice for quantitative analysis 2. Highly sensitive in MRM mode. 3. Relatively cheap 4. Easier to operate and maintain.	1. Mass resolution not sufficient to resolve isobaric interference 2. Reliability of analyte identity is disputable. 3. Low selectivity.
Quadrupole Ion Trap	10,000	50	10^{-15}	1. Best choice for quantitative analysis. 2. Provide reliable, cost-efficient target analyte quantitation and identification 3. Excellent sensitivity and selectivity. 4. Rapid scanning. 5. High resolution and accurate mass. 6. Resolves isobaric matrix interference	1. Require careful tuning and optimization of data acquisition parameters for target analytes which limits number of possible measurements per run 2. Affected by ion-ion interaction. 3. Relatively expensive.
Time of Flight	≥ 100,000 (at m/z 400, FWHM)	< 3	10^{-12} (full scan)	1. Best choice for qualitative analysis 2. High resolving power 3. Wide linear dynamic range 4. High mass accuracy 5. Deconvolution of intact MW for large molecules and complexes possible.	1. No MS^n capability 2. Slower scan speeds compared to other HR-MS. 3. Susceptible to chemical background ions. 4. Limited isotopic fidelity. 5. Limited dynamic range due to detector saturation effects. 6. Poor selectivity and misidentification.
Magnetic Sector	≤ 60,000	~1	10^{-12}	1. High resolving power	1. Expensive

Mass Analyzer	Resolving Power (FWHM)	Mass Accuracy (ppm)	Sensitivity (g)	Advantages	Disadvantages
				2. Wide linear dynamic range	2. Not readily accessible to most laboratories. 3. Available mostly only in specialty laboratories
Orbitrap	280,000 (at m/z 200, FWHM)	~1	10^{-15} (full scan)	1. Deliver qualitative and quantitative analysis in one instrument. 2. High detection analyte specificity 3. Good sensitivity 4. Excellent mass accuracy 5. Fast scan speeds 6. High resolving power 7. Wide linear dynamic range 8. Multiple fragmentation experiments possible (CID, HCD, UV-PD, ETD). 9. Many scan features possible (MS/MS, MS^n, SIM, PRM, DIA, AIF). 10. Ideal for top-down workflows for large molecules. 11. Ideal for qualitative analysis of complex mixtures. 12. Can perform low- or high-resolution experiments separately or concurrently. 13. Can interface with FAIMS analyzer.	1. No MS^n capability 2. Very expensive 3. Requires high level of expertise for operation. 4. Not readily accessible to most laboratories.
MegaOrbitrap	1,000,000 (at m/z 200, FWHM)	~1	10^{-15} (full scan)	1. All advantages listed for Orbitrap above, plus 2. Highest resolving power 3. Scan rate compatible with LC.	1. Very expensive 2. Requires high level of expertise for operation.

Mass Analyzer	Resolving Power (FWHM)	Mass Accuracy (ppm)	Sensitivity (g)	Advantages	Disadvantages
				4. Simpler and compact design. 5. No high field magnet with no cryogens. 6. Capability to perform both targeted and non-targeted analysis on full-spectrum accurate-mass acquisition at good sensitivity.	3. Not readily accessible to most laboratories.
FT-ICR	1,000,000 (at m/z 400, FWHM)	~1	10^{-12} (full scan)	1. Isotopic distribution accuracy 2. High resolving power. 3. Wide intra- and inter-spectrum dynamic range. 4. High sensitivity. 5. Ability to analyze samples of high complexity with strong matrix effect. 6. Ability to analyze samples with low analyte concentration	1. Dependence on cryogens (liquid He and liquid N_2). 2. Need for superconducting magnets of higher field. 3. Very complex and expensive. 4. Logistic constraints in transportation and installation due to large size.

Table 5: Figure of merit for common mass analyzers

3.7.2.1. Quadrupole Time-of-Flight (Q-ToF) Mass Spectrometry

Quadrupole time-of-flight (Q-ToF) mass analyzer is a hybrid instrument that operates in both negative and positive ionization modes with high mass accuracies at ultra-trace levels.[398,450,451] Q-ToF has mass accuracy of 2 ppm and may be limited in its ability to provide indisputable identification of unknown analytes, however, it provides sufficient information for the determination of the exact empirical formula of analytes therefore reducing the potential structures to realistic number.[450]

Isobaric matrix interference with similar retention times overlapping with organoarsenical peaks of interest can be resolved using high resolution instrument like FT-ICR-MS.[450] Some of the applications of Q-ToFMS in elucidation of structure for organoarsenicals include work by McSheehy et al. in the characterization of arsenic species in kidney of clam,[398] Sele et al. in the identification of arsenicals in marine oils,[7] and Arroyo-Abad et al. in the identification and determination of AsLipids in fresh cod liver oil.[423] fish,[421,422] and canned cod liver.[124]

3.7.2.2. Orbitrap™ Mass Spectrometry

Orbitrap™ is an accurate and compact Fourier transform mass analyzer that was first commercialized in 2005 by Thermo Electron as a hybrid instrument (LTQ-Orbitrap) featuring a linear ion trap front-end.[618] Orbitrap mass analyzers employ electrostatic trapping rather than the magnetostatic one characteristic for FT ICR.[646] This precursor instrument has undergone a number of iterative improvements incorporating features like image current detection from FT-ICR-MS, the use of ion trapping in precisely

defined electrode structures, from the radio frequency (RF) ion trap, and pulsed injection and the use of electrostatic fields, from the TOF analyzers.[618]

All these features enables Orbitrap to overcome the major limitations experience by the other techniques like the necessity for a superconducting magnet in FT-ICR-MS, severe limitations on space charge in the RF ion trap, and on dynamic range of detection in TOF analyzers.[618] Amayo et al. concurrently used LC-ICP-MS and Orbitrap mass analyzer for structural assignment of arsenolipids in fish meal,[102] fish oil,[113,433] and fish tissue.[103] The latest version of Orbitrap mass analyzer, MegaOrbitrap has ultra-high resolving power in excess of 1,000,000 within 3s detection time making it compatible with chromatographic separations.[646]

3.7.2.3. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry.

FT-ICR-MS offers resolving power upwards of 10^6 at *m/z* 200 full-width half maximum (FWHM), and mass accuracy (<1 ppm), which is the highest of all existing mass spectrometry techniques.[647] Ions are trapped in a strong magnetic field combined with a weak electric field generating an image current from coherently excited trapped ions that are detected, digitized, and converted using Fourier transform into the frequency domain and then mass spectra.[648] The inherent stability and field uniformity of superconducting magnets that work in synergy with the very high accuracy and dynamic range of frequency measurements has made FT-ICR-MS an ultimate frontrunner in mass resolving power and mass accuracy.[646]

Despite the monoisotopic nature of arsenic, it has a distinct mass defect that FT-ICR-MS is capable of resolving and thus ensuring unequivocal identification of

arsenicals.[450] This is particularly helpful in resolving co-eluting analytes with isobaric interference, which is common in lipophilic organoarsenicals. Pickford et al demonstrated the utility of FT-ICR-MS in the analysis of crudely purified kelp extract,[450] while Rumpler et al. used FT-ICR-MS and Q-ToFMS in structural elucidation of AsLipids in cod liver oil.[17]

3.7.3. Identification by tandem mass spectrometry fragmentation experiments.

Organic arsenicals like AsLipids and AsSugars have distinct fragmentation patterns that can be used together with the high resolving power and accurate mass offered by high resolution mass analyzers for identification and structural assignment of unknowns. For successful fragmentation experiment there are a set of conditions that must be fulfilled: First, there must be chromatographic separation, secondly there must be a soft ionization source, and thirdly, there must be a high-resolution mass analyzer with high resolving power and mass accuracy.

Soft ionization enables mass spectrometry to be used as a separation tool to resolve arsenical peaks by molecular weight. Electrospray ionization (ESI) [17,102,113,420,423] is typically used, although there are other ionization techniques like matrix-assisted laser desorption ionization (MALDI),[17] and fast atom bombardment (FAB).[412]

Electrospray ionization technique coupled with high resolution mass spectrometry (triple quadrupole MS,[612] Q-ToF),[108,124,421–423] Orbitrap [102,103,113,433] and FT-ICR-MS [450] can provide excellent separation of co-eluted components in HPLC and can be utilized for identification of unknown species.[103,452,649] In recent years, ESI-MS/MS has been applied to speciation analysis of Methylarsenicals, AsSugars, and AsLipids.

Selection of distinct precursor ion/product ion transitions enables tandem mass spectrometry methods based on selected reaction monitoring (SRM) also known as parallel reaction monitoring (PRM) to minimize background noise and matrix effect therefore ensuring ultra-fast scan rates, highly sensitive and highly selective detection methods for arsenic speciation. Characteristic fragments for the four common AsSugars detected in positive MS/MS mode are shown in Fig 23. The *m/z* 237 fragment is formed from the loss of the aglycone at C1 and the fragment *m/z* 97 results from additional elimination of the dimethylarsinoyl group alongside a water molecule.[451,412]

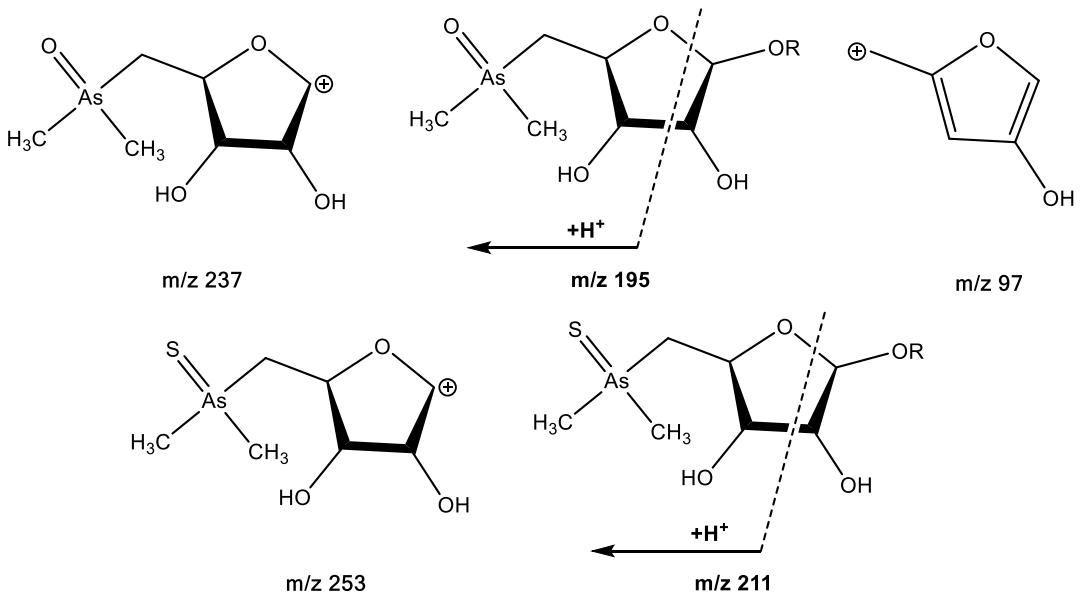


Figure 23: Typical AsSugar and thio-AsSugar fragments detected in tandem mass spectrometry.

Thio-AsSugars display similar fragmentation patterns as their oxo analogues, yielding a structurally diagnostic fragment at *m/z* 253 (see Fig 23) corresponding to the loss of the aglycone,[411,435,628] and *m/z* 97 as a result of loss of a dimethylthionyl moiety together a water molecule.[411,435] The fragment at *m/z* 97 for the sulfate thio-AsSugar may also emanate from the $–OSO_3H$ moiety.[411,513,650] Two additional unique product ions for

thio-AsSugars are observed at *m/z* 107, correlating to AsS^+,[411,519] and *m/z* 91, presumed to result from AsO^+ due to trace amounts of oxygen in the nitrogen drying gas.[519,628,651] Thio-AsSugars exhibit the distinctive sulfur isotope pattern ($^{32}S/^{34}S$) with a mass peak appearing 2 amu higher than the ^{32}S-containing ion and an expected ratio of approximately 23:1.[144]

It is important that peak assignments are verified to eliminate false positives emanating from matrix components with similar SRM transitions as the organoarsenicals.[508,611] A common quality control tool employed is comparison of the standard and analyte peaks retention times by selecting a set of unique transitions for the qualifier and quantifier ion for each analyte and comparing the intensity ratios for the standard and the analyte present in the sample.[508,652]

Other quality control methods include establishment of accurate mass of the molecular ion using HRMS.[611] Several useful tools for SRM optimization exist, which may be considered virtual standards that are derived from numerous fragmentation experiments for purified standards from natural samples.[435,652–655]

The need for complete chromatographic separation is diminished in the absence of critical signal masking by matrix components when using highly unique MRM transitions. However, sufficient chromatographic resolution is required, when DMAsSugar-SO_4 and DMthio-AsSugar, which have similar molecular mass and fragmentation patterns, are present.[653]

In addition, in-source dissociation of DMAsSugar-SO_4,[400,471,611] DMAsSugar-PO_4, and DMThioAsSugar-SO_4 [411,611] to the corresponding AsSugar-OH has been recorded even

at mild cone voltage, leading to AsSugar-OH signal contribution at their corresponding retention times.[471,611] The in-source fragmentation of DMAsSugar-PO_4 occurs marginally resulting in ion formation at *m/z* 409 in likeness to the protonated molecular ion of the sulfate oxo-sugar.[471] Similar fragmentation patterns for oxo- and thio-AsSugars need to be considered. [411,611]

3.8. Standards and Reference materials

The number of organoarsenicals discovered in seafood continues to increase in tandem with advances in instrumentation and analytical protocols. Significant advances have been made in designing highly sensitive and selective separation and detection methods for explicit identification of known and unknown organoarsenicals.[144] Characterization and quantification of organoarsenicals in seafood is impeded by lack of widely accepted analytical protocols, well-characterized and commercially available standards as calibrants and certified reference materials (CRMs) for method validation.[145,146]

Several attempts have been made to indirectly identify and quantify organoarsenicals in marine samples in the absence of standards.[103,452,649] Yu et al. were able to analyze AsSugars in Kelp by fractionation and analysis of individual fractions, using LC-ICP-MS and instrumental neutron activation analysis (INAA).[147] Fractionation is a tedious and time consuming procedure that requires voluminous extraction of natural materials [392,395,396,401] to yield miniscule quantities of pure extracts,[386,387,425,451,656] which may still need to be further characterized.

RP-HPLC coupled simultaneously to ICP-MS (element specific detection) and to HR-MS (molecular structure detection) has successfully been applied in the identification

of AsLipids in marine samples.[7,17,18,108,110,113,124,400] AsSugars have been extracted from various algae sources followed by several purification procedures including SEC, and AE chromatography.[82,386,392,395,396] Characterization and structural determination was done mainly using one- (or two-) [89,395] dimensional ^{1}H and ^{13}C NMR.[82,392,396,397] Additional information for structure determination was obtained using X-ray crystallography,[392,657] XANES or infrared spectroscopy [82] and mass spectrometry.[144]

All these approaches have been instrumental in gaining a better understanding of the nature and proportions of organoarsenicals present in marine diets. Unfortunately, the need for well-characterized standards still endure, especially as a prerequisite for establishment and implementation of regulations. The more practical approach is to synthesize analyte standards, which has successfully been done for several arsenicals mostly for the purpose of confirmation of their identities.[420,421,656]

There are almost two hundred organoarsenicals identified in marine dietary sources and it is practically impossible to attempt to develop standards for all the currently known arsenical analytes. There is need for prioritization in the development of standards using a risk-based approach, especially for organoarsenicals with confirmed toxicities like AsHC 332, AsHC 360 and AsHC 444.[42,44,98,99] and AsFA 362 and AsFA 388,[43,45] These standards should be synthesized for quantification of arsenicals and to support the precise assessment of their toxicity mechanisms and fate in living organisms.

Synthesis of AsSugar-OH, AsSugar-SO_4, and AsSugar-SO_3 and their corresponding thio-analogues has been documented in the literature.[86,656,658–662] The synthetic procedure for AsSugar-OH containing the trimethylated arsonium moiety is lengthy

and challenging involving six to ten reaction steps.[656,659–661] Thio-AsSugars and trimethylated [425] AsSugars can be quantitatively semi-synthesized using synthetic materials or extracted and purified from naturally occurring oxo-analogues as preparatory materials. The synthesis of AsLipids is complex and a number of studies have been published for the preparation of a limited number of AsHCs and AsFAs.[7,42,43,97,116,420,421,425,428,657]

Since arsenic in monoisotopic, the heteroatoms of the synthesized organoarsenicals can be labeled, using for example ^{13}C, ^{2}H etc., to facilitate confirmation of their identity in seafood and for further studies on the biotransformation processes under physiological conditions. They can also be used as internal standard in combination with reliable and robust analytical method for exact quantification of selected organoarsenicals and thus play an important role in the establishment of regulatory limits for these toxic organoarsenicals. With reliable analytical methods and availability of standards, the development of certified reference materials (CRMs) will become a reality.

Presence of AsLipids has been reported in fish oils, [7,17,18,110,113,124] fish tissue, [80,108,423] fish meal,[102] and commercial canned fish liver.[108,124] AsSugar-PLs have been detected in macroalgae,[97,111] while AsSugars have been detected mainly in oyster,[400] clam,[398] and edible macroalgae.[412,451,386,388] These materials provide a good starting point for the development of CRMs.

Only six organic arsenic species are commercially available, with less than ten represented in reference materials. There are at least six seafood-matrix reference materials currently available that give values for at least one organoarsenic species.

Five of the six are only certified for AsB (NMIJ CRM 7402-a, NMIJ 7403-a, DORM-4, TORT-3, and NIES-15), and BCR-627 is only certified for AsB and DMA. NIST SRM 2669 and NIST SRM 3669 are urine reference materials certified for MMA, DMA, TMAO, AsB and AsC, and MMA, DMA and AsB, respectively. Newly released NIST SRM 3232 for Kelp, provides values for DMA as well as three AsSugars: AsSugar-OH, AsSugar-PO_4 and AsSugar-SO_3.[147]

Chapter 4: Method Development for the Determination of Total and Hydrophilic Arsenicals in Seafood.

The work discussed in this chapter has been published in NIST Health Assessment Measurements Quality Assurance Program (HAMQAP): Exercise 5 of 2020. Final report is available at https://doi.org/10.6028/NIST.IR.8343.

4.1. Introduction

A great diversity of biologically relevant arsenic species has been identified in dietary samples. Knowledge of arsenic speciation is important as the chemical form controls its bioavailability, mobility and toxicity.[12] Speciation information of arsenic in seafood is important for food safety as total arsenic alone does not fully address the food safety issues.[93] Studies have shown that arsenic speciation in seafood is complex and the arsenic species have significantly different toxicities.[3]

Due to a lack of toxicity and chronic exposure data for organoarsenical species in humans or other mammals, health risks from exposure to the organoarsenical species are difficult to assess. Most of the adverse effects of arsenic have been documented, but the question about the risk to the individuals exposed to arsenic, especially from seafood, and the level of dose needed to develop these effects still linger.

Several studies have considered potential carcinogenicity from the production of the metabolite DMA^V,[40,41,140,319,320] based on high dose exposure studies in rats to DMA in water [300] or diet.[321] The need to legislate in relation to toxic arsenic in food and to generate more speciated arsenic data was highlighted by European Food Safety Authority (EFSA).[14] Seafood contain up to 100 times more arsenic than rice and

contribute substantially to human dietary exposure to arsenic, which is one of the elements of concern in relation to food safety.[144]

Marine algae and shellfish are the seafood exposure sources with the greatest diversity of arsenicals.[31] Among these arsenic species, the potential for biotransformation upon ingestion varies considerably.[275] Seaweed has been part of human diet, especially in Asia, for centuries and has gained popularity in western countries.[144,147]

Seafood and seaweed are regarded as important parts of healthy diets as they contain several nutrients associated with beneficial health effects [150] and are widely used as dietary supplements. Seaweed are extensively utilized in the food industry and are becoming increasingly commercially available owing to their properties as food additives,[412] their nutritional values [402,412] and suggested medical applications.[402,471] Unfortunately, some seaweed also contain elevated levels of arsenic. For example, *Hijiki* has elevated levels of iAs, which is highly toxic and a known carcinogen.[147]

There is dire need to develop analytical methods that will enable the determination of arsenic species in seafood in their native state. This method should ensure species stability and avoid their interconversion, which calls for a balance between achieving maximum extraction and maintaining species integrity.

In this chapter we are going to discuss the method development for the determination of total arsenic and hydrophilic arsenic species in seafood samples. The choice of geoduck clam as the proxy seafood sample for this study was motivated by it having the greatest diversity of arsenic species.

4.2. Experimental

4.2.1. Sample preparation

Geoduck clam (*Panopea generosa*) harvested from Vegas Hot Spur in Alaska was cryogenically homogenized. Approximately 12 g portions of the homogenized geoduck clam tissue were packaged in pre-cleaned glass jars with polytetrafluoroethylene (PTFE)-lined caps and stored at −80°C until use.

4.2.2. Reagents and standards

ACS grade nitric acid (HNO_3, 69 %–70 %) was from J.T. Baker (Phillipsburg, NJ, USA). ACS HPLC grade methanol (99.99 %) was from Honeywell Burdick Jackson (Muskegon, MI, USA). Puratronic grade ammonium sulfate ($(NH_4)_2SO_4$, 99.9.9 %) and ammonium nitrate (NH_4NO_3, 99.9 %) were purchased from ThermoFisher Scientific (Ward Hill, MA, USA). ACS grade pyridine (99.9 %), laboratory-grade formic acid (90 %), and ACS grade hydrogen peroxide (H_2O_2, 30 %) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). As^{III} in 2 % HCl (998 ± 5 mg/L) and As^{V} in H_2O (1,000 ± 5 mg/L) were purchased from SPEX CertiPrep (Metuchen, NJ, USA). Trimethylarsine oxide (TMAO) was purchased from Wako Chemicals (Richmond, VA, USA). SRM 1566b *Oyster Tissue* (*Crassostrea virginica*) and SRM 3232 Kelp Powder (*Thallus laminariae*) were used as quality control materials. SRM 3030 *Monomethylarsonic Acid Standard Solution*, and SRM 3144 *Rhodium (Rh) Standard Solution* were used as internal standards. SRM 3031 *Dimethylarsinic Acid Standard Solution*, SRM 3033 *Arsenobetaine Standard Solution*, SRM 3034 *Arsenocholine Standard Solution*, SRM 3036 *Arsenic Acid (AsV) Standard Solution*, SRM *3037*

Arsenous Acid (AsIII) Standard Solution, and SRM 3103a *Arsenic (As) Standard Solution* were used as calibrants. All SRMs were obtained from the National Institute of Standards and Technology (NIST). Locally prepared sub-boiling distilled water was used as a solvent in the preparation of samples, standards, and dilute acids.

4.2.2.1. Safety warning

Certain aspects of the sample preparation scheme required working with strong oxidizing acids under conditions of elevated temperature and pressure, which required the use of extracting fume hoods and personal protective equipment.

4.2.3. Instrumentation

An Agilent (Santa Clara, CA, USA) model 8800 triple quadrupole (QqQ) inductively coupled plasma-mass spectrometer (ICP-MS) was used for the determination of total arsenic in sample extracts. A Mettler model AT261 Delta range analytical balance was used in the gravimetric preparation of samples and standards. A CEM (Matthews, NC, USA) Multiwave 3000 microwave digestion system equipped with EasyPrep TFM microwave vessels was used for sample digestion. Advanced vortex mixer and Isotemp oven model 737 F from Fisher Scientific were used for sample extraction. Adams Dynac Centrifuge, Eppendorf centrifuge 5403, PolyScience Dual Action Shaker water-bath and SC-50TH Sonicator were used for sample preparation. A Perkin-Elmer (Shelton, CT, USA) LC system coupled to a Perkin-Elmer model Elan DRCII ICP-MS instrument operating in standard mode was used for arsenic speciation analysis. The LC system consisted of a Peltier-cooled Series 200 autosampler and a Series 200 quaternary pump with a 50 μL injection loop. A Hamilton PRP-X 100 (250 mm × 4.6

mm, 10 μm) anion exchange column (Reno, NV, USA) and a Macherey-Nagel Nucleosil 100-5 SA (250 mm ×4 mm, 5 μm) cation exchange column (Bethlehem, PA, USA) were used for the separation of arsenic species. The mobile phase composition and chromatographic method details are outlined in Table 6.

LC method conditions for As^{III}, MMA, DMA, AsB, AsC and AsSugar-OH separations.	
Columns	
Guard	CC 8/4 Nucleosil 100-5 SA (4 mm x 8 mm)
Analytical	Nucleosil 100-5 SA (250 mm x 4 mm, 5 μm)
Mobile phase	
A: 0.10 mmol/L pyridine + 2% CH_3OH in deionized H_2O, pH 6.2	
B: 30 mmol/L pyridine + 2% CH_3OH in deionized H_2O, pH 3.0	
Gradient program (1.0 mL/min)	
0 min to 4 min	100% A
4 min to 5 min	100% A linear gradient to 100% B
5 min to 20 min	100% B
LC method conditions for As^{V}, TMAO, AsSugar-PO_4, AsSugar-SO_3 and AsSugar-SO_4 separations	
Columns	
Guard	Guard column for PRP X-100 PEEK
Analytical	PRP X-100 PEEK (250 mm x 4.6 mm, 10 μm)
Mobile phase	
10 mmol/L $(NH_4)_2SO_4$ + 10 mmol/L NH_4NO_3, 2% CH_3OH in deionized H_2O, pH 10.0	
Isocratic program (1 mL/min)	
0 min to 50 min	100% A

Table 6: Mobile phase compositions and conditions for separation of arsenic species

4.3. Procedure

4.3.1. Determination of arsenic species

4.3.1.1. Extraction

One jar of geoduck sample was transferred from the -80°C freezer onto a bench in the laboratory for equilibration to room temperature (21°C). After 5 h of thawing and equilibration, approximately 0.5 g sample from the jar was weighed into 50 mL polypropylene centrifuge tubes. The remaining sample was stored in a freezer at -20°C. A 5 mL aliquot of H_2O was added to the sample and accurately massed. The tube was then vortexed at 2500 rpm for 1 min to facilitate thorough wetting of the sample. The sample was then placed in a waterbath maintained at 70°C with continuous shaking at 100 rpm for 4 h. The sample was cooled to ambient temperature before being centrifuged at 10,000 rpm for 20 min. The supernatant was decanted off into 15 mL polypropylene falcon tube. The decanted sample extract was passed through a 0.45μm nylon syringe filter into a 4 mL polypropylene tube that was capped thereafter. About 1 mL of the filtered sample was transferred into a 1.5 mL centrifuge tube and was centrifuged at 15,000 rpm for 5 min at 20°C. For LC-ICP-MS analysis, 0.3 mL of the supernatant was spiked with 0.3 mL of MMA (50 ng/g) internal standard in autosampler vial. The vial was then vortexed for 10 sec. The sample was then analyzed using HPLC-ICP-MS. A standard mixture containing the seven arsenic standards (As^{III}, As^{V}, AsB, AsC, MMA, DMA and TMAO) at 10 ng/g in water was freshly prepared gravimetrically on the day of analysis. Distilled water was used as a procedural blank.

Single point calibration of the seven arsenic standards was used for the quantification of arsenic species.

4.3.1.2. Measurement

For water extraction, measurements were made using LC-ICP-MS using both the Hamilton PRP X-100 anion exchange column and Nucleosil 100-5 SA cation exchange column depending on the arsenic species of interest. For cation exchange separation, 0.1 g of TMAO (0.1 µg/g) internal standard was used. Arsenic was measured at 75 amu in normal mode. Table 6 lists the parameters for the LC method.

4.3.1.3. Determination of moisture content in rice flour and geoduck clam

Since the certified mass fraction values for arsenic in SRM 1568b rice flour are expressed on dry-mass basis, moisture content was determined by weighing four portions of 0.5 g sample of SRM 1568b into weighing vessels. Similarly, six portions of 1 g sample of geoduck clam tissue were transferred into weighing vessels while frozen. The rice and geoduck samples were dried in the oven preheated to 90°C for 2 h and 3 h, respectively. The average of the difference of masses before and after drying was used to determine the moisture content of the samples.

4.3.2. Determination of total arsenic

4.3.2.1. Digestion

Samples were transferred from the -80 °C freezer into a Styrofoam box containing dry ice. Six replicate samples of approximately 1 g each were weighed into acid-cleaned

microwave vessels while frozen. Four SRM 1566b control samples each weighing approximately 0.5 g were transferred into acid-cleaned microwave vessels. Four procedure blanks were prepared in a similar way. The samples were digested with 8 mL of HNO_3, each vessel loosely capped, and left to react overnight in the fume hood at ambient temperature. The vessels were capped the next day after 1 mL of H_2O_2 was added. Microwave digestion was carried out using the following program: 1600 watts power, 25 min ramp and 15 min hold at 220°C. After microwave digestion and cooling, the digests were quantitatively transferred to 60 mL low density polyethylene (LDPE) bottles and diluted to 50 g with sub-boiling distilled water and weighed.

4.3.2.2. Addition of Internal Standard and Spiking Standard

Depending on the arsenic content in the diluted digest, the samples were either further diluted to appropriate analyte mass fraction or used directly for further preparation. A 5 g aliquot of the digest and 0.5 g of a solution containing 0.5 µg/g Rh as an internal standard were transferred to a 60 mL LDPE bottle, and the contents were diluted to 50 g with distilled water to constitute an unspiked sample. A spiked sample was prepared by weighing 25 g of the unspiked sample and 0.5 g of a standard containing 0.75 µg/g As into a 30 mL LDPE bottle. The unspiked and spiked samples, controls and blanks were quantified for arsenic on an ICP-MS by the method of standard addition.

4.3.2.3. Measurement

All measurements were made in tandem mass spectrometry (MS/MS) mode using the spectrum setting of the Agilent 8800 QQQ-ICP-MS. Arsenic was measured at 91 amu as $^{75}As^{16}O^{+}$ while the internal standard Rh was measured on-mass at 103 amu.

4.3.2.4. Determination of moisture content in Oyster tissue

Since the certified mass fraction value for arsenic in Oyster tissue is expressed on dry-mass basis, moisture content was determined by drying 0.5 g of the sample for 5 days over magnesium perchlorate ($Mg(ClO_4)_2$) in a desiccator at ambient temperature until a constant mass was achieved. The difference of the masses before and after drying was used to determine the moisture content of the sample as outlined in the Certificate of Analysis for SRM 1566b. The reported results were corrected for moisture content.

4.4. Results and discussion

4.4.1. Identification of arsenic species in geoduck and kelp

In this work, the identification of arsenic species in both geoduck clam and kelp extracts was achieved by retention time matching using NIST standard reference materials (SRMs), commercial standards, and purified extracts of kelp for AsSugars. We ran the individual standards under the same conditions and noted their retention times. We then mixed the standards and ran them together through the anion exchange column (see Fig. 24) and through cation exchange column (see Fig. 25) to see how well they separate from each other. The cationic species, i.e., AsB, AsC and TMAO co-eluted close to the solvent front, which made it necessary for the samples to be run on a cation exchange column to facilitate their separation.

The potential for overlap between AsB, DMA, and As^{III} with AsSugar-Gly, AsSugar-PO_4, and AsSugar-SO_3, respectively in an anion exchange separation was demonstrated by Gallagher et al.[513] In this study, good separation was observed between AsB and

AsSugar-Gly, and DMA and AsSugar-PO_4, respectively in geoduck. As^{III} was not present in either geoduck or kelp extracts.

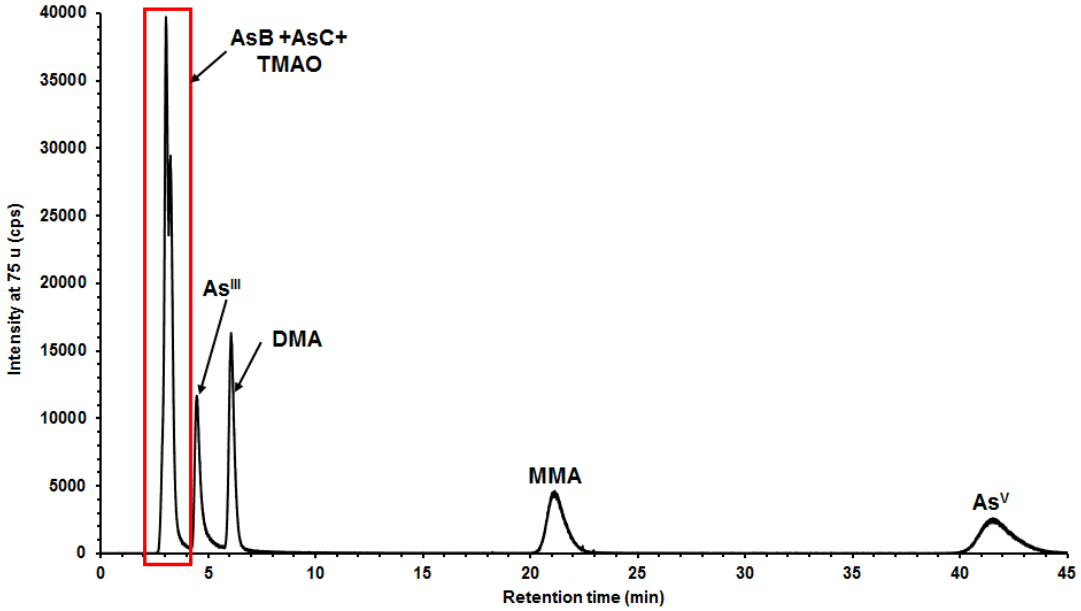


Figure 24: Anion exchange separation of seven arsenic standards showing coelution of species.

From the chromatographic separation, it might appear like the cation exchange would be the best technique for the separation of arsenic species in both geoduck and kelp. However, the most abundant species in both geoduck and kelp are AsSugars, which unfortunately have coelutions, for example, AsSugar-SO_3 and AsSugar-PO_4 coelutes with MMA.[663]

In addition, the run time for anion exchange separation is much longer than for cation exchange, which motivated our decision to run both methods, especially for the determination of As^V, AsSugar-Gly and AsB on the cation exchange column. The cation exchange separation also showed that there was no AsC and TMAO in the geoduck sample, thus the peak seen at the retention time of about 3 min is attributed to AsB.

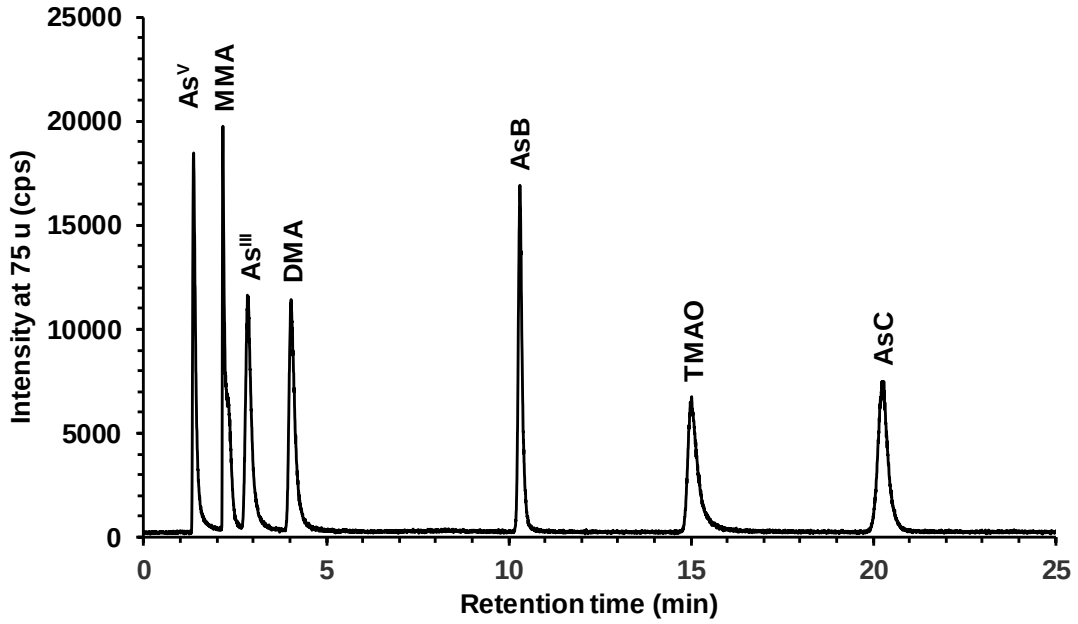


Figure 25: Cation exchange separation of seven arsenic species showing separation of all species.

The anion exchange separation of arsenic species for geoduck and kelp (see Fig. 26) shows good separation of arsenic species, especially the AsSugars. This separation method was adapted from previous work done by Yu et al.,[596] and the arsenic species separations observed in this study were in good agreement with what was previously observed. This is very important because arsenic species identification in this work was based on retention time matching and such agreement in separation profiles validated the identification of species, given that there are no well-characterized and commercially available standards for AsSugars. For further discussion, see section 4.4.3.

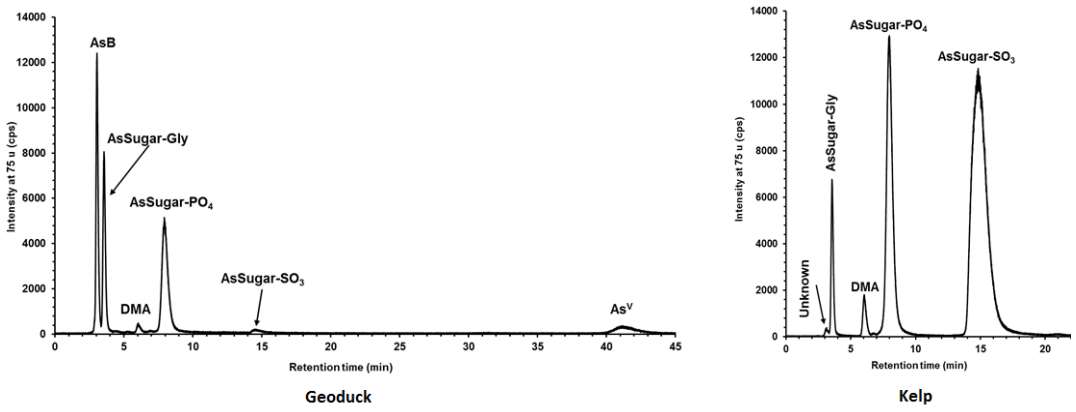


Figure 26: Anion exchange separation of arsenic species in geoduck clam and kelp

4.4.2. Determination of Arsenic species in geoduck and kelp

The determination of arsenic species in geoduck clam (*Panopea generosa*) and kelp (*Thallus laminariae*) involved liquid chromatography separation of the species followed by elemental detection using inductively coupled plasma mass spectrometry (LC-ICP-MS). MMA was used as an internal standard for quantitation, because it is the simplest organic arsenic species, has similar chemical properties to the analytes, and because it is not present in both geoduck and kelp samples. The sample extracts were therefore spiked with MMA prior to analysis. However, enhancement of MMA signal was observed with increasing methanol concentration where methanol/water mixture was used as an extractant (see Fig. 30). To account for this matrix effect, matrix-matched blanks and standards were used.

For determination of arsenic species, the mass fraction of analyte was determined by single point calibration using the formula:

$$x = \frac{I_{smp} \times [Std]}{I_{Std}} \times dil \qquad (1)$$

Where x is the mass fraction of the analyte in the sample; I_{smp} and I_{Std} are the corrected count rate of the sample and standards respectively; [Std] is the concentration of the standard; and *dil* is the overall dilution factor of the sample. The mass fraction of arsenic in the determination of total arsenic was calculated using the method of standard addition formula:

$$x = \frac{usp}{sp-usp} \times \frac{w_{sp}}{w_{smp}} \times C \times dil \quad (2)$$

Where x is the mass fraction of the analyte in the sample; *sp* and *usp* are the internal-standard corrected count rate of the spiked and the unspiked measurement samples; w_{smp} and w_{sp} are the mass of the sample and the mass of the spike solution; *C* and *dil* are the mass fraction of the analyte in the spike solution and the dilution factor of the sample respectively.

The relative proportions of arsenic species in geoduck and kelp are shown in Fig. 27. AsSugars are the predominant organoarsenicals in geoduck and kelp accounting for 34 % and 54 %, respectively of the hydrophilic arsenic species, which agrees well with findings reported in literature by Wolle et al.[459] Yu et al.,[147] and Gallagher et al.,[513] reported three AsSugars in kelp powder (*Thallus laminariae*) extract and ribbon kelp (*Alaria marginata*) extract, respectively. Lai et al.[410] and van Hulle et al.[471] reported four AsSugars in commercially available algal products of Nostoc sp. (*Nostoc commune* var. *flagelliforme*) and *Laminaria japonica* extracts, respectively.

For determination of total arsenic, kelp and geoduck samples were digested in the microwave following treatment with HNO_3 acid and H_2O_2 in order to convert all

arsenic species to iAs^V before measurement because ICP-MS response of arsenic depends on the speciation of the element.[664,146] Extraction was done at 220°C to ensure all the AsSugars present were converted to iAs^V and not DMA, which is reported to be the degradation product of AsSugars subjected to microwave digestion in oxidizing acidic conditions at temperatures below 207°C.[147,403,409,665]

Trivalent inorganic arsenic (iAs^{III}) was not detected in geoduck, while the pentavalent inorganic arsenic (iAs^V) was detected at trace levels. The low molecular weight organoarsenicals like MMA, AsC, TMAO were also not detected in geoduck while DMA was present at very low concentrations. AsB was present in geoduck but not present in kelp. Two AsSugars (AsSugar-Gly and AsSugar-PO_4) were present in geoduck, while in kelp three AsSugars were reported (AsSugar-Gly, AsSugar-PO_4 and AsSugar-SO_3). AsSugar-SO_4 was not detected in both geoduck and kelp.

Inorganic arsenic (iAs^V) and methylated arsenical (DMA), were both at 3 % and 1 % respectively, in both geoduck and kelp samples. The unextractable arsenic portion made a significant portion of both the geoduck and kelp samples standing at 47 % and 44 % respectively. This might be attributed to the fact that the arsenic species identified in this study were hydrophilic. Proximate analysis of geoduck and kelp showed that they have total fat between 1.5% and 3.5%, thus there is a slight possibility of arsenic being present in the lipophilic fraction as arsenolipids.

A residual, non-extractable fraction often remains following speciation analysis, and can contain a significant proportion of total arsenic in some samples.[123,125] The form of arsenic in this fraction remains unclear (see Fig. 27). Seaweed, like kelp, can contain

variable amounts of un-extractable or residual arsenic,[123,126–128] which is thought to be bound to thiol-containing structural compounds.[78] The level of residual arsenic in geoduck agrees with what has previously been reported about the frequently high levels (8–58%),[54,67] in bivalve mollusks. Some residual arsenic may be protein-bound, for which very little is known about their metabolic fate upon consumption.

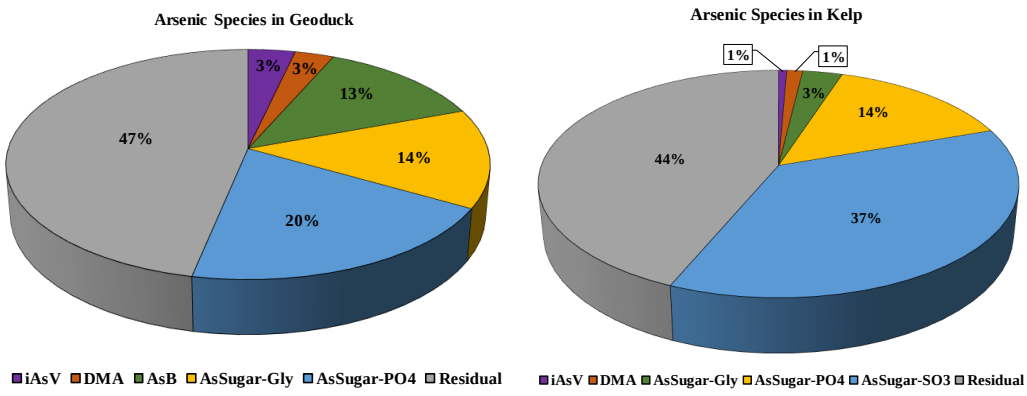


Figure 27: Relative proportions of arsenic species in geoduck and kelp

The un-extracted arsenic species, which may be attached to cell components or proteins,[127] require aggressive extraction conditions that would likely alter their native chemical forms.[130] Since the focus of analysis in this study was unambiguous identification of native arsenicals in the samples, preserving the stability of the species took precedence over quantitative extraction with aggressive conditions. In addition, we considered the effects of solvent polarity on arsenic speciation analysis.

4.4.3. Development of in-house standards

Since there are currently no well-characterized and commercially available AsSugar standards, arsenic species derived from purified extracts from kelp were used in this study. The complete details and experimental design (see Fig. 28) of how the purified

extracts of AsSugars were extracted, fractionated, concentrated, characterized, and quantified are published by Yu et al.[147] Briefly, kelp extract was separated and fractionated based on the arsenic species using liquid chromatography (LC). The fractions were then enriched by evaporation and their arsenic content were determined using instrumental neutron activation analysis (INAA), which resulted in value assignment for the three AsSugars in SRM 3232 without calibration standards.[596] The species in the fractions were identified using electrospray ion-trap time-of-flight mass spectrometry (ESI-IT-ToF-MS).[147] More information of arsenic speciation was obtained by measuring the arsenic species in the digest of kelp and kelp extracts using LC-ICP-MS.

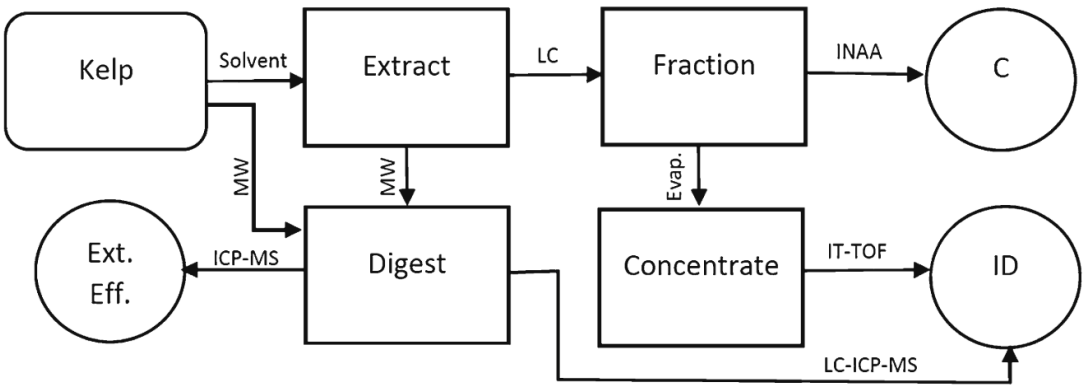


Figure 28: Experimental design for assessing the extraction efficiency of arsenic in kelp, identification of arsenic species, and quantification of arsenosugars in kelp extract. Source: Yu et al., 2015.[147]

4.4.4. Optimization of Extraction Method

4.4.4.1. Effect of solvent composition on extractable arsenic

There is no single method or solvent composition that can extract all arsenic species.[66,127] Geoduck has protein content of 14% – 17% and total fat of 2.8% - 3.7%,[666]

with chances of arsenic being trapped in matrix, therefore the composition of the extractant was systematically altered to evaluate the extraction efficiency. Water and methanol in different proportions have been reported in literature as extractants of choice.[127,667,552] Alberti et al. reported 50% methanol in water to give maximum efficiency.[515]

To obtain optimum extraction conditions using a methanol/water mixture, different compositions of methanol in water were studied. These experiments were conducted in quadruplicates to extract 0.5 g of geoduck with 5 mL of 0%, 25%, 50%, 75% and 100% methanol in water using a waterbath held at 70°C with constant shaking at 100 rpm for four hours.

Yoshinaga et al.[667] and Kuehnelt et al.[552] found that As^{V} was better extracted by water than by methanol/water, which is in agreement with our findings. However, they achieved better extraction yields for AsSugars using 90% and 50% methanol in water respectively, which did not hold true with our findings.

From the experiment it was observed that pure methanol gave the lowest extraction yield while pure water gave the best extraction, with the general trend of decreasing extraction yield with increasing methanol concentration. This is in agreement with what was reported by van Elteren et al.[127] Initially, the highest extraction was observed with solvent composition of 25% methanol in water, (see Fig. 29).

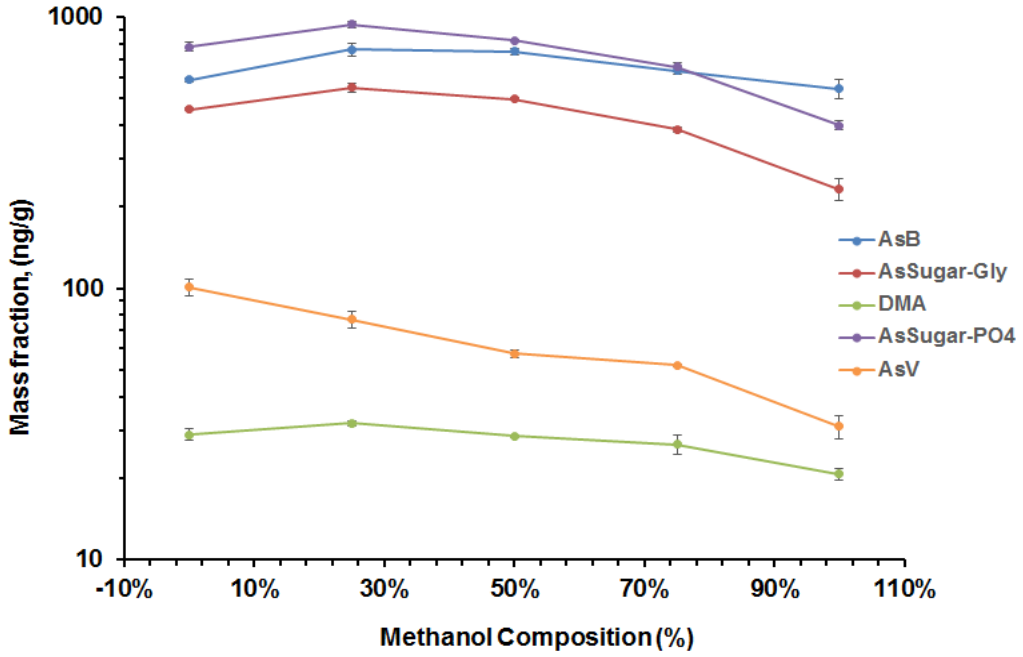


Figure 29: Effect of solvent composition (methanol/water) on extraction efficiency

However, signal enhancement for MMA, which was used as the internal standard, was observed. Therefore, using matrix-matched blanks and standards to account for this matrix effect, it was established that water was indeed the best extractant (see Fig. 30).

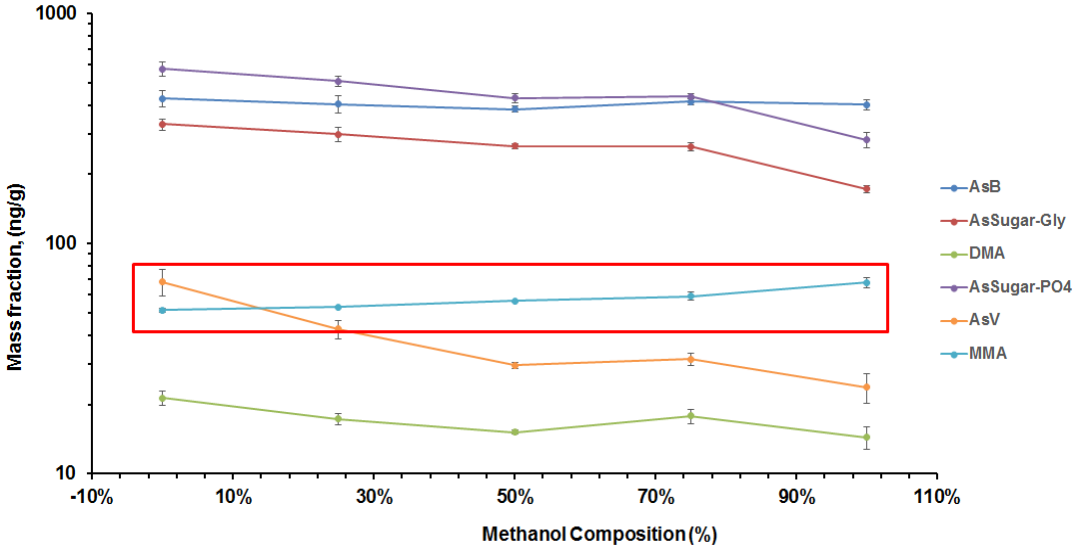


Figure 30: MMA signal enhancement with increase of methanol concentration in the extractant.

4.4.4.2. Effect of extraction methods on extraction efficiency

For comparison of extraction methods, 0.5 g of geoduck sample was extracted with 5 mL water in quadruplicate. Three extraction methods including waterbath, sonication and oven extraction were evaluated. The oven and waterbath were held at 70°C for 4 h, with continuous mechanical agitation at 100 rpm only for waterbath extraction. Unfortunately, the waterbath of the ultrasonic bath could not maintain the temperature of 70°C, therefore the experiment was performed at 30°C. The extraction methods were selected based on the benefits they offer. For example, ultrasonic extraction was selected based on the reported improvement of extraction efficiency, better yields at low temperature, shorter extraction time and low solvent amounts.[668,669] This is because of mass transfer enhancement, cell wall disruption, improved penetration and capillary effects.[670,671] The cavitation process that occurs during sonication causes the rupture of cell walls, consequently enhancing mass transfer and solvent contact with available extractable cell material.[541] However, these benefits were not evident in our experiment because geoduck samples were stored at -80°C, which aided in the disruption of the cell membrane.

The same extraction efficiency using ultrasonic assisted extraction was observed from 30 min to 6 h (see Fig. 31). This experiment demonstrated that extraction efficiency was neither dependent on the sonication process nor the extraction time using ultrasonic power. For oven extraction, the benefit of uniform heat transfer and mixing of sample by convectional current, which enables enhanced contact of sample and extractant was utilized.[542]

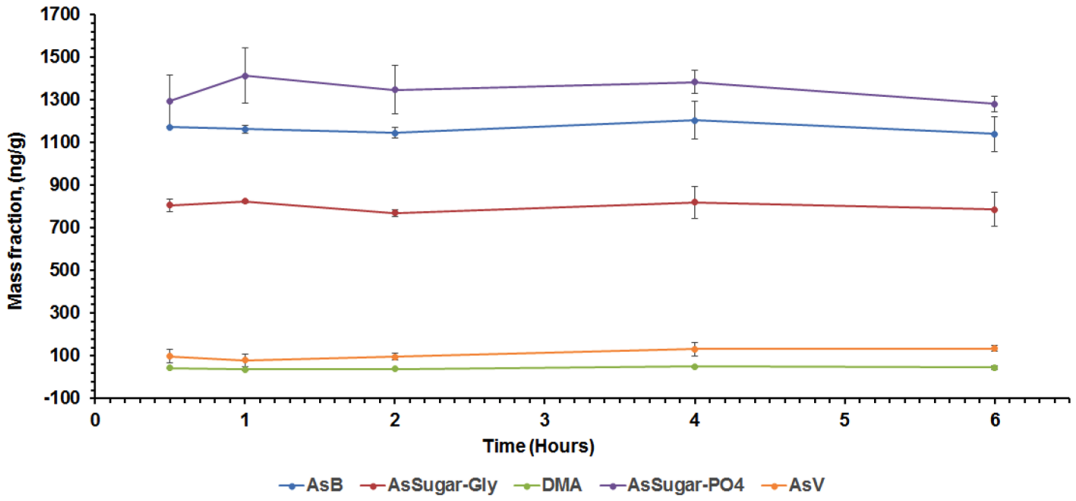


Figure 31: Effect of sonication and extraction time on the extraction efficiency.

Extraction efficiency of sonication was lower compared to the other extraction methods (see Fig. 32). Waterbath extraction gave comparable extraction efficiency to oven extraction, possibly because of better wetting of the sample facilitated by mechanical agitation, high temperatures and convectional current mixing that allowed better solvent contact.

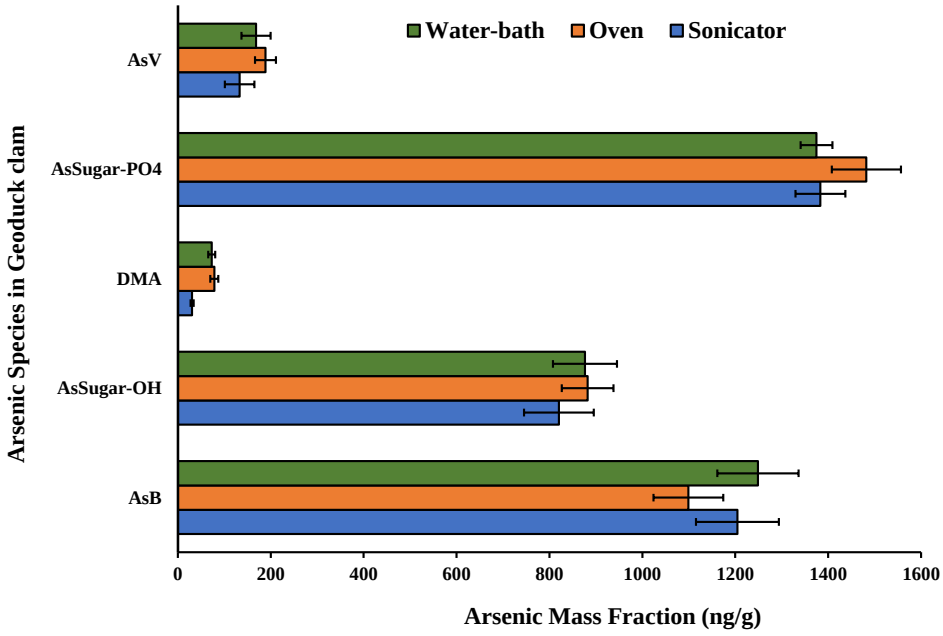


Figure 32: Effect of extraction method on the extraction efficiency.

4.4.4.3. Effect of temperature on extraction efficiency

The effect of temperature on extraction efficiency of arsenicals in geoduck was evaluate at 4°C, 20°C, 50°C, 70°C and 90°C. Experimental data showed no extraction of DMA and As^{V} at 4°C and 20°C (see Fig. 33). The same trend of diminished extraction efficiency was observed for AsB and the two AsSugars within the same temperature range. Maximum extraction is usually observed near the boiling point of the solvent.[12] Since methanol/water mixture and water were the extractants expected to be used with boiling points close to 70°C and 90°C, these temperatures were evaluated. The highest extraction for all arsenicals except for AsB was observed at 70°C. However, the extraction for AsSugars decreased when the temperatures approached 90°C, maybe because of their possible degradation at such high temperatures.

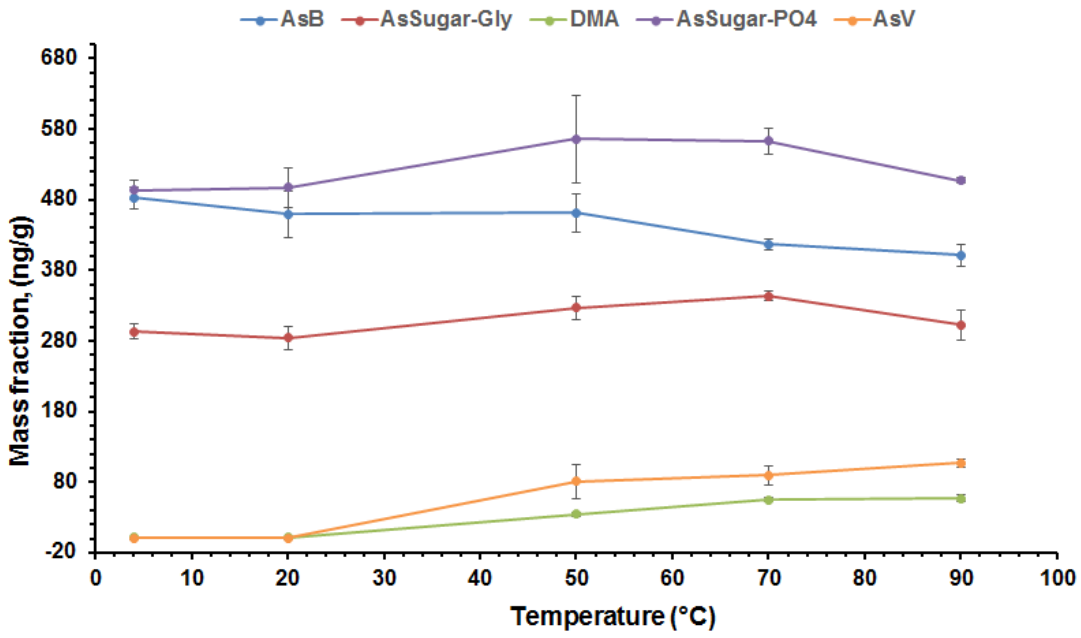


Figure 33: Effect of temperature on extraction efficiency

4.4.4.4. Effect of time on extraction efficiency

Initial assessment was performed on 0.5 g geoduck extracted with 5 mL water in a waterbath maintained at 70°C for 0.5 h, 2 h, 4 h, 6 h and 8 h. The general trend showed an increase in extraction efficiency with increase in extraction time, which seemed to level off at 4 h and further increased towards 8 h. Further evaluation was performed, and the highest extraction was observed at 8 h (see Fig. 34). However, for practical purposes and because two or more sequential extractions are performed on the samples, 4 h was selected as the extraction time for analysis.

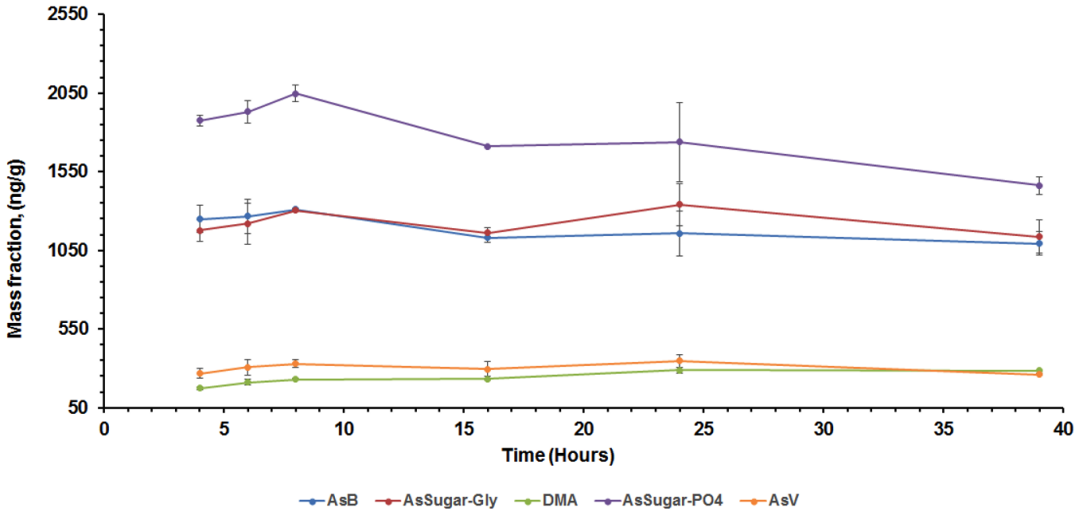


Figure 34: Effect of extraction time on extraction efficiency.

4.4.4.5. Effect of bead beating on extraction efficiency

To assess if arsenic was trapped within the cells of geoduck, bead beating was employed to physically rupture the cell walls and release the content for maximum contact with solvent. 0.5 g of geoduck was extracted with 5 mL water in quadruplicates at ambient temperature for 1 h. Three sets of samples were prepared as follows: beads

only, beads and geoduck, and geoduck only. All the samples were treated the same way. They were vortexed at 2500 rpm for 5 min in a randomized order before addition of water, which was followed by vortexing at 2500 rpm for 2 min.

The samples were allowed to soak for 1 h and then vortexed for 2 min. They were then centrifuged at 10,000 rpm for 20 min and the supernatant decanted off. The supernatant was transferred to a 1.5 mL centrifuge tube and further centrifuged at 15,000 rpm for 5 min before spiking (1:1) with 50 ng/g MMA and analysis on HPLC-ICP-MS. The results for the samples with and without bead beating extraction were comparable (see Fig. 35), demonstrating that bead beating did not improve the extraction efficiency probably because the ultra-low temperatures of -80°C in which the samples are stored caused the cell walls to rupture and made the contents accessible for extraction.

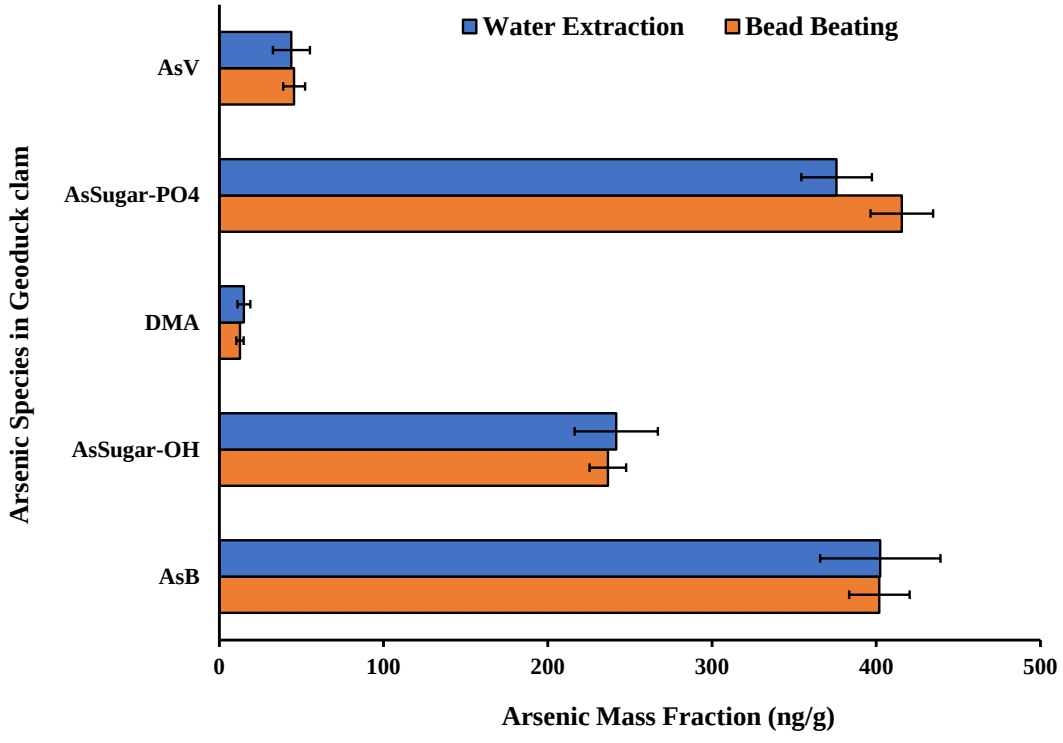


Figure 35: Effect of bead beating on extraction efficiency.

4.4.4.6. Effect of surfactant (SDS) on extraction efficiency.

Arsenicals have divergent ionic characteristics which are pH dependent. Therefore finding a single separation scheme capable of extracting all the arsenicals from the samples in a single run is difficult.[672] Especially, where the sample has arsenic in both the water-soluble and lipid fraction. To develop a single extraction method for analysis of water-soluble and lipid soluble arsenic, sodium dodecyl sulfate (SDS) a surface-active agent (surfactant) was employed. Concerns of surfactants not being amenable with mass spectrometry notwithstanding, the results from the study were inconclusive. For future work, proteolytic surfactants that are amenable with mass spectrometry will be investigated to determine their effect on the extraction efficiency.

4.5. Risk assessment associated with consumption of geoduck and kelp

The mass-balance approach, which was suggested by Feldmann et al.[93] was used to determine the toxic fraction represented by inorganic arsenic, non-toxic fraction represented by AsB, and the potentially toxic fraction, which is the difference between the total arsenic content and the sum of the inorganic and AsB fraction. This involved the determination of the total arsenic content using ICP-MS after mineralization, followed by the determination of iAs as iAs^{V} using ICP-MS after treatment of sample with HNO_3 and H_2O_2 that converts any iAs^{III} in the sample into iAs^{V}, and finally the determination of AsB content using LC-ICP-MS. DMA and all the residual arsenic accounted for the potentially toxic fraction. Determination of arsenic species was semi-quantitative since arsenic is monoisotopic and has no labelled standards available. The schematic representation of this approach has been presented (refer to Fig. 5).

4.6. Conclusion

In this chapter we have utilized the pH-dependent difference in the ionic states of arsenic species to chromatographically separate them using both anion exchange and cation exchange techniques. These two techniques are orthogonal and complementary. They give a better outlook of the ionic hydrophilic arsenic species in seafood. Geoduck was selected as study material due to its great variety of arsenic species. Arsenic species identification was based on retention time matching using commercial standards, SRMs, and purified extracts of AsSugars as calibrants.

We had to modify the acid digestion process so as not to underestimate the total arsenic due to the high content of AsSugars in both geoduck and kelp. This is because at temperatures below 207℃, the AsSugars are converted to DMA, and since ICP-MS responds to the species present and in our case, we were using iAs^{V} as the measurement standard. Incomplete conversion of AsSugars at lower temperatures would have biased our results. There is a substantial amount of arsenic that is unaccounted for and whose identity is currently unknown. There is a possibility that this might be in the form of lipophilic arsenicals and residual arsenic, which must be determined.

Since various factors affect the extraction efficiency of arsenicals from the sample matrix, the following parameters that may affect the extraction efficiency were studied: effect of solvent composition, extraction methods, extraction time, temperature, bead-beating, and surfactant. These studies were important to the optimization of the analytical method for the determination of hydrophilic arsenicals in geoduck (a seafood), which will be deployed in the study of the other seafood samples. Details of

the application of the optimized method in the identification, characterization and quantification of total arsenic and hydrophilic arsenic species will be reported in Chapter 5. This method was also applied in the characterization and quantification of total and arsenic species in seafood (wild-caught and aquacultured shrimps and salmons), that were used in the NIST Health Assessment Measurements Quality Assurance Program (HAMQAP): Exercise 5 of 2020.[673] Final report is available at https://doi.org/10.6028/NIST.IR.8343.

Lack of reliable information on the organoarsenical species in seafood has been the crucial missing link to understanding exposure and informing regulatory practices. Access to matrix-based reference materials for validation of measurement protocols will generate accurate and reliable speciation data, while addressing the challenge of lack of widely accepted analytical methods. This will lead to more informative epidemiological studies on toxicity and exposure assessment for organoarsenicals in seafood that will inform regulatory practices. Knowledge garnered from this research will contribute to shaping future research on toxicity and inform the development of standards and new reference materials, especially for food, biological and environmental sample.

Chapter 5: Determination of Total Arsenic and Hydrophilic Arsenic Species in Seafood.

Work discussed in this chapter is published in the Journal of Food Composition and Analysis. *J. Food Comp. Anal.* ***2021****, 96, (103729), 1-11.*

5.1. Introduction

Seafood encompass a heterogenous group of aquatic organisms not only from the marine environment but also freshwater, including shellfish like mollusks and crustaceans, and all types of finfish.[149] Even though there is consistent evidence of health benefits from modest seafood consumption, there exist possible risks and adverse effects associated with seafood, especially arsenic that is inherently present as a contaminant, particularly organic arsenic species.[150] This has raised concerns regarding the consumption of aquatic foods.

Seafood and seaweed are the major dietary sources of total arsenic in humans, with organic arsenic being the dominant species.[47] However, exceptions have been reported with elevated levels of iAs, for example, in edible seaweed Hijiki (*Hizikia fusiformis*),[273] freshwater fish from Thailand,[274] and blue mussel from Norway.[55] Marine algae and shellfish are the seafood exposure sources with the greatest diversity of arsenicals.[31]

Arsenic toxicity, bioaccumulation and mobility are largely dependent on the chemical form and the extent of methylation.[39] Typically, the lower the oxidation number, the higher the toxicity and the higher the methylation, the lower the toxicity. iAs species are categorized as non-threshold Class 1 carcinogens, while simple methylated

arsenicals such as MMA and DMA are Class 2B having intermediary toxicity.[10] AsB, a tetraalkylated compound present in fish and the principal dietary source of arsenic exposure for humans, is nontoxic because it is practically eliminated unchanged in the urine by humans.[20] Studies suggest that AsSugars exhibit no acute cytotoxicity or mutagenicity, even though these compounds may be metabolized within the human body to form potentially toxic metabolites like DMA.[40]

Microalgae are the primary iAs accumulators in the marine environment and represent an important stage in arsenic migration through the food chain.[47] It is generally understood that iAs, which is mainly absorbed in the form of As^V from seawater, is metabolized by the algae to a variety of organic arsenic species, especially AsSugars.[48] Elevated levels of AsSugars (12 µg/g to 84 µg/g dry weight) have been detected in seaweed leading to considerable interest with regard to their toxicological behavior.[419] Human exposure to AsSugars is relatively high in Asia based on their diet that is rich in seaweed.[453] Fortunately, there is no indication for acute and chronic toxicity related to seaweed ingestion from epidemiological studies.[40,238]

Despite the nutritional benefits associated with spirulina, they have the capacity to bioaccumulate not only essential elements but also toxic elements such as arsenic.[674] Since these algal products are frequently consumed in high quantities and over an extended period of time by humans as dietary supplements, this increases the consumer's risk of critical exposure to toxic arsenic and therefore potentially serious health effects.[675] Consequently, it is imperative that arsenic present in spirulina and the dietary supplements derived from them are quantified as a preliminary step to evaluating their toxicological relevance.[674]

The toxicity mechanism of iAs and methylated arsenicals (MMA and DMA) is well established and understood; however, the same does not hold true for the other organoarsenicals like AsSugars. It is therefore critical from a risk-based perspective to determine the arsenic species present in the various aquatic foods and their relative proportions consumed by humans to enable more accurate toxicological assessments.[255] This will provide key data that is necessary for establishing health advisories to limit consumption of particular types of seafood and seaweed.

The aim of this study was to survey the total arsenic and arsenic species in the food chain of common aquatic species, to contribute to the growing body of knowledge on arsenic speciation. This information is of toxicological relevance to the establishment of arsenic exposure metrics like provisional tolerable weekly intake (PTWI) and allowable dietary intake (ADI), which are paramount in the setting of appropriate regulatory limits. In this study we also assessed the stability of arsenic species to establish their integrity during extraction using typical measurement procedures to contribute to the current understanding of the extraction conditions.

In this study, we surveyed the total arsenic content and relative proportions of inorganic arsenic and hydrophilic organic arsenic species in different seafood and seaweed samples cutting across the marine food chain from microalgae represented by spirulina powder (*Spirulina platensis*), macroalgae represented by kelp powder (*Ascophyllum nodosum*), shellfish represented by geoduck clam (*Panopea generosa*), wild caught shrimp (*Farfantepenaeus aztecus*) and aquacultured shrimp (*Litopenaeus vannamei*), and finally finfish represented by wild-caught and aquacultured Coho salmon (*Oncorhynchus kisutch*). We evaluated the extraction methods for the arsenic species

that give the maximum extraction efficiency without species interconversion after assessing the stability of the arsenicals under the extraction conditions (see Fig. 36).

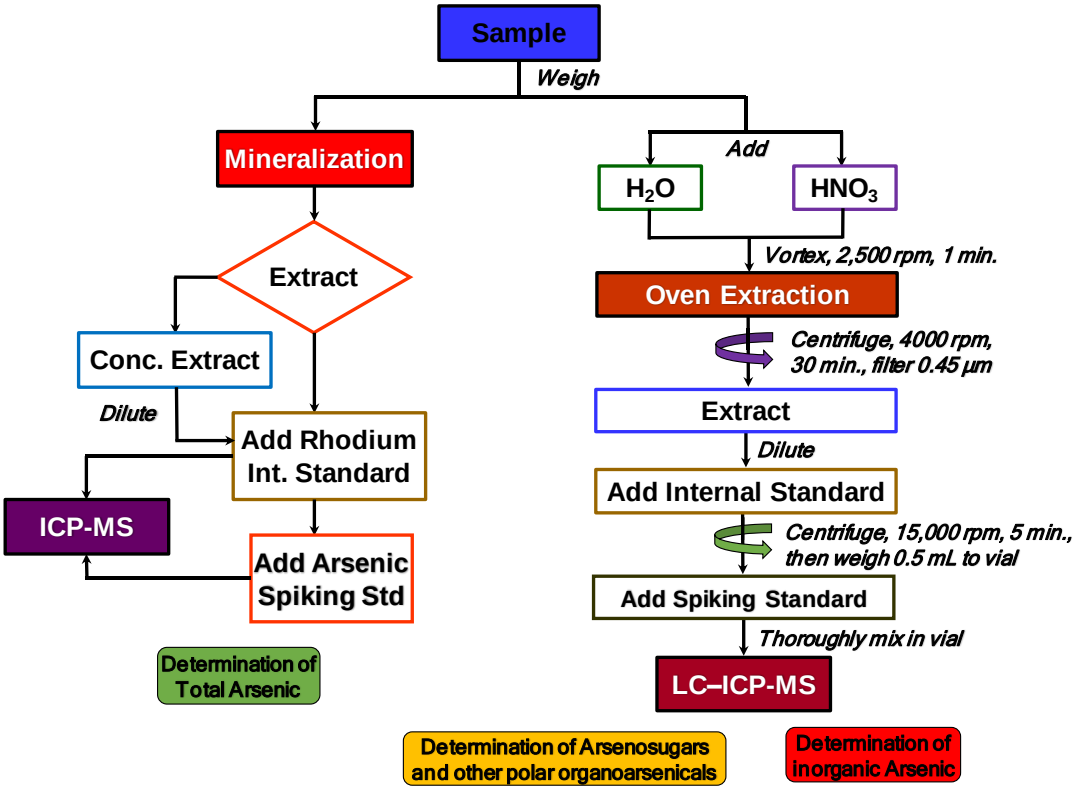


Figure 36: Analytical procedure for the determination of total arsenic using ICP-MS and hydrophilic arsenicals using anion exchange (AE) and cation exchange (CE) LC-ICP-MS in seafood.

5.1. Experimental

5.1.1. Sample Collection and Preparation

Spirulina (*Spirulina platensis*) purchased from Herb Store USA and Atlantic kelp powder (*Ascophyllum nodosum*) purchased from Essential wholesale and labs were homogenized, filtered, and packaged into packets. Each packet contained approximately 5 g of the powder sample sealed in a nitrogen flushed plastic bag, which was sealed inside a nitrogen flushed opaque air-tight aluminized mylar with two

packets of silica gel to protect the material from moisture and light. To prevent mold growth during long-term storage, the packets of spirulina and kelp powder were irradiated to an absorbed dose of 5.9 kGy to 7.6 kGy and then stored in corrugated boxes at controlled ambient temperature (20 °C ± 2 °C).

Geoduck clam (*Panopea generosa*) harvested from Vegas Hot Spur in Alaska was cryogenically homogenized. Approximately 12 g portions of the homogenized geoduck clam tissue were packaged in pre-cleaned glass jars with polytetrafluoroethylene (PTFE)-lined caps for storage at -80 °C until use. Wild-caught brown shrimp (*Farfantepenaeus aztecus*) were trawl-caught off the coast of Charleston, South Carolina while aquacultured white leg shrimp (*Litopenaeus vannamei*) were obtained from Boligee, Alabama. The shrimp were deheaded, peeled and chopped before they were cryogenically homogenized at the Hollings Marine Laboratory (HML). The effectiveness of the cryomilling procedure was monitored using Laser diffraction particle size analysis before 6 g to 8 g portions of the fresh frozen powder was bottled in pre-cleaned glass jars with PTFE-lined caps for storage at -80 °C until use. Both the wild-caught and aquacultured Coho Salmon (*Oncorhynchus kisutch*) were procured from reliable sources and were verified by the Marine Forensics division of the National Oceanic and Atmospheric Administration (NOAA). The materials were processed to include only the edible portions. The Coho Salmon were deheaded, scaled, fileted and chopped before they were cryogenically homogenized at the NIST Reference Material Production Facility in Charleston, South Carolina. The effectiveness of the cryomilling process was assessed for both the wild-caught and aquacultured material using Laser-diffraction particle size analysis to ensure the

material was homogeneous based on the particle size. Thereafter, 6 g to 8 g portions of the fresh frozen powder was bottled in pre-cleaned glass jars with PTFE-lined caps for storage at -80 °C until use.

5.1.2. Reagents and standards

ACS grade nitric acid (HNO_3, 69% - 70%) was from J.T. Baker (Phillipsburg, NJ, USA). ACS HPLC grade methanol was from Honeywell Burdick Jackson (Muskegon, MI, USA). Puratronic grade ammonium carbonate ($(NH_4)_2CO_3$, 99.999%) was purchased from ThermoFisher Scientific (Ward Hill, MA, USA). ACS grade pyridine (99.9%), Acetonitrile (99.98%), laboratory grade formic acid (88%), and ACS grade hydrogen peroxide (H_2O_2, 30%) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Trimethylarsine oxide (TMAO) was purchased from Wako Chemicals (Richmond, VA, USA). SRM 1566b *Oyster Tissue (Crassostrea virginica)*, SRM 1568b *Rice Flour*, SRM 1947 *Lake Michigan Fish Tissue*, SRM 3232 *Kelp Powder (Thallus laminariae)*, and SRM 3669 *Arsenic Species in Frozen Human Urine* were used as quality control materials. SRM 3030 *Monomethylarsonic Acid Standard Solution*, and SRM 3144 *Rhodium (Rh) Standard Solution* were used as internal standards. SRM 3031 *Dimethylarsinic Acid Standard Solution*, SRM 3033 *Arsenobetaine Standard Solution*, SRM 3034 *Arsenocholine Standard Solution*, SRM 3036 *Arsenic Acid (AsV) Standard Solution*, SRM 3037 *Arsenous Acid (AsIII) Standard Solution*, and SRM 3103a *Arsenic (As) Standard Solution* were used as calibrants. All SRMs were obtained from the National Institute of Standards and Technology (NIST). Locally prepared sub-boiling distilled water was used as a solvent in the preparation of samples, standards, and dilute acids.

5.1.2.1. Safety warning

Certain aspects of the sample preparation scheme required working with human urine, as well as strong oxidizing acids under conditions of elevated temperature and pressure, all of which required the use of extracting fume hoods, personal protective equipment, and adherence to biohazard handling procedures.

5.1.3. Instrumentation

An Agilent (Santa Clara, CA, USA) model 8800 triple quadrupole (QQQ) inductively coupled plasma mass spectrometry (ICP-MS) was used for the determination of total arsenic in sample extracts. A Mettler model AT261 Delta range analytical balance was used in the gravimetric preparation of samples and standards. A CEM (Matthews, NC, USA) Multiwave 3000 microwave digestion system equipped with EasyPrep TFM microwave vessels was used for sample digestion. Advanced vortex mixer and Isotemp oven model 737F from Fisher Scientific were used for sample extraction. A Perkin-Elmer (Shelton, CT, USA) LC system coupled to a Perkin-Elmer model Elan DRCII ICP-MS instrument operating in standard mode was used for arsenic speciation analysis. The LC system consisted of a Peltier-cooled Series 200 autosampler and a Series 200 quaternary pump with a 50 µL injection loop. A Hamilton PRP-X 100 (250 mm x 4.6 mm, 10 µm) anion exchange column (Reno, NV, USA) and a Macherey-Nagel Nucleosil 100-5 SA (250 mm x 4 mm, 5 µm) cation exchange column (Bethlehem, PA, USA) were used for the separation of arsenic species. The mobile phase composition and chromatographic method details are outlined in Table 7.

LC method conditions for As^{III}, MMA, DMA, AsB, AsC and AsSugar-OH separations.	
Columns	
Guard	CC 8/4 Nucleosil 100-5 SA (4 mm x 8 mm)
Analytical	Nucleosil 100-5 SA (250 mm x 4 mm, 5 µm)
Mobile phase	
A: 0.10 mmol/L pyridine + 2% CH_3OH in deionized H_2O, pH 6.2	
B: 30 mmol/L pyridine + 2% CH_3OH in deionized H_2O, pH 3.0	
Gradient program (1.0 mL/min)	
0 min to 4 min	100% A
4 min to 5 min	100% A linear gradient to 100% B
5 min to 20 min	100% B
LC method conditions for As^V, TMAO, AsSugar-PO_4, AsSugar-SO_3 and AsSugar-SO_4 separations	
Columns	
Guard	Guard column for PRP X-100 PEEK
Analytical	PRP X-100 PEEK (250 mm x 4.6 mm, 10 µm)
Mobile phase	
A: 20 mmol/L $(NH_4)_2CO_3$ + 2% CH_3OH in deionized H_2O, pH 10.0	
Isocratic program (1 mL/min)	
0 min to 50 min	100% A

Table 7: Mobile phase compositions and conditions for separation of arsenic species.

5.2. Procedure

The experimental design of this work is shown in Fig. 37. For the determination of total As, mineralization was achieved by microwave-assisted acid decomposition to obtain digests that were spiked with 0.5 µg/g Rh gravimetrically prepared from SRM 3144 *Rhodium (Rh) Standard Solution* as the internal standard. The digest containing the internal standard was split into two approximately equal aliquots. One of the aliquots was spiked with 0.75 µg/g As^V gravimetrically prepared from SRM 3103a *Arsenic (As)*

Standard Solution for the purpose of calibration by the method of standard addition. For As speciation analysis, two methods were employed, i.e. acid extraction of iAs that is converted and measured as As^{V} based on European Standard EN 16802:2016, and water extraction method. For a summary of the analytical procedure (see Fig. 36).

5.2.1. Determination of Total Arsenic

5.2.1.1. Digestion

Samples were transferred from the -80 °C freezer into a Styrofoam box containing dry ice. Five replicate samples of approximately 0.5 g to 1 g each were weighed into acid-cleaned microwave vessels while frozen. Four SRM 1566b control samples each weighing approximately 0.5 g were transferred into acid-cleaned microwave vessels. In a similar way, four SRM 1947 control samples each weighing approximately 1 g were transferred into acid-cleaned microwave vessels while frozen. Four procedure blanks were prepared in a similar way.

The samples were digested with 8 mL of HNO_3, each vessel loosely capped, and left to react overnight in the fumehood at ambient temperature. The vessels were capped the next day after 1 mL of H_2O_2 was added. Microwave digestion was carried out using the following program: 1600 watts power, 25 min ramp and 15 min hold at 220°C. After microwave digestion and cooling, the digests were quantitatively transferred to 60 mL low density polyethylene (LDPE) bottles and diluted to 50 g with sub-boiling distilled water and weighed.

5.2.1.2. Addition of Internal Standard and Spiking Standard

Depending on the As content in the diluted digest, samples were either further diluted to appropriate analyte mass fraction or used directly for further preparation. A 5 g aliquot of the digest and 0.5 g of a solution containing 0.5 µg/g Rh as an internal standard were transferred to a 60 mL LDPE bottle, and the contents were diluted to 50 g with distilled water to constitute an unspiked sample. A spiked sample was prepared by weighing 25 g of the unspiked sample and 0.5 g of a standard containing 0.75 µg/g As into a 30 mL LDPE bottle. The unspiked and spiked samples, controls and blanks were quantified for As on an ICP-MS by the method of standard addition.

5.2.1.3. Measurement

All measurements were made in tandem mass spectrometry (MS/MS) mode using the spectrum setting of the Agilent 8800 QQQ-ICP-MS. Arsenic was measured at 91 amu as $^{75}As^{16}O^{+}$ while the internal standard Rh was measured on-mass at 103 amu.

5.1.1. Determination of moisture content in Oyster tissue

Since the certified mass fraction value for arsenic in Oyster tissue is expressed in dry-mass basis, moisture content was determined by drying 0.5 g of the sample for 5 days over magnesium perchlorate ($Mg(ClO_4)_2$) in a desiccator at ambient temperature until constant mass was achieved. The difference of the masses before and after drying was used to determine the moisture content of the sample as outlined in the Certificate of Analysis of SRM 1566b. The results reported below were corrected for moisture content.

5.1.1. Arsenic speciation analysis

5.1.1.1. Extraction

Samples were transferred from the -80 °C freezer into a Styrofoam box containing dry ice. Approximately 0.5 g to 1 g sample was weighed into a 50 mL Falcon tube while frozen. For acid digestion method, four 1 g portions of SRM 1568b samples as controls were weighed into four 50 mL Falcon tubes. A 5 mL solution containing 0.2 mol/L HNO_3 and 6% by volume H_2O_2 in water was added to each tube, and water was used to bring the overall added solution to 10 g.

For the water extraction method, four 0.5 g portions of SRM 3232 Kelp Powder samples were weighed into four 50 mL Falcon tubes. Four 1 g portions of SRM 1568b as controls were prepared similarly. Water was added to bring the overall added solution to 10 g. Four procedural blanks were prepared similarly.

The samples and the blanks were tightly capped and vortexed at 2500 rpm for 1 min then the caps were loosened and transferred to the oven preheated to 95 °C. The samples and blanks were removed from the oven after 3 h. After the temperature was equilibrated with the room temperature at 21 °C, the samples were centrifuged at 4000 rpm in a centrifuge for 30 min. The supernatant was transferred to 15 mL Falcon tubes.

5.1.1.2. Addition of Internal Standard and Spiking Standard

The decanted extracts were passed through 0.45 µm Nylon syringe filters and an unspiked sample was prepared by weighing the filtrate into a 4 mL polypropylene tube containing 0.1 g of 1 µg/g MMA serving as the internal standard. A spiked sample for

acid extraction method was prepared by weighing 0.5 g of the unspiked sample into a 0.75 mL polypropylene autosampler vial containing 0.1 g of 0.08 µg/g As^{V} for the purpose of calibration by the method of standard addition. In the water extraction method, a spiked sample was prepared by weighing 0.5 g of the unspiked sample into a 0.75 mL polypropylene autosampler vial containing 0.1 g of 0.08 µg/g As^{V}, 0.08 µg/g As^{III} and 0.11 µg/g DMA for the purpose of quantification.

5.1.2. Determination of AsSugars

AsSugars were separately determined from the water extracts by external calibration method using locally purified and characterized AsSugar standards that were gravimetrically prepared. MMA was employed as an internal standard for quantification while SRM 3232 was used as a control.

5.1.3. Measurement

For acid digestion, measurements were made using LC-ICP-MS with Hamilton PRP X-100 anion exchange column while those for water extraction were made using LC-ICP-MS with both the Hamilton PRP X-100 anion exchange column and Nucleosil 100-5 SA cation exchange column depending on the arsenic species of interest. For cation exchange separation, a different suite of internal standard and spiking standard was used i.e. 0.1 g of 0.1 µg/g TMAO internal standard and 0.1 µg/g AsB as spiking standard. Arsenic was measured at 75 amu in normal mode. Table 7 lists the parameters for the LC method.

5.1.4. Determination of moisture content in rice flour and geoduck tissue

Since the certified mass fraction value for arsenic in SRM 1568b rice flour is expressed on dry-mass basis, moisture content was determined by weighing four portions of 0.5 g sample of SRM 1568b into weighing vessels. Similarly, six portions of 1 g sample of SRM 2983 geoduck clam tissue were transferred into weighing vessels while frozen. The rice and geoduck samples were dried in the oven preheated to 90 °C for 2 h and 3 h, respectively. The difference of the masses before and after drying was used to determine the moisture content of the samples.

5.1.Results and discussion

5.1.1. Arsenic species stability

For the arsenic species stability study, the native standards with concentrations of about 200 ng/g of AsB, AsC, MMA, DMA, As^{III}, As^{V}, TMAO, AsSugar-OH, AsSugar-SO_3 and AsSugar-PO_4 were individually prepared by the dilution of the respective NIST SRMs, commercial TMAO standard, and well-characterized AsSugars purified extracts.[147] The diluted standards were then subjected to water and acid extraction conditions outlined in Fig. 36 to study the effects of the extraction procedures on the arsenic species integrity. The native standards and the water and acid extracts were then analyzed using both anion exchange (AE) and cation exchange (CE) LC-ICP-MS. The peak areas of the water and acid extracts as a percentage of the original native standards were calculated and were used to determine the arsenic species' stability.

The results revealed that AsB, AsC, MMA, DMA, TMAO and As^{V} were not affected by either the water or acid extraction procedures under the study conditions. However, there was marked influence of acid extraction conditions on the integrity of As^{III} and all the AsSugars species (Fig. 37). The AsSugars standards were fractionated from kelp extract and were not entirely pure. The procedure employed to determine the exact concentration of the individual AsSugars in the fractions using INAA and LC-ICP-MS is described.[147] All the AsSugars remained stable under water extraction conditions.

The observation showed that AsSugars underwent acidic hydrolysis at elevated temperature that cleaved off the aglycone moieties at C1 to yield AsSugar 254 (DMAsSugarHydroxy), an aglycone free AsSugar, which confirms what was reported in literature by Gamble et al.[405] Initially when the acid extracts were run through the anion exchange column, it showed that all the AsSugars were converted to AsSugar-OH, which elutes close to the solvent front of the AE column.

However, when the same acid extracts were run through the CE column it revealed the presence of another arsenic species eluting at a different retention time from that of AsSugar-OH. Based on what is reported in literature,[405] the peak was postulated to be that of AsSugar 254, which elutes close to the solvent front of the anion exchange column in likeness with AsSugar-OH. The acid extracts and the AsSugar-OH standard were thus separated using cation exchange chromatography to confirm their identities.

At the experimental conditions with mobile phase pH of 6.2, both AsSugar-OH and AsSugar 254 are neutral because they do not dissociate and thus lack the pH dependent aglycone functionality that influence their retention in the AE column. Therefore, they

both are unretained and are eluted close to the solvent front of the column and cannot be effectively separated by AE chromatography.

Purified extract of AsSugar-OH had unknown arsenic species present in its native state, which accounted for about 2% of the total arsenicals. The unknown arsenic species content did not significantly change following water extraction. However, after acid digestion, AsSugar-OH disappeared and a new species probably AsSugar 254 appeared at a lower level than the initial AsSugar-OH.

The amount of unknown arsenic species increased significantly to about 20% of the total arsenic content in the sample extract as shown in Fig. 37A. There was also a slight increase in the amount of DMA in the acid extract of AsSugar-OH. A similar trend was observed in AsSugar-SO_3 and AsSugar-PO_4 shown in Fig. 37B and 37C, respectively, even though they had much lower levels (<1%) of initial unknown arsenic species. There was no statistical difference between the amounts of the other AsSugars that were present as impurities in both the native form and in the water extracts of the AsSugar purified extracts that were studied in these experiments.

DMA was found stable under the acidic condition; however, a possibility cannot be eliminated of DMA being a product of the decomposition of other arsenic species. In this work, the amount of DMA increased with acid extraction of purified AsSugars extracts but was not observed with water extraction where the amount remained unchanged after the extraction as shown in Fig. 37.

The acid extraction method additionally converted all the three AsSugars to yield about 20% of unknown arsenic species on the account of the peak area of the unknowns. The

mechanism that governs the decomposition of AsSugars to DMA under acidic experimental conditions at elevated temperatures is not well understood. AsSugars, with a dimethylarsinoyl group attached to a ribofuranoside, are trialkylated arsenicals analogous to TMAO that is susceptible to nucleophilic attack.[676]

Rabb et. al.[676] in their study demonstrated the stepwise demethylation of TMAO under acidic conditions using concentrated HCl at elevated temperatures to yield lower methylated arsenicals such as DMA and MMA, and eventually As^{V}. They observed that it was not possible to drive this reaction to completion with the HNO_3/H_2O_2 mixture, and they postulated Cl^- to be critical for the demethylation reaction.[676] In addition, results from gas-phase reaction of AsSugars under oxidizing acidic conditions produce compounds detected using mass spectrometry at *m/z* 237 resulting from the loss of the aglycone group at C1.[451]

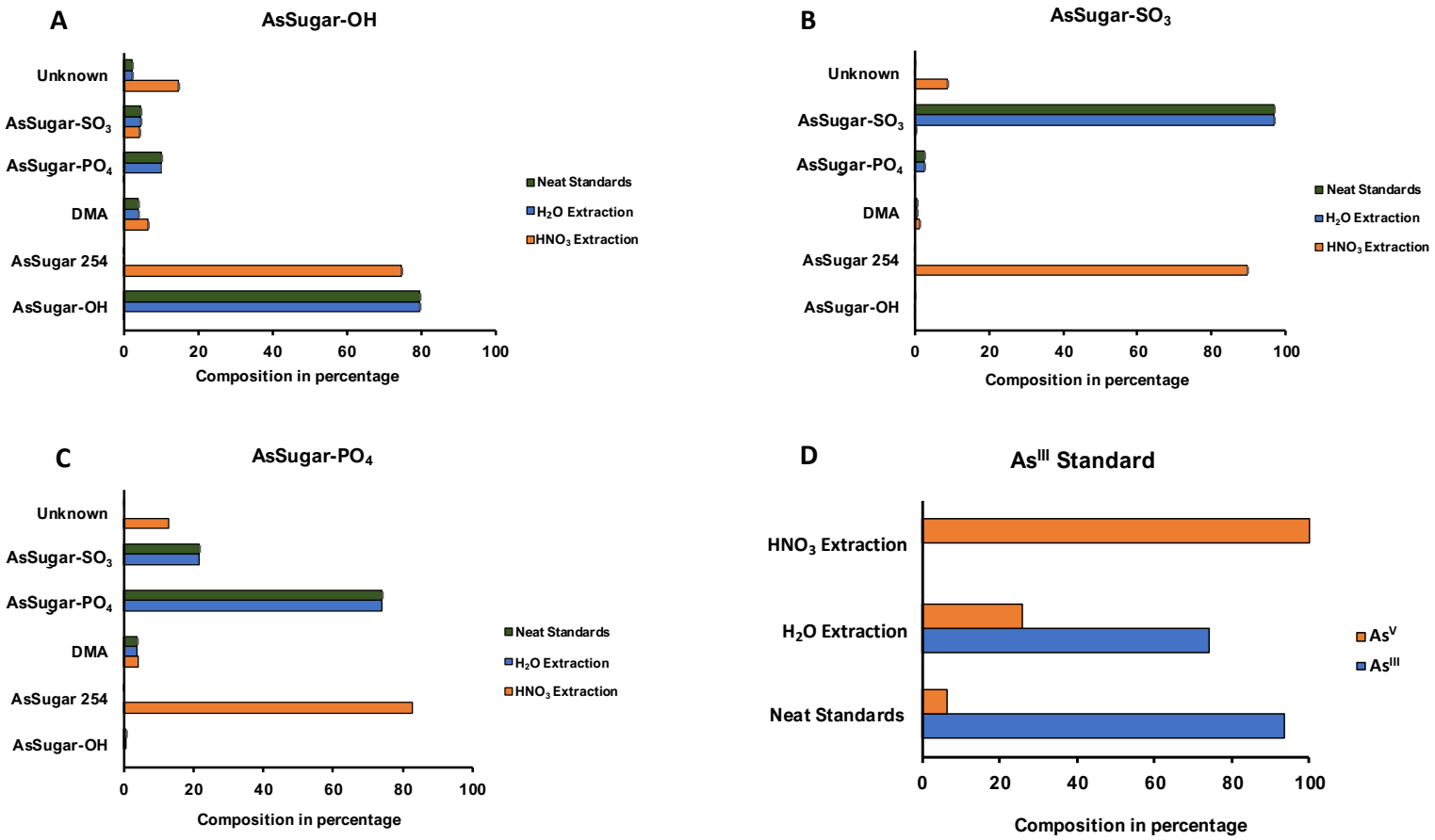


Figure 37: Mass fraction in percentage of arsenic species in native standards and after water extraction and acid extraction of the standards.

The presence of molecular ion with *m/z* 97 suggests that DMA had been cleaved off from the aglycone free 5-membered ring of AsSugars with additional loss of H_2O molecule, while the DMA moiety is then detected at *m/z* 122, as shown in Fig 38.[412] These evidences support that DMA is a byproduct of the decomposition of AsSugars.

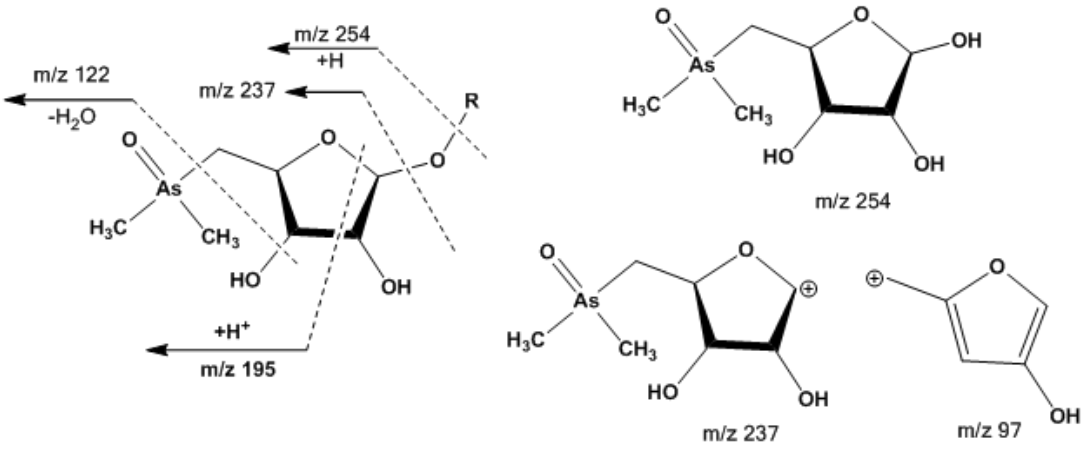


Figure 38: Typical AsSugar fragments after acid digestion detected in tandem mass spectrometry

Elevated amounts of DMA in acid extracts of geoduck clam tissue (Fig. 39B) may arise from the degradation of AsSugars, which are the major arsenic species. Moreover, while the peaks of MMA and As^V are baseline resolved, the peak of DMA is overlapped by peaks to the right resulting from AsSugars (Fig. 39B). Therefore, the measurement of DMA was performed using the water extraction method which gives a baseline resolved DMA peak (Fig. 39D). This justified the use of water extraction for determination of DMA, especially for samples with high levels of AsSugars even though the acid extraction method gave higher extraction efficiencies. While absent in the water extract (Fig. 39C), unknown arsenic species were found in the acid extract of spirulina (Fig. 39A). The unknown arsenic species in acid extracts of spirulina (Fig. 39A) likely resulted from the degradation of AsSugars (Fig. 39C) based on the stability

study of AsSugars above and supported by the comparative profile of Geoduck extract samples (Fig. 39B and 39D) that are known to contain AsSugars.

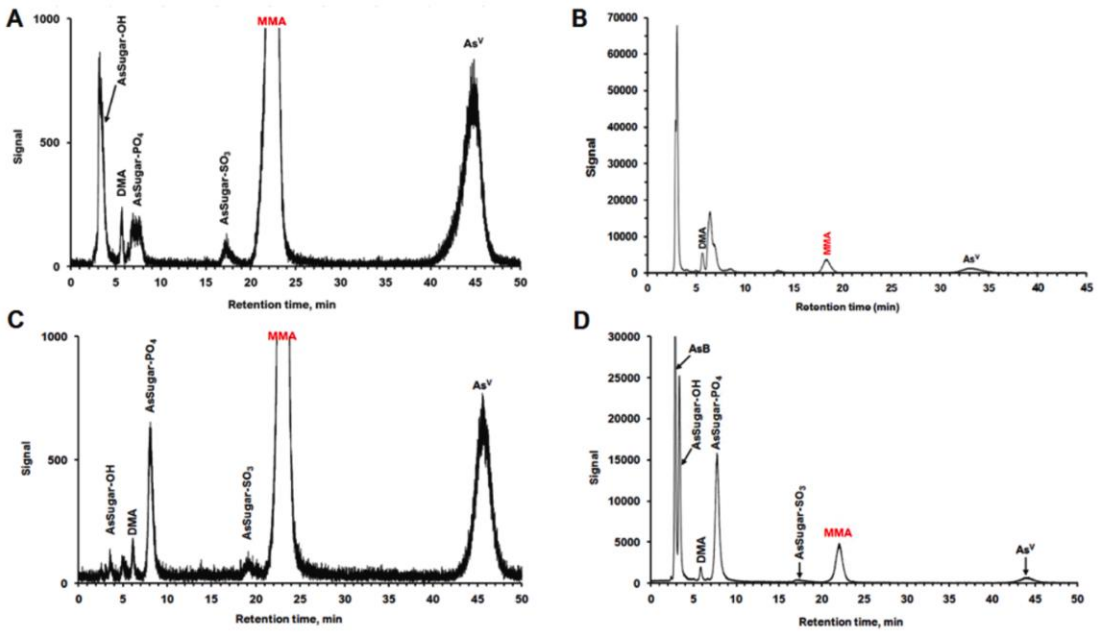


Figure 39: Anion exchange LC-ICP-MS of [A] spirulina powder acid extract, [B] geoduck clam tissue acid extract, [C] spirulina powder water extract spiked with MMA and [D] geoduck clam water extract.

SRM 3037 *Arsenous Acid (AsIII) Standard Solution* and SRM 3036 *Arsenic Acid (AsV) Standard Solution* were used in the study of iAs species stability as As^{III} and As^{V} standards respectively. The results show that the measurement sample of As^{III} was comprised of about 5% As^{V}. The amount of As^{V} in the As^{III} standard increased to about 25% after water extraction while acid extraction resulted in the complete conversion of As^{III} to As^{V} (Fig. 37D). The results indicate that As^{III} is not stable and it tends to be oxidized to As^{V}, especially in the presence of oxidizers such as HNO_3. Therefore, owing to the instability of As^{III} in its native form and under the other experimental conditions, it is not feasible to accurately determine As^{III} directly using either of the extraction methods.

On the other hand, As^{V} in SRM 3036 *Arsenic Acid (AsV) Standard Solution* remained stable under all the experimental conditions, i.e. in the native state, throughout water and acid extraction. These results show that using the water extraction method under the experimental conditions would not give an accurate assessment of the relative proportions of the two inorganic arsenic species in the study materials owing to the conversion of As^{III} to As^{V}. This study therefore validated stability of As^{V} under the acid extraction conditions and supported the approach for the complete conversion of As^{III} to As^{V} for the quantification of iAs. The results of this study served as the guiding principle for the choice of the extraction procedures.

5.1.2. Mass fraction of total Arsenic

The mass fraction of As was calculated according to the method of standard addition as follows:

$$x = \frac{usp}{sp-usp} * \frac{w_{sp}}{w_{sa}} * c * dil \qquad (3)$$

where x is the mass fraction of the analyte in the sample; *sp* and *usp* are the internal-standard-corrected count rate of the spiked and the unspiked measurement samples; w_{sa} and w_{sp} are the mass of the sample and the mass of the spike solution; c and *dil* are the mass fraction of the analyte in the spike solution and the dilution factor of the sample, respectively. The mass fractions of the total arsenic in common seafood are shown below (see Fig. 40).

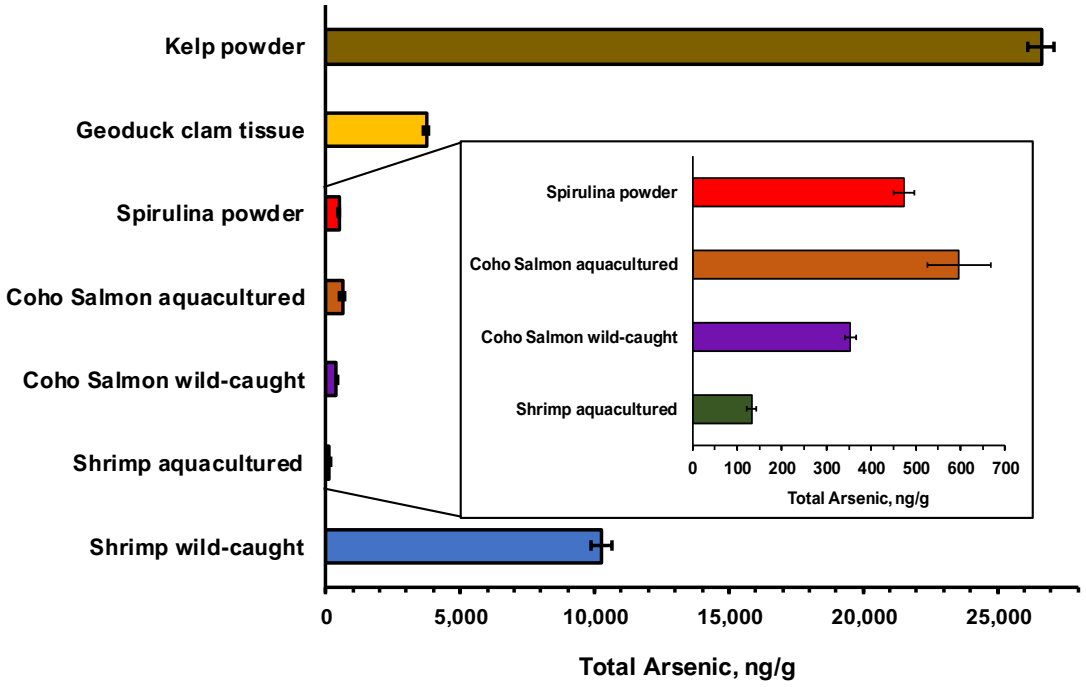


Figure 40: Mass fraction of total arsenic in common seafood. Error bars are uncertainty associated with the determination of each value (n = 10, 95% CI).

Quantification of total arsenic was achieved by the method of standard addition using Rhodium as an internal standard. The mass fractions of total arsenic, arsenic species, and recoveries of hydrophilic arsenic species as a proportion of total arsenic in the studied materials are shown in Table 8. All the results are reported on an as received basis apart from those of spirulina and kelp which are reported on a dry mass basis. Total arsenic values ranged from 133 ng/g ± 11 ng/g (mean ± s, n = 10) in aquacultured shrimp to 26,630 ng/g ± 520 ng/g (mean ± s, n = 10) in kelp, which is consistent with results published by Wolle et al.,[130] and Taylor et al.[31] The presence of high levels of As in seaweed products, and its ability to form potentially toxic species has raised concern about possible human health impacts of seaweed consumption.[419]

Total As content in both wild-caught and aquacultured salmon was generally lower, although the amount in aquacultured salmon was almost double the amount present in

the wild-caught counterpart. This might be attributed to the fact that they are pelagic and thus their feeding habits in the open sea do not expose them to intake of significant amounts of arsenic. However, the level of total As in geoduck clam as compared to salmon was between 6 to 10 times higher depending on the source. This may be because geoduck clam is a demersal marine filter-feeder that consumes algae and phytoplankton that are arsenic accumulators.[31,140]

There was significant difference in the total As content in the wild-caught and aquacultured shrimp with the total As level in wild-caught shrimp being almost 100 times higher than that present in the aquacultured shrimp although the shrimps were from different species. This massive difference may be more of a result of salinity, where AsB is absorbed and retained as an osmolyte,[14] than of the difference in species. Almost certainly the total arsenic difference between shrimp has very little to do with species and almost everything to do with their marine versus freshwater habitat and differences in diet.

Shrimp is the most consumed seafood in the US by a wide margin, according to the National Fisheries Institute's Annual Top Ten list.[677] Pricing of shrimp at import is determined by weight, species and provenance, including whether the food is from aquacultured or wild-caught sources. Each of these characteristics can be falsified, resulting in an inflated payout, encouraging unsustainable practices that would adversely affect the US domestic seafood economy.[678] The anticipated difference in the total arsenic content between the aquacultured and wild-caught seafood is therefore expected to be used as a tool to better identify fraudulent trade items at import and

provide a standardization tool for typical analyses conducted by inspection laboratories.

Findings from this study also agree with what has been reported in the literature where seaweed has the highest total arsenic concentrations in the marine food web,[14] shellfish have higher arsenic content than finfish, and demersal fish contain more arsenic than pelagic fish,[53] with significant variations between and within species.[31,49–51] The accuracy of the measurement was verified by method validation using SRM 1566b *Oyster Tissue* and SRM 1947 *Lake Michigan Fish Tissue* that represented dried seafood with high total arsenic content and frozen seafood with low arsenic content in as-received samples respectively. The measured values were within the range of certified values (Fig. 41A and 41B).

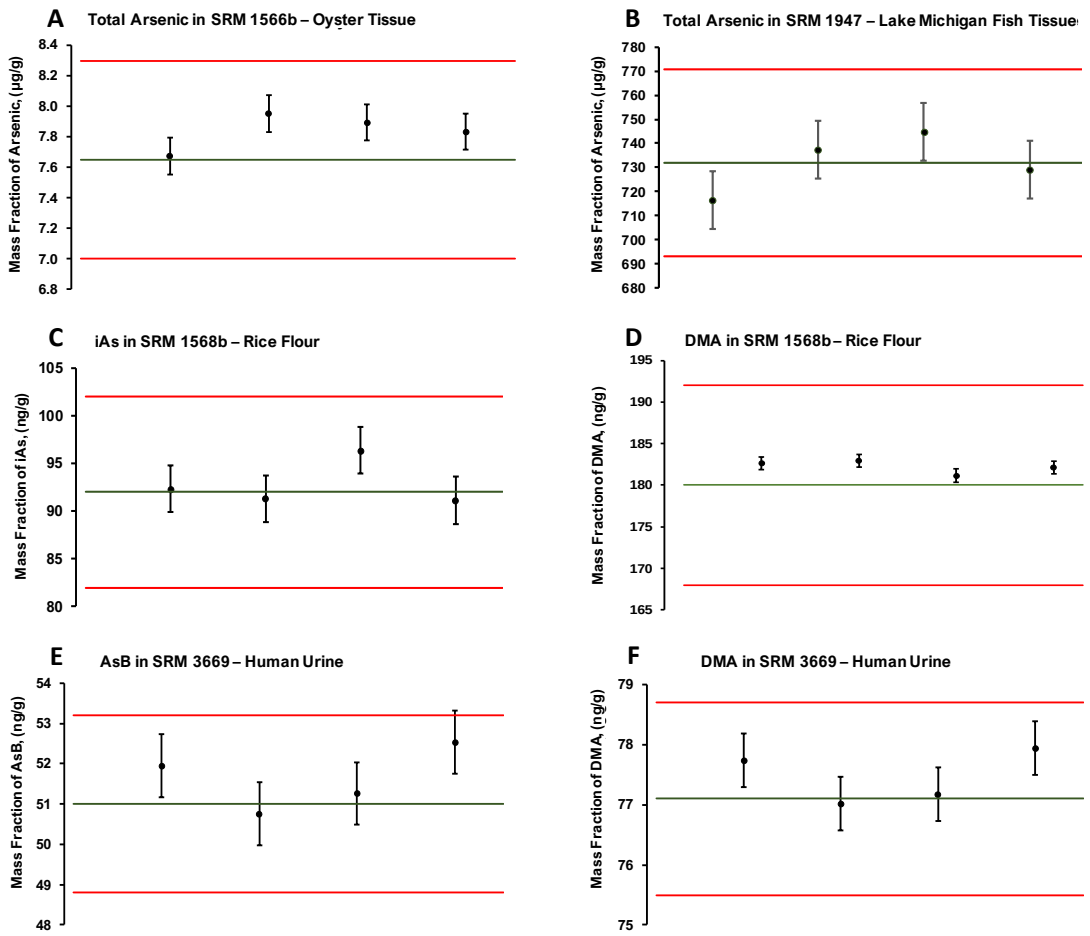


Figure 41: Control samples for AsB, DMA, iAs and total Arsenic in Urine, Rice, Oyster Tissue and Fish Tissue. The dots are mass fractions of arsenic species, error bars are uncertainties expressed as 1 SD n=4, green line is the certified and the red lines are the uncertainties related to the certified value at 95% CI.

5.1.3. Mass fraction of arsenic species

For arsenic speciation analysis, two methods of extraction were used. For the analysis of iAs, an extraction method using dilute nitric acid, based on European Standard (EN 16802:2016) was used. This method was applied to the determination of iAs as As^{V}. The oxidizing acidic conditions used in the method, as described in section 5.1.1 above, has the potential to decompose and alter the integrity of the other arsenic species present in the samples, especially AsSugars.

Sample ID	Total As (ng/g)	AsV (ng/g)	AsB (ng/g)	DMA (ng/g)	AsSugar-OH (ng/g)	AsSugar-PO_4 (ng/g)	AsSugar-SO_3 (ng/g)	AsSugar-SO_4 (ng/g)	Unknown (ng/g)	Recovery %
Shrimp (WC)	$1.025*10^4 \pm 370$	< 0.005	9458 ± 112 (2)	18.9 ± 1.2 (2)	< 0.2	< 1.5	< 1.0	< 0.2	770	92
Shrimp (AQ)	133 ± 11	< 0.005	10.4 ± 0.6 (3)	2.9 ± 0.2 (3)	< 0.2	< 1.5	< 1.0	< 0.2	120	10
Salmon (WC)	354 ± 12	< 0.005	165 ± 3 (3)	25.2 ± 0.8 (3)	< 0.2	< 1.5	< 1.0	< 0.2	160	54
Salmon (AQ)	597 ± 71	< 0.005	473 ± 7 (3)	13.3 ± 0.3 (3)	< 0.2	< 1.5	< 1.0	< 0.2	110	82
Spirulina powder	473 ± 23	265 ± 12 (4)	< 0.005	11.8 ± 1.6 (4)	< 0.2	144 ± 3 (4)	< 1.0	< 0.2	52	89
Geoduck clam	3733 ± 110	201 ± 16 (6)	491 ± 10 (6)	122 ± 9 (6)	528 ± 17 (4)	743 ± 25 (4)	64.3 ± 1.8 (4)	< 0.2	1600	58
Kelp powder	$2.663*10^4 \pm 520$	< 0.005	< 0.005	680 ± 16 (4)	6820 ± 150 (4)	1532 ± 21 (4)	5473 ± 76 (4)	$1.083*10^4$ ± 140	1300	95

Table 8: Total arsenic and arsenic species in common seafood.[1]

[1] For total arsenic, the uncertainties are expressed at 95% CI (n=10). For arsenic species, the uncertainties are expressed as 1 standard deviation with replication numbers in parenthesis. **WC** means wild-caught and **AQ** means aquacultured

For the determination of the other hydrophilic arsenicals, a water extraction method was employed as it preserved the integrity of the arsenic species. All the marine samples were screened for the presence of MMA, which was not detected in any of the study materials. Therefore, MMA was selected as an internal standard to be used in the quantification of the arsenic species.

5.1.3.1. Inorganic arsenic

Toxic iAs was detected only in spirulina at 265 ng/g ± 12 ng/g (mean ± s, n = 4) and in geoduck clam samples at 201 ng/g ± 16 ng/g (mean ± s, n = 6). These samples were also extracted using the water extraction method for comparison of the extraction efficiencies. The water extraction method gave iAs content of 254 ng/g ± 3 ng/g (mean ± s, n = 4) in spirulina and 72 ng/g ± 11 ng/g (mean ± s, n = 6) in geoduck clam tissue. The extraction methods did not show any statistically significant effect on results of iAs in spirulina powder and rice flour, however, there was a marked difference of iAs results in geoduck clam tissue (see Fig. 42).

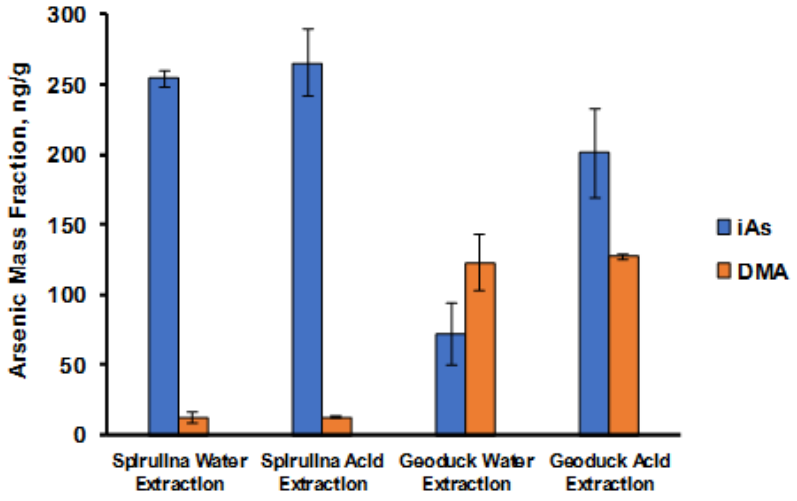


Figure 42: Comparison of extraction of iAs and DMA in geoduck clam and spirulina using acid and water extraction methods with error bars representing uncertainty at 95% CI.

The acid extraction method gave three-fold extraction efficiency as compared to the water extraction for geoduck clam tissue samples, supporting that extraction with dilute nitric acid is a more robust procedure for a more conservative estimate of iAs. The accuracy of the measurement was verified using SRM 1568b *Rice Flour* and the measured values for both the acid extraction and water extraction methods, as results were within the range of the certified value (Fig. 43).

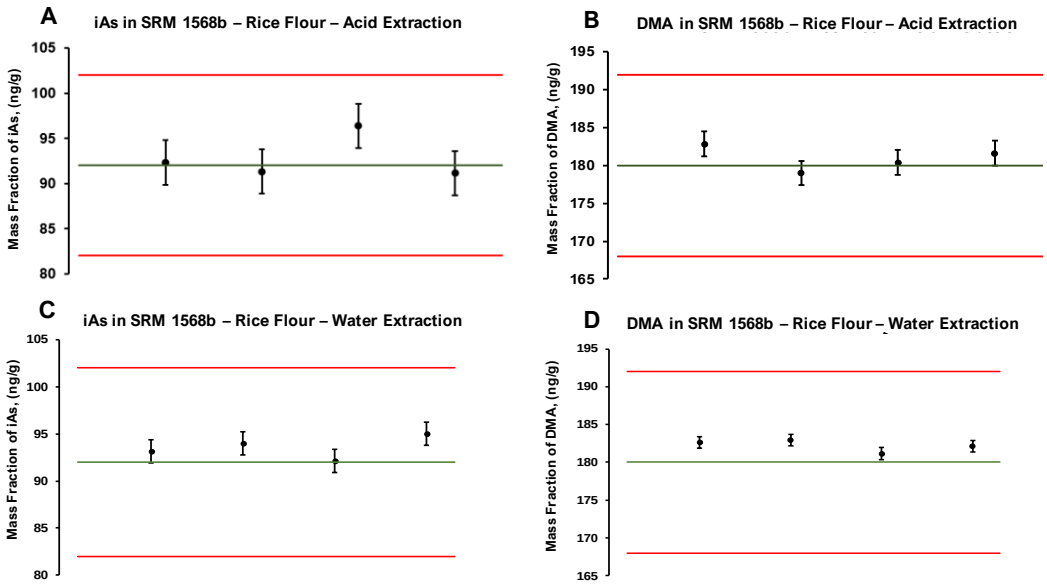


Figure 43: Mass fraction of control samples extracted using acid and water extraction methods. The dots are mass fractions of arsenic species, error bars are uncertainties expressed as 1 SD n=4, green line is the certified value and the red lines are the uncertainties related to the certified value at 95% CI

Of concern is the level of toxic iAs in spirulina which accounts for about 56% of the total arsenic content (Fig. 44). Even though spirulina is not a typical food item, it is extensively used as a dietary supplement with high consumption rates for prolonged periods.[675] Dietary supplements are regulated as food for label claim accuracy and for safety. In this case where they pose risk to the health and safety of consumers, it may be prudent to provide consumption guidelines by notifying the consumers of the

inherent risks. Dietary supplements are mostly recommended and marketed to the vulnerable segment of the society due to their presumed health benefits. It is, therefore, important to monitor the consumption patterns of spirulina and probably consider adding it to the list of food products for which the maximum permissible iAs content is established.

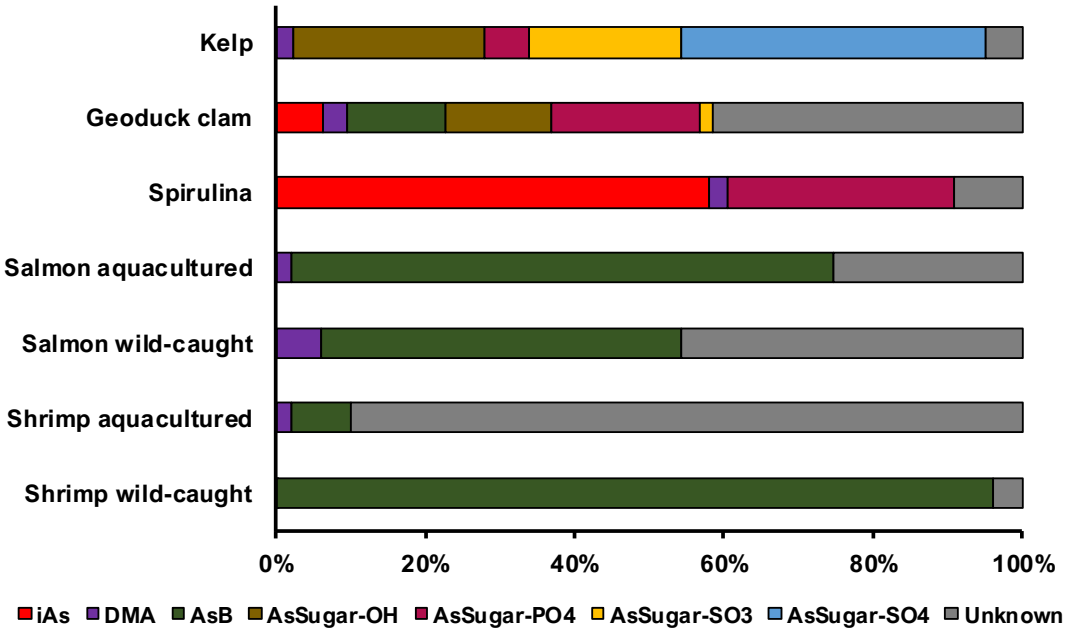


Figure 44: Mass fraction of arsenic species in various edible marine species.

Ronan et al. reported iAs in all 25 kelp (*A. nodosum*) samples.[47] The reported levels were less than 1% of the total arsenic content and lower than 1 µg/g.[47] In this study, iAs was not detected in the kelp (*A. nodosum*). However, iAs was found at 79.2 ng/g ± 0.4 ng/g (mean ± s, n= 4) in SRM 3232 *Kelp Powder* (*Thallus laminariae*), for which the SRM does not have an assigned value. Krishnakumar et al. reported results for iAs in 26 samples of seafood including bivalves, cuttlefish, shrimp and finfish from the Arabian gulf,[679] where nine of the samples showed iAs level below the detection limit of 0.01 µg/g on dry mass basis. iAs in the other samples ranged between 0.01 µg/g and

0.83 µg/g accounting for below detection limit and 1.84% of total arsenic content ranging between 11 µg/g and 117 µg/g on a dry mass basis. In this study, iAs was not detected in any of the seafood samples other than geoduck clam tissue at 0.20 µg/g ± 0.02 µg/g (mean ± s, n = 6) in as-received basis.

5.1.1.1.Mass fraction of other arsenic species

Mild extraction procedure was applied for the extraction of hydrophilic arsenicals using only water as an extractant in order to maintain arsenic species integrity, especially for AsSugars. The relative proportions in percentage of the arsenic species in the seafood and seaweed studied are shown in Figure 44. Hydrophilic arsenicals account for between 10% and 95% of the total arsenicals in the study materials.

Microalgae, macroalgae and bivalves displayed the greatest variety of arsenic species, which is in agreement with what was reported in the literature by Taylor et al.[31] DMA is the only arsenic species present in all the food types that were studied at trace levels with proportions relative to total arsenic content ranging from 0.2% in wild-caught shrimp to 7% in wild-caught salmon. For all the other samples in this study, DMA accounted for between 2% and 3% of the total arsenic content.

Elevated levels of DMA in urine have been reported following consumption of diets rich in AsSugars and AsLipids.[336,416,419] The uncertainty concerning the threat to seafood consumers exposed to organic arsenic and the dose required to trigger potential health effects is still an ongoing concern. The extent of dietary exposure to different arsenic species vary from country to country and is dependent on the types of food, the amounts of the food consumed per serving, the frequency of consumption of the food

types, the amount of arsenic species present in the food and the metabolic transformation and elimination mechanisms from the body amongst others.[40] It is therefore important to consider the cumulative effects of consumption of the different food types containing different arsenic species.

AsB was present in all higher trophic organisms tested but was absent in spirulina and kelp (Fig. 45). AsB accounted for 13% of total arsenic in geoduck clam, 8% in aquacultured shrimp, 92% in wild-caught shrimp, 79% in aquacultured salmon, and 47% in wild-caught salmon. Based on projected exposure level to dietary arsenic and the expected metabolism, it seems unlikely that dietary arsenic can significantly promote arsenic-associated carcinogenic effects. This is because the bulk of arsenic in seafood exists as AsB which is toxicologically inert with LD_{50} > 10,000 µg/g, and is rapidly eliminated from the body intact in the urine.[20]

Several studies have however shown that aerobic GIT microflora,[366,680] and microorganisms from marine sources [273,681] have the potential to biotransform AsB into more toxic arsenicals, like DMA, DMAA (dimethylarsinoylacetate), and TMAO. These observations suggest that AsB may not be as recalcitrant as was previously thought. Work by Lai et al. on the feed trial involving blue mussel gave credence to this concept.[682]

The dominant arsenic species in seaweed and geoduck clam are AsSugars but they were absent in salmon and shrimp. AsSugars contents were determined in water extracts using an external calibration method with standards from the purified kelp extracts and MMA as internal standard. AsSugar-SO_4 standard was not available for this work.

However, the slopes of the calibration curves obtained for AsSugar-OH, AsSugar-PO_4, and AsSugar-SO_3 were all in good agreement, suggesting the instrument response to AsSugars are similar. The similarity of the structures of AsSugar-SO_3 and AsSugar-SO_4 further support the approach, which assumes that the ICP-MS response for arsenic is independent of the AsSugar species and that the chromatographic recoveries of calibrants and analytes are the same. Therefore, the calibration curve for AsSugar-SO_3 was used for the quantification.

The only AsSugar present at quantifiable level in spirulina is AsSugar-PO_4. AsSugar-OH and AsSugar-SO_3 were present but were below the limit of detection (Fig. 45B). All the four commonly found AsSugars i.e. AsSugar-OH, AsSugar-PO_4, AsSugar-SO_3 and AsSugar-SO_4 were present in the kelp sample, with AsSugar-SO_4 as the dominant species (Fig. 45C).

Human exposure to AsSugars is relatively high in Asia because of the more frequent use of seaweed in cooking (e.g., Nori, one of the products used to wrap Sushi), which typically contains arsenic levels as high as 100 μg/g dry weight. As a result, dietary intake of arsenic can reach 1 mg/day in Japan,[683] far exceeding the WHO guideline of 0.15 mg/day. Fortunately, there is no indication for acute toxicity although there is a possibility of AsSugars having slight chronic toxicity.[238]

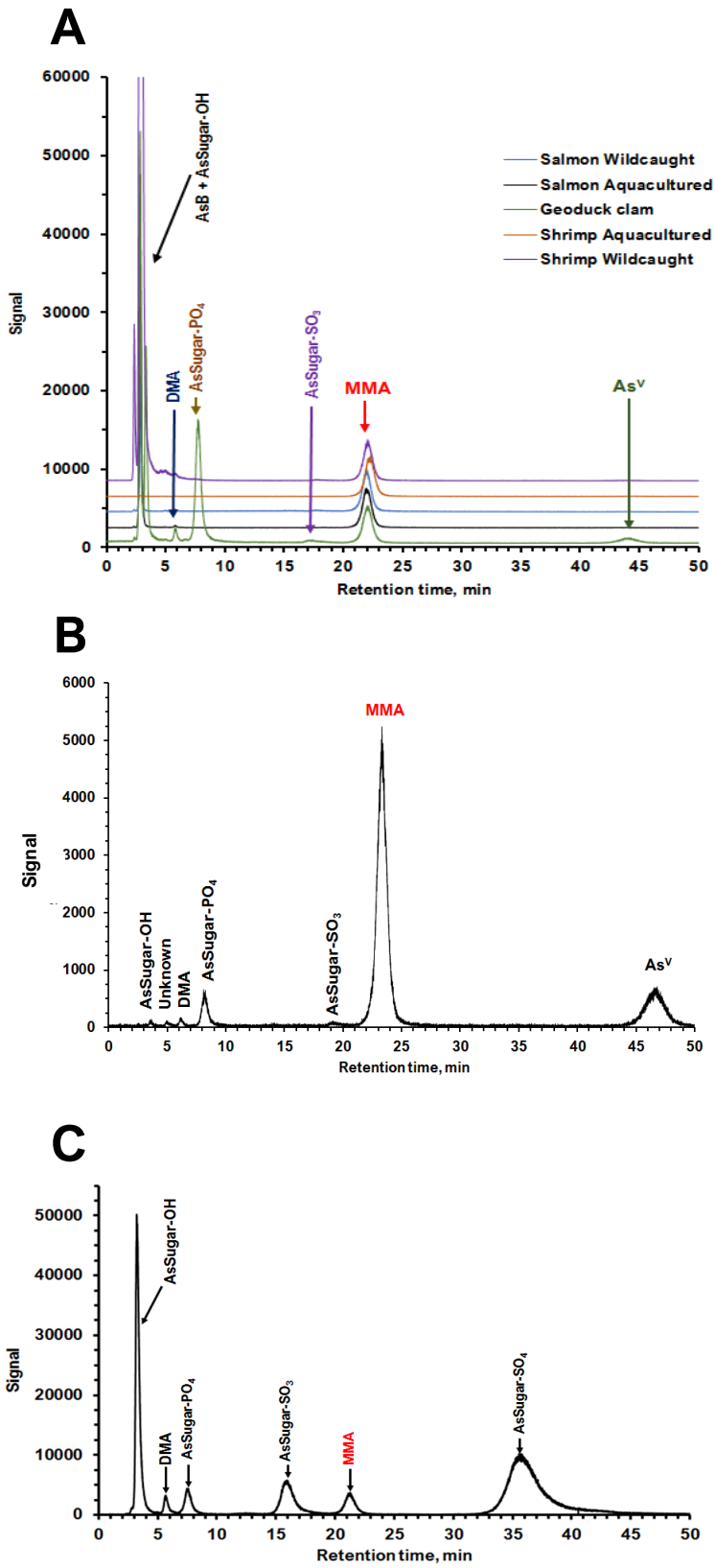


Figure 45: AE separation of arsenic in [A] seafood, [B] spirulina and [C] kelp spiked with MMA

5.2. Conclusion

Results from this study show that kelp, wild-caught shrimp, and geoduck clam have the highest total arsenic content of 26.63 µg/g ± 0.52 µg/g, 10.25 µg/g ± 0.37 µg/g and 3.73 µg/g ± 0.11 µg/g respectively. All the other seafood samples in this study had total arsenic content below 0.60 µg/g. The toxic iAs contents in these seafood samples, on the other hand, range from <0.005 ng/g to 265 ng/g. Therefore, total arsenic as a measure of toxicological significance of arsenic is misleading.[41]

In the absence of speciation analysis, assuming that the total arsenic content is 100% iAs is most protective for human health. It is, however, incorrect to conclude that kelp, wild-caught shrimp, and geoduck clam are dangerous from the standpoint of total arsenic, while nearly all arsenic therein are nontoxic. For example, 92% of the total arsenic in wild-caught shrimp is AsB, which is known to be toxicologically inert.[42] The major arsenic species in kelp (about 93%) and geoduck clam (about 36%) were AsSugars, which are not known to exhibit acute and chronic toxicity.[15]

Potentially toxic levels of iAs were detected only in spirulina and geoduck clam. Although the total arsenic content in spirulina (473 ng/g) was relatively low, the mass fraction of iAs (265 ng/g) on a dry mass basis accounted for 56% of the total arsenic content. The mass fraction of iAs (201 ng/g) on an as received basis relative to the total arsenic content accounts for about 5% in geoduck clam. These observations underpin the importance of arsenic speciation analysis in the determination of the arsenic species of toxicological relevance in the different food types. DMA was established to be a byproduct of decomposition of AsSugars in acid extracts of samples known to contain

AsSugars like spirulina, geoduck clam and kelp. Acid extraction should therefore not be used to determine DMA in samples that are known to contain AsSugars.

There is need for further study of the acid extracts using alternative detection techniques such as tandem mass spectrometry (LC-MS/MS), which provide necessary molecular information for the explicit identification of the unknown arsenic species such as the aglycone free arsenosugar (AsSugar 254) that coelutes with AsSugar-OH in AE chromatography.

In this study we have reported the hydrophilic arsenicals which forms the major proportion of the arsenic species in the study materials, except for aquacultured shrimp. Since not all arsenic species were extracted in the aqueous phase in all the study materials, there is need to characterize and quantify the unknown arsenic fraction in order to establish the level of risk.

The samples should be extracted with nonpolar solvents to establish the mass fraction of the lipophilic arsenicals and the non-extractable residual arsenic, which is important for a more complete toxicological profile of these food types. Lipophilic arsenic species are mostly soluble in organic solvents which are not amenable with ICP-MS, the preferred detection technique for arsenic. This class of arsenicals should therefore be analyzed using complementary detection techniques like LC-MS/MS,[43] which is can identify and quantify the novel lipophilic organic arsenicals in the absence of well-characterized standards.

Chapter 6: Structural elucidation and annotation of arsenolipids in seafood based on *in silico* tandem mass spectrometry.

6.1. Introduction

Lipophilic organic arsenic compounds generally known as arsenolipids (AsLipids) are novel species of interest that are present at relatively high natural levels in seafood and seaweed.[96] In seafood, AsLipids comprise up to 70% of the total arsenic content,[18] which accounts for between 0.3-3.6 μg As g^{-1} dry weight. The highest amounts are found in fatty fish,[80] like herring (*Clupea harengus*), cod (*Gadus morhua*), capelin (*Mallotus villosus*), and mackerels (*Scomber scombrus*) and seaweed,[96] like Hijiki (*Hizikia fusiformis*) and Wakame (*Undaria pinnatifida*).

AsLipids elicit research interest due to their novel structures, their likely role in membrane biochemistry, and since they exist in common seafood with potential health concerns based on arsenic toxicity.[21] Despite the diversity of AsLipids occurring in seafood, studies on toxicity, toxicodynamics and toxicokinetics have so far, been explicitly examined only for AsHCs, AsFAs, and AsPCs. AsHCs were found to be highly toxic in *in vitro* and *in vivo* studies, causing toxicity in the low micromolar range in human liver cancer cells (HepG2),[42] and neurons[324] comparable to iAs.[42,44,98–101] However, the molecular modes of action regarding their toxicity as well as their metabolism in the liver still remain unclear.[98]

In contrast, AsFAs caused 10–20 times lower toxicity than AsHCs in HepG2 cells although their cellular bioavailability was only 2.5–5 times lower.[43] Even though their cytotoxic effect in human liver cells are lower than iAs and they do not show any

genotoxic effects, the risk to human health from AsFA 362 and AsFA 388 cannot be excluded.[43,45] Toxicological assessment of AsPCs in HepG2 cells indicate that their toxicity might be largely governed by their arsenic fatty acid content and suggest a multifaceted human metabolism of food derived complex AsLipids.[684] Speciation analysis revealed biotransformation of AsPCs to a series of AsFAs in culture medium.

AsHCs and AsFAs could be detected in the milk of nursing Norwegian mothers,[685] and studies with the fruit fly (*Drosophila melanogaster*),[44,101] and skipjack tuna (*Katsuwonus pelamis*) [686] indicate that AsHCs are able to cross the blood brain barrier. Consistent with those results, AsHCs were able to cross an *in vitro* blood–brain barrier model,[99] and the Caco-2 intestinal barrier model.[45]

Collectively the data suggested that the higher lipophilicity of the AsHCs compared to AsFAs contributes to their greater toxicity.[43] Combining existing knowledge on AsLipids, it is apparent that these might be a concern for human health, while systemic bioavailability, biotransformation and toxicological characterization of emerging complex AsLipids in seafood has not yet been addressed.

Advances in the biochemistry and toxicological studies of AsLipids has been hindered by challenges related to their identification, separation and analysis,[16] their trace amounts in marine samples as compared to polar arsenicals,[11] limited knowledge on the stability of their analytes in the course of ordinary sample preparation steps,[457] and lack of well-characterized and commercially accessible standards and quantitative analytical methods.[96] Improved knowledge with regards to chemical structures,

amounts present, bioavailability, and toxicity of the specific AsLipids is necessary for a more comprehensive risk assessment of these arsenic species present in seafood.[31]

As far as structural elucidation for the novel AsLipids is concerned, NMR spectroscopy technique has been used.[97] However, this technique requires copious amounts of sample at high concentration, which is not feasible for AsLipids that are present in ultra-trace amounts in some seafood. This would require extraction, fractionation, and cleanup of analytes from large amounts of sample, which would further be concentrated before analysis. This is costly both in terms of effort and time.

Most of the known AsLipids have been identified by analytical methods based on separations by liquid chromatography (LC) and the complementary use of ICP-MS and ESI-MS for element specific detection and structural elucidation, respectively.

The currently known AsLipids have either been identified from chromatographically fractionated extracts mostly based on tandem mass spectrometry and extrapolation of structural knowledge from lipidomics or from indirect confirmation of structures by comparison to synthesized AsLipids that have been fully characterized by NMR spectroscopy and molecular mass spectrometry.[687]

Several AsHCs, AsFAs and AsPCs have been synthesized for confirmation of the respective AsLipids identities.[7,116,122,420,421,425,428,429] With the ever-increasing number of new AsLipids compounds, it is practically impossible to keep up with the synthesis of pure standards for confirmation of their identities.

Although the current approaches have been effective and successful, they require expensive instrumentation and in-depth knowledge of lipidomics and synthetic chemistry, which is still beyond the reach of many laboratories. The need for authentic standards and reference materials for confirmation of proposed structures and development of a database for the novel class of compounds still endures.

Two AsHCs and five AsSugar-PLs have been identified and quantified in CRM NMIJ 7405-a (*Hijiki*), which had already been certified for total arsenic and As^{V}.[96] While this has been a great contribution to the identification and quantification of AsLipids, a lot more effort is still needed to cover more measurement space. There are many classes of AsLipids lacking authentic standards and reference materials that are vital for their unequivocal identification and quantification.

The need for a tool for the prediction and identification of the novel lipophilic compounds from marine based dietary sources that are known to contain arsenic species and whose identities are still unknown is apparent. Therefore, a cheap, robust, and readily accessible method for the identification of these novel classes of lipophilic compounds is required.

Searching for compounds in molecular structure databases is limited to compounds that are present in such databases. Since the structures of most of the lipophilic arsenicals are still unknown and their metabolic transformation is yet to be fully elucidated, a plausible approach is to apply non-targeted analysis of these analytes. Metabolite identification for non-targeted analysis of AsLipids has been hampered by the lack of experimentally collected reference spectra from tandem mass spectrometry (MS/MS).

To accomplish this, we used Competitive Fragmentation Modeling for Metabolite Identification (CFM-ID) software version 3.0 to predict ESI-MS/MS spectra from known chemical structures reported in literature to aid in compound identification via MS/MS spectral matching. CFM is a probabilistic generative model for the MS/MS fragmentation process that uses machine learning techniques to learn its parameters from reliable high quality data.[688] The model estimates the likelihood of any given fragmentation event and predicts the peak to be observed.

In this chapter, an account of the process of development of a spectral library based on *in silico* fragmentation data generated using CFM-ID for putative identification of the novel lipophilic organic arsenic species in marine based dietary sources for which authentic standards and spectral libraries are not available is presented.

Since there is no database for novel AsLipids, the CFM-ID 3.0 model was trained to generate *in silico* fragmentation MS/MS spectra using extant AsLipids experimental molecular structural information reported in literature. CFM-ID 3.0 is linked to the METLIN database, which has lipids fragmentation data. Therefore, linking this information with knowledge gleaned from the AsLipids *in silico* MS/MS spectral data will enable the prediction of the novel lipophilic organic arsenic compounds in seafood.

This approach adds value to the body of knowledge by 1). contributing to the development of a library for rapid screening and identification of unknown lipophilic organoarsenicals whose toxicological impact is of interest but is yet to be fully elucidated; 2). eliminating the need to generate empirical data from all the compounds required to develop a database, especially without authentic standards thus saving time;

3). enabling the extension of extraction procedure to achieve higher extraction efficiencies, since this will allow for the use of organic solvents that are not amenable with LC-ICP-MS, which is the limiting factor in the identification and quantification of AsLipids; 4). expediting the characterization and certification of candidate reference materials; and 5). accelerating the development of synthetic standards by providing a complementary cheap, robust, and reliable approach for their characterization.

6.2. Methods

An overview of the workflow employed for generating the comprehensive AsLipids screening database is depicted below (see Fig. 46).

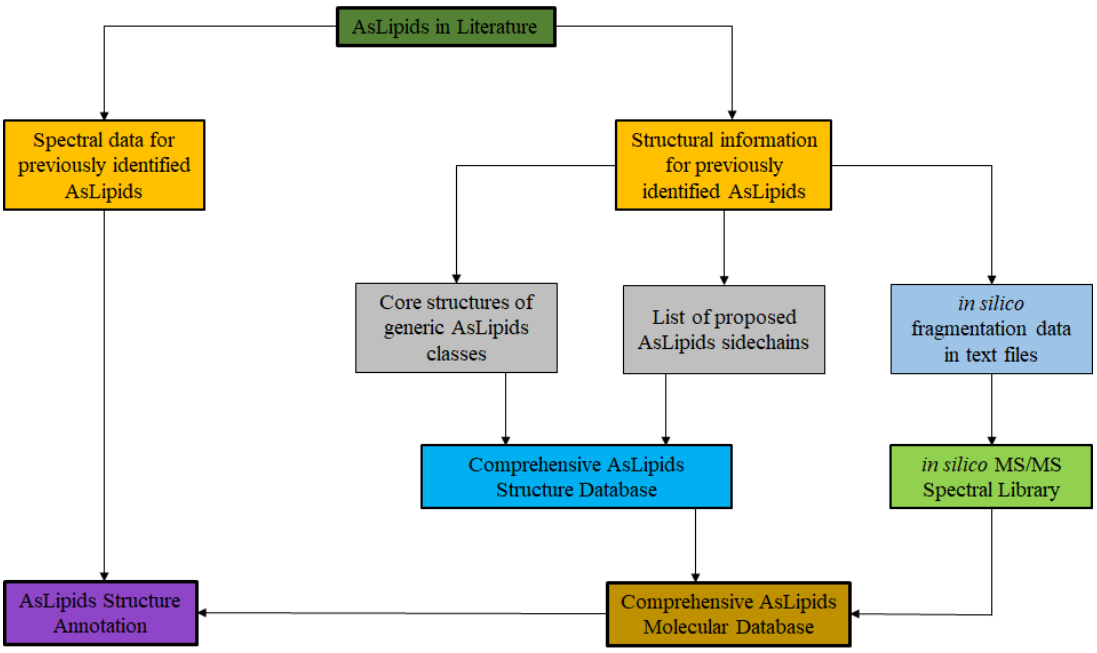


Figure 46: Workflow for generating AsLipids structure and *in silico* MS/MS library.

6.2.1. Literature search and structure determination

Novel structures for organic arsenicals that were reported in peer-reviewed literature were extracted, capturing the images, structure description, and elemental formula for the proposed structure, when provided by the authors in the manuscript or supporting information. The structures were determined from the reported information, and compounds were not included where there was insufficient evidence of structural identification, such as incomplete or unstable structures (*i.e.*, carbon radical).

Using ChemDraw 19.1, the individual compound structures were drawn and converted into their simplified molecular-input line-entry system (SMILES) and their International Chemical Identification (InChI) structural notation for further analysis.

6.2.2. *in silico* fragmentation mass spectrum

For each literature-based organic arsenic structure, an *in silico* fragmentation mass spectrum was generated using CFM-ID 3.0. Some compounds could not be generated using the online interface of CFM-ID 3.0. Therefore, *in silico* fragmentation mass spectra were also generated using CFM-ID 2.4 with an R-based interface.

For each compound, three different fragmentation mass spectra were predicted at low (10V), medium (20V), and high (40V) fragmentation energies. Exportable formats for all *in silico* fragmentation mass spectra were captured and stored in both positive and negative ionization modes.

6.2.3. Comparison to literature spectra

After *in silico* fragmentation mass spectra were generated for all literature-based organic arsenicals, the *in silico* spectra were compared with any fragmentation mass spectra that was provided in the literature for structure elucidation. This approach was employed to validate the generated spectra. To accomplish this, the *in silico* fragmentation mass spectra from all three fragmentation energy levels were tabulated in Excel and visually compared to mass spectral images from the respective literature.

The presence of specific *m/z* fragment ions were compared, not the individual intensity levels, and only *m/z* values that are specifically annotated in the mass spectral image. No extrapolation or interpolation of unlabeled *m/z* values were done, limiting the user interpretation of the comparison.

6.2.4. Analysis of fragmentation mass spectral patterns

Using R, the *in silico* fragmentation mass spectra for the literature-based organic arsenic species were compiled and transformed into two matrices; the first matrix consisted of the structures (columns) with the annotated *in silico* fragments from all fragmentation energy levels (rows), and the second matrix consisted of the structures (columns) with the implied neutral losses (rows), as determined by the mass difference of the precursor ion *m/z* and the individual *in silico* fragment ion *m/z* values. These matrices were created separately for positive and negative ionization modes.

6.2.5. AsLipids structure prediction

The literature-based organic arsenicals could be organized by specific class structures (*e.g.*, arsenic-containing triglycerides), resulting in 10 different organic arsenical classes (see Table 9). Generic structures were created with the common base structure and R_1, R_2, and R_3 notation for specific repeating substructures (*e.g.*, fatty acids, hydrocarbons, terpenes). An R script was developed to generate the SMILES notation for all possible class structures with repeating substructures, as well as monoisotopic masses for each generated compound.

SNo.	Class	SMILES	R Group		
			R1	R2	R3
1.	AsHCs	C[As](C)([R1])=O	Hydrocarbon		
2.	AsFAs	C[As](C)([R1])=O	Reverse Fatty Acid		
3.	TMAsFOHs	C[As^{+}](C)(C)[R1]	Fatty Alcohol		
4.	DMAsFOHs	C[As](C)([R1])=O	Fatty Alcohol		
5.	AsPCs	O=P(OCC[As^{+}](C)(C)(C)(OCC([R1])C[R2])O	Fatty Acid	Fatty Acid/ OH	
6.	AsPEs	O=P(OCC[N+]([H])([H])([H])(OCC([R2])C[R1][As](C)(C)=O)O	Fatty Acid	Fatty Acid/ OH	
7.	AsSugar-PLs	O=P(OCC(COC1OC(C(C1O)O)C[As](C)(C)=O)O)(OCC([R2])C([R1])O	Fatty Acid	Fatty Acid/ OH	
8.	AsSugar-Phytol	OC(C(OC1[R2])C[As](C)(C)=O)C1[R1]	CH_3O / OH	Terpene	
9.	AsTAG	[R3]C([R2])[R1][As](C)(C)=O	Fatty Acid	Fatty Acid	Fatty Acid
10.	AsDAG	[R3]C([R2])[R1][As](C)(C)=O	Fatty Acid	Fatty Acid	OH

Table 9: Generic Structure for the development of predicted AsLipids structures.

0

6.2.6. Development of AsLipids *in silico* library search engine

AsLipids *in silico* library search engine is a cross platform open-source software. The back end consists of MySQL server (see Fig. 47 for database structure) while the front end is a web-based graphical user interface developed in Python's Flask web framework. The pre-requisite open-source software for the search engine are:

1) **MySQL database** (https://www.mysql.com/products/community/)

2) **Python version 3.6** or greater with the following python packages installed: ***flask***, ***flask_mysqldb***, ***matplotlib***, ***sklearn***, ***numpy***, and ***fmpld3.***

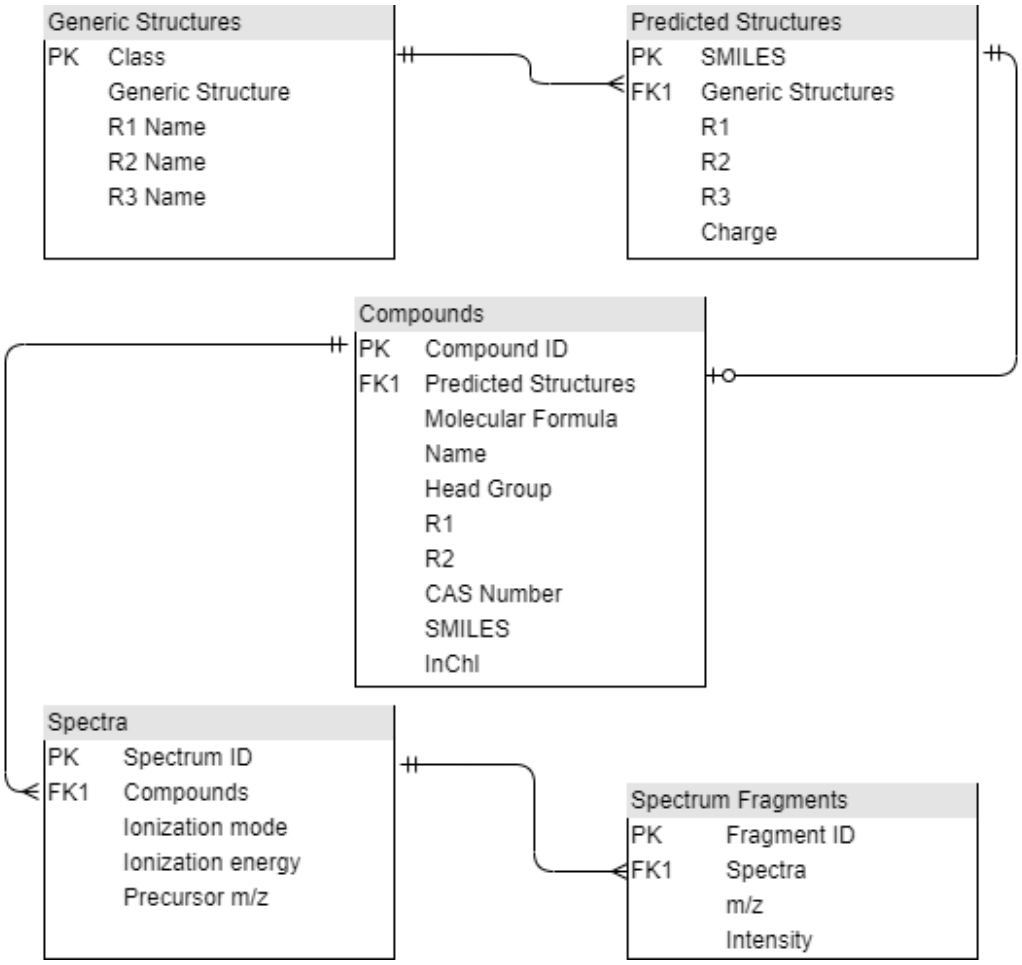


Figure 47: Schema of the Relational Database structure.

The predicted *in silico* fragmentation spectral data generated by CFM-ID 3.0 software and R-scripts combined with extant AsLipids metadata stored in Excel files was inserted into MySQL database with a custom data parser written in Python programming language.

6.3. Results and Discussion

6.3.1. Literature search and mass spectrum extraction of extant AsLipids

The existence of arsenic in lipid extracts of fish and algae was originally reported by Lunde in 1968,[15] but their structures remained unknown. The first structure of AsLipids was presented by Morita and Shibata in 1988 through their classic study using ^{1}H NMR and identified AsSugar-PL 958 in a marine alga, Wakame (*Undaria pinnatifida*).[97] Literature search of about 40 publications showed more than 200 AsLipids discovered in various marine samples and distributed between ten lipid classes (Table 10).

95% of the AsLipids reported in literature provided molecular formulae and chemical structures that were confirmed by one of the following approaches (see Table 10): The most commonly employed approach was by accurate mass measurement using a combination of reversed-phase liquid chromatography coupled to inductively coupled plasma mass spectrometry and high-resolution electrospray mass spectrometry (RP-LC-ICP-MS/HR-ESI-MS).[7,17,80,98,102,103,105–107,112,114,115,117,124,422,423,433,457]

SNo.	Sample	Total Arsenic, µg As/g	AsLipid, µg As/g	AsLipid Speciation	Techniques	References
1.	Cod-liver oil	5	1	6 AsFAs	HPLC-ICP-MS HPLC-ESI-MS MALDI-HRMS FT-ICR-MS	Rumpler, A. et al. 2008
2.	Capelin oil	12	8.4	3 AsHCs	ICP-MS HPLC-ESI-MS MALDI-HRMS FT-ICR-MS ^{13}C-NMR	Taleshi, M. S., et al. 2008
3.	Capelin oil	-	-	3 AsHCs	GC/MS	Raber, G., et al. 2009
4.	Tuna fish	5.9	2.36	3 AsHCs 2 AsFAs	ICP-MS HPLC-ICP-MS HPLC-ESI-MS	Taleshi, M. S., et al. 2010
5.	Canned cod-liver tissue			3 AsHCs	GC-ICP-MS GC-MIP-AES GC-EI-qMS HPLC-ToF-MS	Arroyo-Abad, U., et al. 2010
6.	Capelin fish meal	2.95 ± 0.11	0.88 (MeOH) 0.35 (Hexane)	3 AsHCs 3 AsFAs	HPLC-ICP-MS HPLC-ESI-MS	Amayo, K. O., et al. 2011
7.	Cod-liver oil and capelin oil	5 - 11		3 AsHCs 3 AsFAs	HPLC-ICP-MS	Ruiz-Chancho, M. J., et al. 2012

SNo.	Sample	Total Arsenic, µg As/g	AsLipid, µg As/g	AsLipid Speciation	Techniques	References
8.	Two species of brown algae (Wakame and Hijiki)	40 ± 3 (Wak) 113 ± 5 (Hiji)	2.7 ± 0.2 (Wak) 1.8 ± 0.08 (Hiji)	11 AsPLs 3 AsHCs	HPLC-ICP-MS HPLC-ESI-MS	Garcia-Salgado, S. et al. 2012
9.	Capelin oil	4.10 ± 0.13	3.87 ± 0.27	3 AsFAs 7 AsHCs 2 TMAsFOHs	HPLC-ICP-MS HPLC-ESI-MS	Amayo, K. O., et al. 2013
10.	Fresh cod-liver	1.53 ± 0.02	1.44 ± 0.24	16 AsFAs 4 AsHCs	HPLC-ICP-MS HPLC-ESI-qToFMS	Arroyo-Abad, U., et al. 2013
11.	Herring fillet (*Clupea harengus*)	5.77 ± 0.3	3.67 ± 0.2	13 AsFAs 3 AsHCs	HPLC-ICP-MS HPLC-ESI-TOF-MS	Lischka, S., et al. 2013
12.	Commercial fish oils	5.9 – 8.7		3 AsHCs 1 AsFAs 1 AsPLs	GC-ICP-MS GC-MS/MS HPLC-ESI-TOF-MS	Sele, V., et al., 2013
13.	Commercial fish oils	1.6 – 12.5		3 AsHCs 2 AsFAs 1 TMAsFOHs 1 AsPLs	HPLC-ICP-MS HPLC-ESI-TOF-MS	Sele, V., et al., 2014
14.	Northeast Arctic cod (*Gadus morhua*)	2.1 - 240	1.8 – 16.4	3 AsHCs 5 AsFAs	HPLC-ICP-MS HPLC-ESI-TOF-MS	Sele, V., et al. 2015

SNo.	Sample	Total Arsenic, µg As/g	AsLipid, µg As/g	AsLipid Speciation	Techniques	References
15.	Brown alga (*Saccharina latissimi*)	79 ± 1.4	3.2 ± 0.20	6 AsHCs 2 AsFAs 13 AsPLs	HPLC-ICP-MS HPLC-ESI-MS	Raab, A., et al. 2013
16.	Cod-liver oil	12.15		13 AsFAs 3 AsHCs	HPLC-ICP-MS HPLC-ESI-Orbitrap-MS	Amayo, K. A. et al. 2014a
17.	Muscle-tissue of four commercial fish species	4.87 ± 0.19 to 6.00 ± 0.04	2.33 ± 0.18 to 4.79 ± 0.15	3 AsFAs 5 AsHCs	HPLC-ICP-MS HPLC-ESI-Orbitrap-MS	Amayo, K. A. et al. 2014b
18.	Canned cod-liver oil	2.56 ± 0.01 to 5.50 ± 0.03	2.51 ± 0.02 to 5.38 ± 0.02	12 AsFAs 5 AsHCs	HPLC-ICP-MS HPLC-ESI-TOF-MS	Arroyo-Abad, U., et al. 2014
19.	CRM NMIJ 7405-a (Hijiki)	35.6 ± 0.9	3.42 ± 0.05	2 AsHCs 5 AsPLs	HPLC-ICP-MS HPLC-HR-ESI-Orbitrap-MS	Glabonjat, R. A. et al., 2014
20.	Blue whiting oil	2.16		4 AsFAs 7 AsHCs	HPLC-ICP-MS HPLC-ESI-MS	Taleshi, M. S. et al., 2014a
21.	Fish and algae	Synthesized		4 AsFAs 3 AsHCs	HPLC-ICP-MS HPLC-ESI-TOF-MS ^{13}C-NMR	Taleshi, M. S. et al., 2014b
22.	Liver of Northeast Arctic cod (*Gadus morhua*)	2.1 to 240	1.8 to 16.4	3 AsHCs 5 AsFAs	HPLC-ICP-MS HPLC-ESI-TOF-MS	Sele, V. et al., 2015

SNo.	Sample	Total Arsenic, µg As/g	AsLipid, µg As/g	AsLipid Speciation	Techniques	References
23.	Herring fillet (*Clupea harengus*), cod liver (*Gadus morhua*) and canned cod-liver	Synthesized		1 AsFAs (264) 1 AsHCs (374)	HPLC-ICP-MS HPLC-ESI-TOF-MS ^{1}H and ^{13}C-NMR	Arroyo-Abad, U., et al. 2016a
24.	Freshwater fish: bream (*Abramis brama*), ide (*Leuciscus idus*), asp (*Aspius aspius*), pike (*Esox lucius*)	0.09 - 0.16		4 AsFAs 2 AsHCs	HPLC-ICP-MS HPLC-ESI-TOF-MS	Arroyo-Abad, U., et al. 2016b
25.	Peruvian anchoveta (*Engraulis ringens*) fish oil	129	77.8	2 AsHCs 6 AsFAs	HPLC-ICP-MS HPLC-ESI-MS	Pereira, E. R., et al. 2016
26.	Herring Caviar (*Clupea harengus*)	0.8	0.64	5 AsPCs 7 AsFAs 6 AsHCs 1 AsPEs	HPLC-ICP-MS HPLC-ESI-MS	Viczek, S. A., et al. 2016
27.	Unicellular green alga (*Dunaliella tertiolecta*)	Synthesized		Arsenic-containing cyclic ethers	ESI-HRMS	Guttenberger, N., et al. 2016

SNo.	Sample	Total Arsenic, µg As/g	AsLipid, µg As/g	AsLipid Speciation	Techniques	References
28.	Fish oil	Synthesized (25)	25	2 AsFAs 1 AsHCs	HPLC-ICP-MS HPLC-ESI-MS ^{1}H and ^{13}C-NMR	Khan, M., et al., 2016
29.	Unicellular green alga (*Dunaliella tertiolecta*)	1500	10	AsSugPhytol	HPLC-ICP-MS HPLC-ESI-MS ^{1}H and ^{13}C-NMR	Glabonjat, R. A., et al. 2017
30.	Fish oil (proposed natural constituent)	Synthesized		2 AsTAGs	HPLC-ICP-MS HPLC-ESI-MS ^{1}H and ^{13}C-NMR	Guttenberger, N., et al. 2017
31.	Herring, Capelin and Blue Whiting fish meal and edible seaweed Dulse	4.5 (Herr) 3.9 (Cap) 18.0 (BW) 7.9 (Dulse)	0.5 – 1.2	6 AsFAs 3 AsHCs 2 TMAsFOH 4 AsPCs 1 AsPEs 2 AsPLs	HPLC-ICP-MS HPLC-ESI-MS	Petursdottir, A., et al. 2018
32.	Two brown algae (*Alaria esculenta* and *Saccharina latissimi*)	53 – 127 (SL) 43 – 116 (AE)	2.9 – 12.5 (SL) 3.7 – 8.7 (AE)	7 AsHCs 1 TMAsFOHs 14 AsPLs	HPLC-ICP-MS HPLC-ESI-MS	Petursdottir, A., et al. 2019
33.	17 Food composites	0.004 – 0.233	0.0033 – 0.004	7 AsHCs 6 AsPLs	HPLC-ICP-MS HPLC-ESI-MS	Al Amin M., et al., 2018

SNo.	Sample	Total Arsenic, µg As/g	AsLipid, µg As/g	AsLipid Speciation	Techniques	References
34.	18 seafood samples	3.3 – 191	0.006 – 0.241	3 AsHCs 2 AsFAs	HPLC-ICP-MS HPLC-ESI-MS	Al Amin M., et al., 2019
35.	Green alga Coccomyxa (*Trebouxiophyceae, Chlorophyta*)		1480 (*C. onubensis*) 2130 (*C. elongate*)	39 AsTAGs 15 AsPCs 8 AsPEs 6 AsPIs 2 AsPGs	HPLC-ICP-MS HPLC-ESI-MS	Rezanka, T., et al., 2019
36.	Edible brown alga Kombu (*Saccharina japonica*)	44.9 – 55.0	4.4 – 5.8	5 AsHCs 16 AsPLs (8 di-acyl and 8 mono-acyl)	HPLC-ICP-MS HPLC-ESI-MS ESI-HR-MS-MS	Yu, X., et al. 2018

Table 10: Arsenolipids identification in Seafood

Other approaches additionally utilized chemical derivatization in combination with accurate mass measurement using LC-ICP-MS/ESI-MS,[96,113] while others employed a combination of mass spectral data with degradation and derivatization experiments, partial synthesis and Nuclear Magnetic Resonance (NMR) spectroscopy.[119]

In some cases, confirmation was achieved by synthesis of standards that were characterized by HR-ESI-MS,[81,118,122,420,421] ESI-Q-TOF-MS,[7,80,105,111,120,121,124,421–423] GC/MS,[109,111] MALDI-FT-ICR-MS,[18] and NMR.[97,116,420,421,429]

In the absence of standards, confirmation of chemical structures was achieved by comparison with non-arsenic containing lipids founds in natural food sources, like fish oil.[81,122] Some research groups performed accurate mass measurement of a certified reference material (CRM) from the national metrology institute of Japan (CRM NMIJ 7405-a) to confirm the identities of arsenic species.[120,121,432]

Glabonjat et al.[119] confirmed the structures of AsSugar-Phytols using a combination of mass spectral data with degradation and derivatization experiments, partial synthesis, and NMR spectroscopy. Arroyo-Abad et al.[108] identified and confirmed the structures of AsHCs using a combination of ICP-MS, microwave induced plasma atomic emission spectrometry (MIP-AES), and Electron Impact quadrupole mass spectrometry (EI-qMS) after GC separation, while Ruiz-Chancho et al.[104] confirmed the identities of AsLipids in fish oil by comparison of the arsenic species with those published in literature. Sele et al.[110] on the other hand confirmed the identities of AsHCs in fish oils by performing accurate mass measurement using a combination of GC-ICP-MS, GC-MS/MS and Q-TOF-MS.

Almost 20% of the publications reporting on identification and characterization of AsLipids did not provide mass spectral data. For those that provided the mass spectral data, it was digitized by capturing the screenshots of the spectra which were saved in an Excel file. The following additional information was enumerated: the compounds ID, their elemental formula, the *m/z* of the precursor ions, the approximate relative intensities of all the ions in the mass spectra, and any additional information provided, like the proposed elemental composition of the product ions and the citations for the publications. The spectral data was compared with the *in silico* fragmentation data to validate the predicted spectral data.

6.3.2. Generation of predicted spectra for extant AsLipids

Identification of known metabolites is based on independent measurements of authentic standards. However, for novel and unknown molecules, which have not yet been chemically characterized, such standards and compound structural databases may not be available. Therefore, novel approaches beyond facile spectral comparison are required. Access to compound databases and tandem mass spectrometry data can play an important role in the identification of AsLipids.

Even though molecular structure databases such as PubChem and Chemspider are orders of magnitude larger than spectral libraries, they are still limited to compounds whose molecular structures have been previously identified and stored in such databases. Therefore, our approach to addressing this analytical challenge was to develop a spectral library using *in silico* fragmentation and machine learning of known AsLipids for structural elucidation and identification of unknown AsLipids.

A computationally derived mass spectral library for AsLipids with *in silico* generated structures was developed. To accomplish this, structural information, and experimental mass spectral data for all known AsLipids that are published in literature was extracted. Using ChemDraw 19.1, we obtained the SMILES notation of the chemical structures. The SMILES format of the chemical structures was used as input to train the CFM-ID 3.0 software to predict the compounds' ESI-MS/MS spectral data.

CFM-ID 3.0 is linked to METLIN database,[689] which has a collection of lipid structural and spectral information, but has very limited collection of structural information for AsLipids. CFM-ID 3.0 produced ESI-MS/MS spectra at low (10V), medium (20V), and high (40V) collision energy. The CFM-ID 3.0 predicted spectra output was graphically displayed and was available in a peak list format for ease of download as text files. The text files were saved as a list of 'mass intensity' pairs, each corresponding to a peak in the spectra for the AsLipids in both positive and negative ESI mode.

To generate a simple coherent single file that enables pattern recognition, the predicted *in silico* fragmentation text files from different compounds in the different AsLipids classes were combined using an R-script into two matrix files of positive and negative ESI modes. The matrix of fragmentation data for the individual AsLipids class was analyzed for pattern recognition and the results were saved in the matrix analysis summary sheet on the same Excel files, which presents a list of unique and common fragments for each AsLipids class.

These unique fragments are vital in helping to reduce the search space for unknown AsLipids and aid in their classification into specific compound classes that have

common basic structures. This is important because it is a key step in the identification of the unknown AsLipids and may even play a vital role in their toxicological studies.[684] New and unknown AsLipids metabolites have been encountered while undertaking toxicity studies. It is therefore imperative that their identities are established because they may be of toxicological significance.

To validate the *in silico* fragmentation data, the extant AsLipids experimental mass spectral data was collated in an Excel file showing their: compound identification, molecular formula, *m/z* of their precursor-ions, product-ions with their relative intensities, citations, and the published mass spectra. This data was then compared with the *in silico* AsLipids spectral data. There was good agreement between the *in silico* spectral data and the reported experimental spectral data.

The *in silico* tandem mass spectral data show great potential for filling the gap for the lacking AsLipids molecular database. The combination of the predicted compounds with the *in silico* MS/MS spectral data provides a molecular database on which the unknown experimental MS/MS spectra may be searched against for their identification. The spectral library is deployed on a web-based platform for ease of use and ready access to users with access to the Internet.

6.3.3. Creation of the searchable molecular database

High-throughput small molecule identification in complex samples typically requires the comparison of experimental features, like *m/z* or chromatographic retention times, to corresponding reference values in libraries in order to build evidence for the presence of a particular molecule.[690] Compound libraries can be generated experimentally

through analysis of authentic reference materials and standards or through *in silico* calculation of chemical properties for the prediction of analytical features.

Experimental analysis is the gold standard approach for building libraries, mainly because of the assumed lower associated variance of the derived properties from modern analytical platforms, which provides higher levels of confidence in compound identification and assignment.[691,692] However, most compounds, as is the case for AsLipids, are not commercially available as authentic reference materials, cannot be isolated or easily synthesized, or are still unknown.[693,694] Further, the experimental approach to building of libraries is costly and time consuming. In contrast, *in silico* methods can yield reference values rapidly, facilitating the generation of larger libraries than can reasonably be achieved using experimental approaches.[690]

The current trend is towards the development of purely computationally derived mass spectral libraries with large compound libraries such as PubChem or Chemspider utilized for input structures. However, since in our case AsLipids are not currently available in such libraries, we had to develop our own library of predicted compounds.

To this end, we initially generated a list of AsLipids that were categorized into ten different classes (see Table 10), based on their structural features and authors' grouping, including their citation with the aim of updating the current knowledge of AsLipids available in public repositories.

We further noticed the dearth of structural information on AsLipids in literature and public repositories, since only a few AsHCs and AsFAs existed in the PubChem

database. However, the bulk of the AsLipids, especially the complex ones like AsPCs, AsSugar-PLs and AsSugar-Phytols, were not available.

This discovery motivated the decision to develop the list of known and published AsLipids for ease of access and reference. This list is an important starting point towards developing a comprehensive public repository for AsLipids and is subject to continuous update as new information on AsLipids becomes available.

Literature review revealed ten classes of AsLipids based on their structural features, i.e., head groups and their sidechains. To predict structures for the different AsLipids classes, there was need to establish their generic structures in which the different sidechains were to be inserted. A scaffold of the core structures for the different classes of AsLipids was generated using ChemDraw 19.1 and was saved in SMILES format.

Different classes of sidechains, i.e., alkyl, acyl, terpenoid and fatty alcohols, were identified for which a list of possible sidechain structures was developed, and they were saved in their SMILES format in an Excel file. An R-scripts was prepared to combine the generic structures (see Table 9) for the different AsLipids classes with their possible sidechains to generate a list of predicted AsLipids presented in SMILES format.

The CFM-ID 3.0 spectrum prediction utility provides for the input of a neutral molecule, to which a proton is either added or removed by the program to form a $[M+H]^+$ or $[M-H]^-$ molecular ion, according to the input specifications of the ionization mode, whether positive or negative. Consequently, some of the AsLipids classes, especially the ones that already had a positive charge like As-PCs, As-PEs, and

TMAsFOHs were not able to be fragmented using CFM-ID 3.0 and thus had to be processed using CDM-ID 2.4 software with an R-based interface.

To avoid combinatorial explosion of the number of structures generated, we applied constraints. For example, we constrained the length of the alkyl carbon chains to C4 – C34, the double bonds in a single alkyl chain to 0 – 6 (mainly C18), and removed the trans-conformational structures, which are not commonly found in nature and are not reported in literature, leading to 78 possible alkyl sidechains.

Similar rules were applied to the acyl, terpenoid and fatty alcohol sidechains restricting the carbon chain lengths to C4 – C28, C4 – C20 and C4 – C30 respectively, leading to 63, 33 and 84 possible sidechains. Using this strategy, we reduced the AsTAGs from over one million possible structures to 250,046 structures and a total of 270,581 AsLipids predicted structures.

The generated list of predicted AsLipids structures was curated by physically checking the accuracy of a few selected predicted chemical structures in each AsLipids class. Some structural errors were discovered mostly related to the double-bond configuration for the unsaturated sidechains because R programming language doesn't work well with forward slashes (/), and thus ignored them while generating the final SMILES format of the predicted structures. This resulted in the predicted structures generating error messages in ChemDraw 19.1 during the validation of compound structures. The R script was edited, and the error was corrected.

The other source of error was related to the orientation of the inserted sidechains. There was a need to indicate directionality of fatty acids (reverse fatty acids) as SMILES

requires this directional notation for some classes of AsLipids. Therefore, a new set of fatty acid sidechains was generated with reversed orientation, which were inserted in the correct orientation to the generic structures.

The list of AsLipids predicted structures in SMILES was provided in table format in an Excel file. Additional meta data like the compound identification information typically used in literature, their head groups, and sidechains all in SMILES format is provided. Although the list of predicted AsLipids structures is by no means comprehensive, it is important because it provides a library of possible input of AsLipids structures that can be queried for the identification of unknown AsLipids.

It must be noted that not all the computationally generated structures exist in nature, while other potentially existing structures may have been missed due to the constraints applied. CFM-ID predicts the MS/MS spectra for candidate compounds computationally, which allows matching against any compound in a queried database, even if that database does not contain the spectrum.[688]

6.3.4. Searching the spectral library

The spectral library has a web-based graphical user interface that enables searches by compound or by unknown spectrum (see Fig. 48 below).

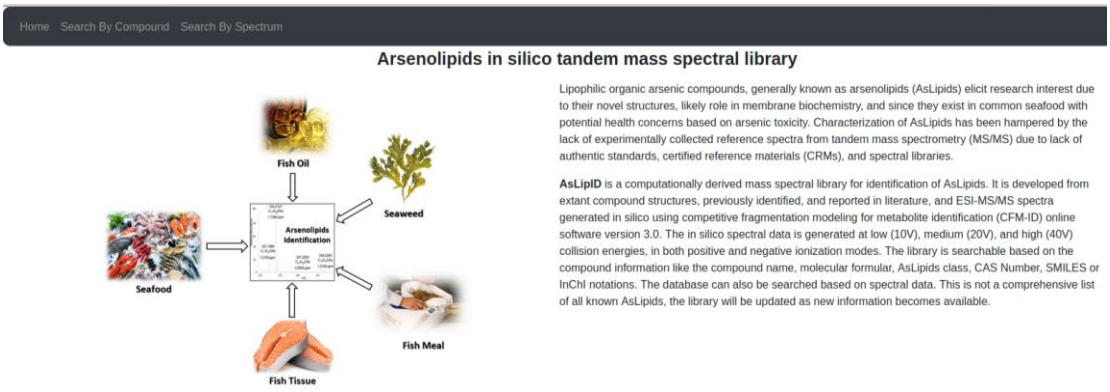


Figure 48: Arsenolipids *in silico* web-based mass spectral library home page

6.3.4.1. Search by Compound

Compound search can be performed by typing in search parameter and choosing one of the six search filters (see Fig. 49 below).

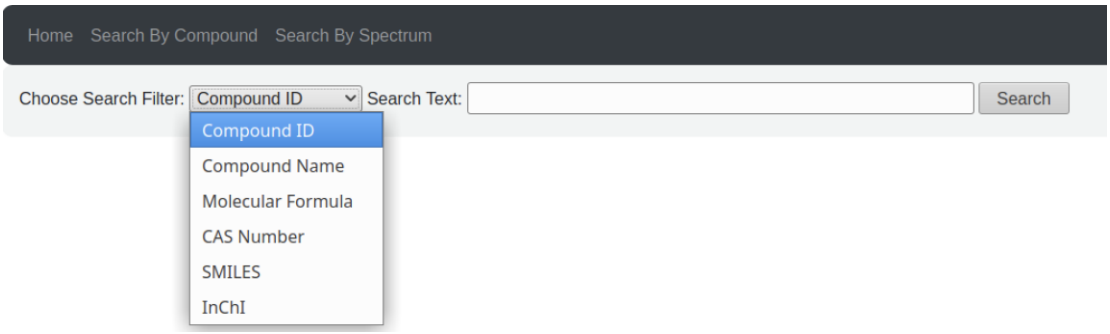
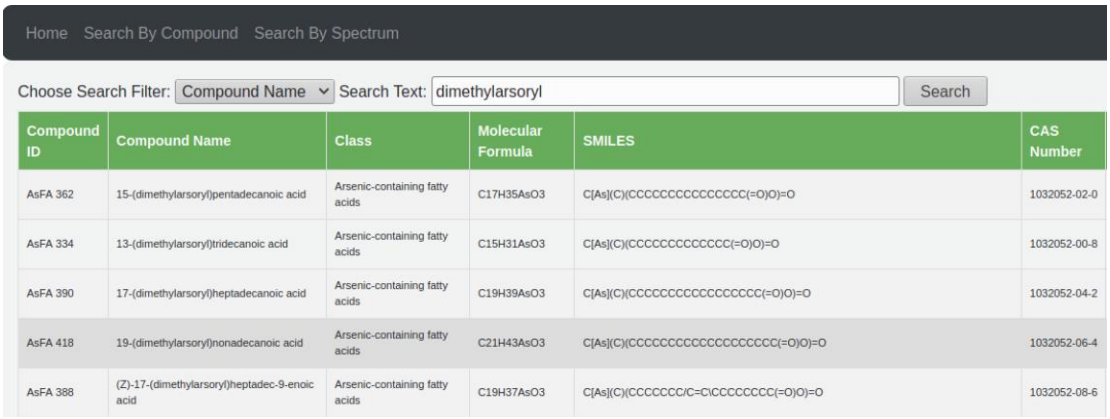


Figure 49: Web-based graphical user interface of search based on compound.

While filtering search based on Molecular Formula, CAS Number, SMILES and InChI provides an exact match of search input, searching on Compound ID and Compound name allows for partial search text matching. For example, searching for "dimethylarsoryl" and filtering on compound name will produce all compounds containing the word "dimethylarsoryl" in their name (see Fig. 50 below).



Compound ID	Compound Name	Class	Molecular Formula	SMILES	CAS Number
AsFA 362	15-(dimethylarsoryl)pentadecanoic acid	Arsenic-containing fatty acids	C17H35AsO3	C[As](C)(CCCCCCCCCCCCCCC(=O)O)=O	1032052-02-0
AsFA 334	13-(dimethylarsoryl)tridecanoic acid	Arsenic-containing fatty acids	C15H31AsO3	C[As](C)(CCCCCCCCCCCCC(=O)O)=O	1032052-00-8
AsFA 390	17-(dimethylarsoryl)heptadecanoic acid	Arsenic-containing fatty acids	C19H39AsO3	C[As](C)(CCCCCCCCCCCCCCCCC(=O)O)=O	1032052-04-2
AsFA 418	19-(dimethylarsoryl)nonadecanoic acid	Arsenic-containing fatty acids	C21H43AsO3	C[As](C)(CCCCCCCCCCCCCCCCCCC(=O)O)=O	1032052-06-4
AsFA 388	(Z)-17-(dimethylarsoryl)heptadec-9-enoic acid	Arsenic-containing fatty acids	C19H37AsO3	C[As](C)(CCCCCCC/C=C\CCCCCCCC(=O)O)=O	1032052-08-6

Figure 50: Output of partial search text matching.

Since the compound ID is composed of the short form of the compound class and the molecular weight, searching by compound ID in partial search text matching also enables filtering of search by compound class or molecular weight. For example, searching by AsHC would give a list of all arsenic-containing hydrocarbon compounds in the library, while searching a number like 362 will give you all the compounds with a similar molecular weight found in the library. To narrow your search to the exact compound one must provide the full compound ID. For example, ASHC 362.

6.3.4.2. Search by Spectrum

Spectral matching search can be performed by providing an unknown spectrum and matching it to one that is found in the library. The format of the unknown spectrum needs to be a set of fragmentation *m/z* with corresponding intensity values on each line separated by a white space (see Fig 51) as shown below.

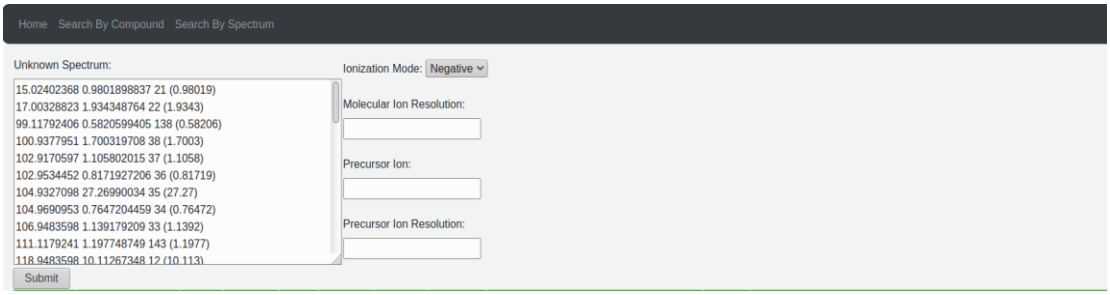


Figure 51: Web-based graphical user interface of search based on unknown spectrum.

Four filtering options are provided for searching by spectrum as follows:

6.3.4.2.1. Ionization Mode

This is a required field that indicates whether the spectrum was obtained in positive or negative ionization mode.

6.3.4.2.2. Molecular Ion Resolution

This is an optional field and will search to account for variable decimal point rounding in spectrum *m/z* values. If a value is provided, the search will be performed on the *m/z* in the range *m/z* - Molecular Ion Resolution and *m/z* + Molecular Ion Resolution.

6.3.4.2.3. Precursor Ion

This is an optional field. Entering precursor ion *m/z* will help narrow down the spectral search to spectra with the specified precursor *m/z* value.

6.3.4.2.4. Precursor Ion Resolution

This is an optional field that sets the lower and upper range for the precursor *m/z* values to be searched against. The precursor m/z search will be limited based on the specified tolerance for the precursor *m/z*.

Results based on spectral search are displayed in a tabular form (see Fig. 52). Clicking on the Compound ID, which is an active link, will display a visual representation of the unknown spectrum alongside the one found in the database (see Fig. 53).

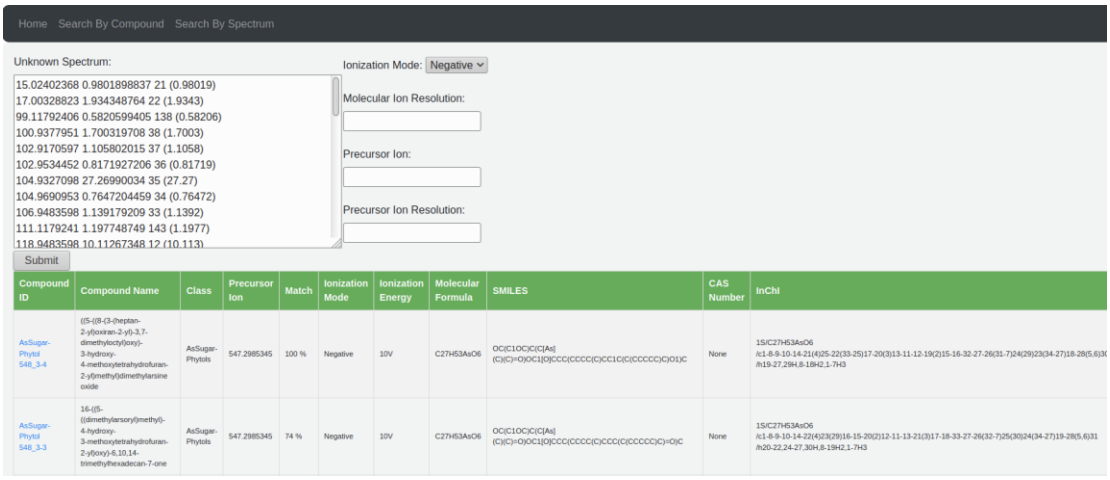


Figure 52: Output of database search based on spectrum showing the list of possible compounds.

The visual comparison of the matching spectrum from the library alongside the unknown spectrum is interactive by allowing the spectrum to be zoomed in and out for closer analysis.

Figure 53: Butterfly diagram showing how the unknown spectrum compares with *in silico* spectrum with associated metadata.

6.4. Conclusion

Typically, AsLipids are identified after reversed-phase separation using gradient elution and fractionation. Prior to identification, the AsLipids are extracted and detected into fractions of the sample extracts by the *m/z* 75 (As^+) signal of the ICP-MS.

AsLipids are separated into fractions according to their polarity and elution order in the reversed-phase column. The early eluting AsLipids species are usually more hydrophilic short carbon chain AsFAs, followed by medium chain highly saturated AsFAs, which are hard to separate chromatographically, but can be distinguished by mass spectrometry. This is followed by the more hydrophobic AsHCs mixed with some long chain AsFAs, the last eluting lipophilic organic arsenic species are usually the complex AsPCs, AsPEs, AsSugar-PLs and AsTAGs. The AsLipids are then identified based on their exact mass and product ion spectra gathered by ESI-MS or MALDI-MS. This process requires expensive instrumentation and high level of technical competence, which is not accessible to most laboratories.

In this work, we have demonstrated the development and utility of a comprehensive AsLipids structural database, which includes both the predicted and extant AsLipids reported in literature, and an accompanying *in silico* MS/MS spectral library. This tool will greatly enhance tandem mass spectrometry identification of these novel lipophilic organoarsenicals in marine and environmental samples by providing a complementary AsLipids library search.

The technique for development of the tool is transferable and applicable to other similar fields. More than 270,000 AsLipids structures were predicted and were used to generate

an *in silico* spectral search engine, which is in good agreement with the experimental data that is reported in literature. This provides a cheaper means of identification for these novel class of arsenic compounds employing their tandem mass spectral data.

This work, as is currently presented, serves as a proof of concept of the operation and functionality of the AsLipids search engine. The tool presented here is deployed using a web-based graphical user interface (GUI) that enhances the usability and accessibility of the system, since it will be readily available online.

Due to time constraints and challenges in accessibility to the laboratory owing to the COVID-19 pandemic, the envisioned acquisition of experimental mass spectral data to further validate and extend the AsLipids library was not possible. For this reason, secondary data that is published in literature was used. There was good agreement between the predicted and experimental mass spectral data. There is, however, still a need to collect data for further validation and extension of the mass spectral library.

Chapter 7: Future Perspectives.

7.1. Analytical Considerations

Seafood is a complex matrix and as such extraction of arsenic species in their native state poses the greatest challenge. There is no single method for extraction of all arsenic species and the extraction efficiency achieved for arsenic species, especially AsLipids in seafood is quite low. The prevalence of complex AsLipids, including AsPCs, AsPEs, and AsTAGs, has likely been underestimated in food due to the lability of these compounds once they are extracted from biological matrix.[106,114,122] In contrast to AsHCs and AsFAs, both of which are stable,[457] these complex AsLipids are presumed to be degraded by hydrolysis, in the presence of traces of water. This is due to a species dependent stability of the glycerol-ester bonds depending on the nature of the bound fatty acid.[684] More studies on the extraction conditions of AsLipids are required.

There is need to identify and quantify the arsenic species in the 'residual arsenic' fraction, which comprises a major proportion of total arsenic present and because this may be of toxicological relevance. Sample preparation issues are complicated as there is no widely accepted instrumental analytical method for the determination of arsenic species.

7.1.1. Extraction recovery

Oily matrices present a major challenge in the analysis of lipophilic arsenic species. Extraction recoveries of almost 60% of the total arsenic content have been reported in fatty seafood,[7,105,110] which emphasizes the need for more efficient sample extraction

methods. However, it must be recognized that a delicate balance must be maintained between desired higher extraction efficiency and arsenic species' integrity.

Improvement in the sample cleanup techniques may also allow access to more information from the lipophilic fraction of the sample extracts. Most of the work on the analysis of lipophilic arsenicals has employed liquid-liquid extraction (LLE), primarily with acetonitrile and methanol as extractants. These organic solvents have not produced complete extractions. There is still information to be gleaned from the hexane extracts of fatty and oily fish that is used to defat the fish prior to traditional analysis.

The dependence on HPLC-ICP-MS for the detection and quantification of arsenic has been a limiting factor due to signal enhancements associated with the use of common organic solvents, like hexane, which makes it difficult to optimize the separation and limits the detection. A detection technique that is not dependent on ICP-MS can allow use of other organic solvents, which may result in more selective extraction of lipophilic arsenic species. Also, a longer solvent interaction time may increase the extraction recovery of such lipophilic arsenic species in polar solvents.

Use of surfactants, though they are not amenable with currently employed detection techniques, may increase the extraction efficiency. In this work, we used SDS for the extraction of arsenic from geoduck and kelp, resulting in a general improvement in extraction efficiencies. The results were inconclusive since the enhancement in extraction efficiency could not be solely attributed to the use of the SDS surfactant. The solvent compositions, especially a combination of SDS and methanol/water mixture,

had a signal enhancing effect in geoduck but the same combination had a negative effect on kelp. This was not investigated further.

Since most seafood are high in protein, the use of proteolytic surfactants, which are amenable with mass spectrometry, may enhance the extraction of arsenic species, especially for those that are tightly bound to the proteins in the sample matrix (arsenopeptides). This might constitute a significant portion of the total arsenic since iAs^{III} forms strong sulfhydryl bonds with the amino acids that contain sulfur moieties, such as methionine. Aggressive extraction methods are therefore required to break such strong bonds and hence release the bound arsenic species.

Since toxicity is dependent on the chemical state of the arsenic species and given that trivalent arsenic species are known to be more toxic than their pentavalent counterparts, it may be prudent to attempt to establish the identity and metabolic fate of the residual arsenic. Residual arsenic, which is presumed to be in trivalent form, has unknown toxicity and it is important to establish its toxicological profiles in order to evaluate the actual risk associated with consumption of seafood.

7.1.2. Identification tool for novel arsenic species

Several non-polar lipophilic arsenicals have previously been detected, but not conclusively identified. Therefore, an improvement in the sample extraction procedure for such lipophilic arsenic species will remove interfering matrix components and concentrate them, thus facilitating their structural elucidation. This will involve an assessment of the various compositions of extraction solvents and cleanup procedures for the extracts. The use of ICP-MS for the detection and quantification of arsenic

species is a limiting factor, since it is the preferred element-specific detector but is not amenable with organic solvents used to solubilize arsenic species in lipid matrix.

The current approach for the identification of lipophilic arsenicals circumvents the dependence on the polarity of the extraction solutions, through the simultaneous use of electrospray ionization coupled to tandem (MS/MS) mass spectrometry and ICP-MS. This approach was first used in 2008 for the identification and quantification of AsLipids. The cost of instrumentation used, and the level of competence required to perform these tasks is beyond the reach of most laboratories, thus limiting the use of the technique. In addition, lack of publicly available spectral databases for the lipophilic compounds to facilitate their identification has impeded progress.

In this work, the development of an *in silico* library for identification of the novel lipophilic arsenic species without the need for experimental data and reference standards has been demonstrated. This library complements the extant measurement infrastructure using LC-ICP-MS/ESI-MS/MS for the identification and quantification of lipophilic arsenic species and allows the flexibility for further extension to incorporate new compounds to be identified in future. The library can further be enriched by validating the *in silico* spectral data with experimental data, obtained from analysis of standards and CRMs, and as more information becomes available.

To the best of our knowledge, there has only been one attempt to develop an *in silico* mass spectral database for lipophilic arsenic species. Coniglio et al.,[695] developed an *in silico* mass spectral database for AsSugar-PLs using Excel. This work presents a working prototype that demonstrates the proof of concept with regards to the operation

and functionality of the AsLipids search engine. The system deployed is web-based, which enhances its usability and accessibility, since online presence will ensure its availability to many users with access to the Internet.

7.2. Standards and certified reference materials (CRMs) needs

There are no commercially available standards, especially for lipophilic arsenicals. Therefore, the standards that have been used to date to confirm the identities of the novel arsenic species have been synthesized and characterized using NMR spectroscopy and tandem mass spectrometry. The synthesis of these compounds has been limited by safety concerns.

Commercially available standards of AsLipids and AsSugars are necessary for method development and optimization as well as for quality assurance. Currently, synthesis of arsenolipids appears to be the best option to address the need for commercially available analytical standards.

7.2.1. Synthetic and isotopically labeled standards needs

It is almost impossible to synthesize standards for all known arsenicals. The best approach is to synthesize the standards for arsenicals with known toxicities with their labeled counterparts. Due to the monoisotopic nature of arsenic, there are no isotopes that would enable the use of isotopically labeled internal standard approaches to quantitation. Instead the carbon and hydrogen heteroatoms of organic arsenic species can be labeled with ^{13}C and ^{2}H to achieve this purpose.

Labelled standards are important for the positive identification of unknown arsenic species, and their quantification. Concurrent use of these synthetic standards can be useful in overcoming coelutions, isobaric and polyatomic interferences associated with quantification of organic arsenic species using ICP-MS.

7.2.2. Matrix-matched CRMs needs

There currently are no matrix-matched CRMs available for lipophilic arsenicals in seafood and there are very few seafood CRMs with certified values for organic arsenic species. The available CRMs have certified values only for simple low molecular weight hydrophilic methylated arsenic species like DMA, AsB and AsSugars.

Few CRMs have certified values for higher molecular weight lipophilic arsenic species. Since matrix plays a major role in arsenic speciation, there is need to consider developing naturally incurred matrix-matched CRMs for proper commutability of the certified value when used in the laboratory.

7.3. Risk Assessment considerations

Current risk assessment of dietary exposure to arsenic is entirely based on inorganic forms and maximum allowable limits have been established for inorganic arsenic only.[696] This has largely been due to lack of relevant data i.e., arsenic speciation data and their concentrations in seafood, their toxicity and metabolism. Occurrence data for other arsenic compounds are needed and this is particularly true for arsenolipids, owing to their widespread presence in seafood (i.e. potential for human exposure) and emerging evidence regarding potential adverse effects.

Aside from iAs that is a well-characterized carcinogen, other organic arsenic species, e.g., AsHCs, AsFAs and AsPCs, present in seafood have been characterized and have been found to show some level of toxicities. Preliminary data show that some of the arsenolipids are cytotoxic to human cells,[42,44] and can cross the blood-brain barrier of the fruit fly.[101,697] Although only a few results are available so far, the suggestion has been made that bio-accessibility of arsenolipids, and perhaps toxicity as well, increases with the increasing lipid character displayed by the arsenolipids.[43,45,698]

This underscores the need for arsenic speciation analysis to ascertain the actual risk associated with consumption of seafood. Dietary studies have been performed to establish the consumption patterns and to identify the major sources and levels of human exposure to arsenic.

7.3.1. Toxicity studies

Estimation of arsenic toxicity requires the identification and quantification of the individual arsenic species. Since many new lipophilic arsenic species have been identified, and the list keeps growing in tandem with advances in technology, there is need for more toxicity studies on these new compounds to understand the potential sources of risk. Toxicity studies should not be limited to the identified organic arsenic species but should also be extended to their metabolites.

It has been established that most arsenicals are not acutely toxic, but their toxicity may emanate from metabolic transformations so understanding the fate and metabolism of organoarsenicals is essential. There are still many organic arsenic species with unknown toxicities, and they are assumed to be nontoxic because of the benign nature

of AsB, which is the most abundant organic arsenic species in seafood. However, these compounds are not known to be nontoxic, which justifies the need for toxicity studies on these new compounds.

7.3.2. Regulatory framework

All this information eventually needs to be systematically collated and evaluated with the objective of establishing regulatory limits, especially for organic arsenic species in seafood, which currently do not have set limits. Regulatory limits should be set for monitoring the arsenic species that are known to demonstrate toxicities. This information should also act as important input in guiding the development of analytical methods for speciation analysis, standards, and CRMs for seafoods.

7.4. Conclusion

There are at least six seafood-matrix reference materials currently available that have values for organoarsenic species. Five of the six are only certified for AsB (NMIJ CRM 7402-a, NMIJ 7403-a, DORM-4, TORT-3, and NIES-15), and BCR-627 is only certified for AsB and DMA. Newly released NIST SRM 3232 for Kelp, provides values for DMA as well as three AsSugars: AsSugar-OH, AsSugar-PO_4 and AsSugar-SO_3.[147] This calls for more work in the development of CRMs for seafood, which has been one contribution of this work.

All the samples employed in this study are either candidate reference materials (RMs) or materials meant for use in the NIST coordinated quality assurance programs (QAP). The measurements performed in this study contributes to the certification process for

the reference materials or property value assignment for the QAP materials. This work contributes to the development of the much needed CRMs for arsenic species, especially organic arsenic species, in seafood.

The quality assurance program in the form of interlaboratory comparisons plays an important role in providing an additional level of confidence in measurement results. It also acts as a tool for assessing the robustness of the analytical protocols by evaluating the equivalence of measurement results in the absence of a widely accepted method of analysis as is the case for arsenic speciation analysis.

The concurrent use of the synthetic standards and labeled synthetic standards as internal standards in combination with reliable and robust analytical methods for quantification of arsenic species will play an important role in the establishment of regulatory limits. In addition, with access to reliable analytical methods and availability of standards, the development of matrix-matched CRMs from common seafood will become a reality.

Bibliography

(1) Francesconi, K. A.; Edmonds, J. S. Arsenic and Marine Organisms. In *Advances in inorganic chemistry*; Sykes, A. G., Ed.; 1997; pp 147–189.

(2) Cullen, W. R.; Reimer, K. J. Arsenic Speciation in the Environment and Humans. *Chem. Rev.* **1989**, *89*, 713–764.

(3) Wang, Y. Y.; Wang, S.; Xu, P.; Liu, C.; Liu, M.; Wang, Y. Y.; Wang, C.; Zhang, C.; Ge, Y. Review of Arsenic Speciation, Toxicity and Metabolism in Microalgae. *Rev. Environ. Sci. Biotechnol.* **2015**, *14*, 427–451.

(4) Rahman, M. Azizur., Hasegawa, H., Lim, R. P. Bioaccumulation, Biotransformation and Tropic Transfer of Arsenic in Aquatic Food Chain: Review. *Environ. Res.* **2012**, *116*, 118–135.

(5) Baeyens, W.; Gao, Y.; de Galan, S.; Bilau, M.; van Larebeke, N.; Leermakers, M. Dietary Exposure to Total and Toxic Arsenic in Belgium: Importance of Arsenic Speciation in North Sea Fish. *Mol. Nutr. Food Res.* **2009**, *53* (5), 558–565.

(6) Schoof, R. A.; Yost, L. J.; Eickhoff, J.; Crecelius, E. A.; Cragin, D. W.; Meacher, D. M.; Mezel, D. B. A Market Basket Survey of Inorganic Arsenic in Food. *Food Chem. Toxicol.* **1999**, *37*, 839–846.

(7) Sele, V.; Sloth, J. J.; Holmelid, B.; Valdersnes, S.; Skov, K.; Amlund, H. Arsenic-Containing Fatty Acids and Hydrocarbons in Marine Oils - Determination Using Reversed-Phase HPLC-ICP-MS and HPLC-QTOF-MS. *Talanta* **2014**, *121*, 89–96.

(8) Molin, M.; Ulven, S. M.; Meltzer, H. M.; Alexander, J. Arsenic in the Human Food Chain, Biotransformation and Toxicology - Review Focusing on Seafood Arsenic. *J. Trace Elem. Med. Biol.* **2015**, *31*, 249–259.

(9) Thomas, D. J.; Bradham, K. Role of Complex Organic Arsenicals in Food in Aggregate Exposure to Arsenic. *J. Environ. Sci. (China)* **2016**, *49*, 86–96.

(10) IARC. *A Review of Human Carcinogens. Arsenic, Metals, Fibres and Dusts. Lyon: IARC Monographs 100*; International Agency for Research on Cancer (IARC): Lyon, 2012; Vol. 100.

(11) Sele, V.; Sloth, J. J.; Lundebye, A. K.; Larsen, E. H.; Berntssen, M. H. G.; Amlund,

H. Arsenolipids in Marine Oils and Fats: A Review of Occurrence, Chemistry and Future Research Needs. *Food Chem.* **2012**, *133*, 618–630.

(12) Maher, W. A.; Ellwood, M. J.; Krikowa, F.; Raber, G.; Foster, S. Measurement of Arsenic Species in Environmental, Biological Fluids and Food Samples by HPLC-ICPMS and HPLC-HG-AFS. *J. Anal. At. Spectrom.* **2015**, *30*, 2129–2183.

(13) Popowich, A.; Zhang, Q.; Chris Le, X. Arsenobetaine: The Ongoing Mystery. *Natl. Sci. Rev.* **2016**, *3*, 451–458.

(14) EFSA. Scientific Opinion on Arsenic in Food. *EFSA J.* **2009**, *7*, 1351.

(15) Lunde, G. Analysis of Arsenic in Marine Oil by Neutron Activation: Evidence of Arseno Organic Compounds. *J. Am. Oil Chem. Soc.* **1968**, *45*, 331–332.

(16) Schmeisser, E.; Goessler, W.; Kienzl, N.; Francesconi, K. A. Direct Measurement of Lipid-Soluble Arsenic Species in Biological Samples with HPLC-ICPMS. *Analyst* **2005**, *130* (6), 948–955.

(17) Rumpler, A.; Edmonds, J. S.; Katsu, M.; Jensen, K. B.; Goessler, W.; Raber, G.; Gunnlaugsdottir, H.; Francesconi, K. A. Arsenic-Containing Long-Chain Fatty Acids in Cod-Liver Oil: A Result of Biosynthetic Infidelity? *Angew. Chemie - Int. Ed.* **2008**, *47* (14), 2705–2707.

(18) Taleshi, M. S.; Jensen, K. B.; Raber, G.; Edmonds, J. S.; Gunnlaugsdottir, H.; Francesconi, K. A. Arsenic-Containing Hydrocarbons: Natural Compounds in Oil from the Fish Capelin, Mallotus Villosus. *Chem. Commun.* **2008**, *39*, 4706–4707.

(19) Maulvault, A. L.; Anacleto, P.; Barbosa, V.; Sloth, J. J.; Rasmussen, R. R.; Tediosi, A.; Fernandez-Tejedor, M.; van den Heuvel, F. H. M.; Kotterman, M.; Marques, A. Toxic Elements and Speciation in Seafood Samples from Different Contaminated Sites in Europe. *Environ. Res.* **2015**, *143*, 72–81.

(20) Kaise, T.; Wanatabe, S.; Itoh, K. The Acute Toxicity of Arsenobetaine. *Chemosphere* **1985**, *14*, 1327–1332.

(21) Francesconi, K. A. Arsenic Species in Seafood: Origin and Human Health Implications. *Pure Appl. Chem.* **2010**, *82* (2), 373–381.

(22) European Commission. *Reports of Experts Participating in Task 3.2.1.1 for Scientific Co-Operation.*; European Commission Directorate-General Health and Consumer

Protection., 2004.

(23) Dixon, B. F. H. The Biochemical Action of Arsonic Acids Especially as Phosphate Analogues. *Adv. Inorg. Chem.* **1997**, *44*, 191–228.

(24) Geiszinger, A.; Goessler, W.; Pedersen, S. N.; Francesconi, K. A. *Arsenic Biotransformation by the Brown Macroalga Fucus Serratus*; 2001; Vol. 20, pp 2255–2262.

(25) Yancey, P. H.; Clark, M. E.; Hand, S. C.; Bowlus, R. D.; Somero, G. N. Living with Water Stress : Evolution of Osmolyte Systems. *Science (80-.).* **1982**, *217* (4566), 1214–1222.

(26) Clowes, A. L.; Francesconi, K. A. Uptake and Elimination of Arsenobetaine by the Mussel Mytilus Edulis Is Related to Salinity. *Comp. Biochem. Physiol. Part C* **2004**, *137*, 35–42.

(27) Gailer, J.; Irgolic, K. J. The Ion-Chromatographic Behavior of Arsenite, Arsenate, Methylarsonic Acid and Dimethylarsinic Acid on the Hamilton PRP-X100 Anion-Exchange Column. *Appl. Organomet. Chem.* **1994**, *8*, 129–140.

(28) Larsen, E. H.; Francesconi, K. A. Arsenic Concentrations Correlate with Salinity for Fish Taken from the North Sea and Baltic Waters. *J. Mar. Biol. Assoc. United Kingdom* **2003**, *83*, 283–284.

(29) Volkman, J. K.; Barrett, S. M.; Blackburn, S. I.; Mansour, M. P.; Sikes, E. L.; Gelin, F. Microalgal Biomarkers: A Review of Recent Research Developments. *Org. Geochem.* **1998**, *29* (5-7–7 pt 2), 1163–1179.

(30) Van Mooy, B. A. S.; Fredricks, H. F.; Pedler, B. E.; Dyhrman, S. T.; Karl, D. M.; Koblížek, M.; Lomas, M. W.; Mincer, T. J.; Moore, L. R.; Moutin, T.; et al. Phytoplankton in the Ocean Use Non-Phosphorus Lipids in Response to Phosphorus Scarcity. *Nature* **2009**, *458* (7234), 69–72.

(31) Taylor, V. F.; Goodale, B.; Raab, A.; Schwerdtle, T.; Reimer, K.; Conklin, S.; Karagas, M. R.; Francesconi, K. A. Human Exposure to Organic Arsenic Species from Seafood. *Sci. Total Environ.* **2017**, *580*, 266–282.

(32) Davis, M. A.; Signes-pastor, A. J.; Argos, M.; Slaughter, F.; Pendergrast, C.; Punshon, T.; Gossai, A.; Ahsan, H.; Karagas, M. R. Assessment of Human Dietary Exposure to Arsenic through Rice. *Sci. Total Environ.* **2017**, *586*, 1237–1244.

(33) Hoffmann, T.; Warmbold, B.; Smits, S. H. J.; Tschapek, B.; Ronzheimer, S.; Bashir, A.; Chen, C.; Rolbetzki, A.; Pittelkow, M.; Jebbar, M.; et al. Arsenobetaine: An Ecophysiologically Important Organoarsenical Confers Cytoprotection against Osmotic Stress and Growth Temperature Extremes. *Environ. Microbiol.* **2018**, *20*, 305–323.

(34) Gilbert-Diamond, D.; Emond, J. A.; Baker, E. R.; Korrick, S. A.; Karagas, M. R. Relation between in Utero Arsenic Exposure and Birth Outcomes in a Cohort of Mothers and Their Newborns from New Hampshire. *Environ. Health Perspect.* **2016**, *124* (8), 1299–1307.

(35) Davis, M. A.; Signes-Pastor, A. J.; Argos, M.; Slaughter, F.; Pendergrast, C.; Punshon, T.; Gossai, A.; Ahsan, H.; Karagas, M. R. Assessment of Human Dietary Exposure to Arsenic through Rice. *Sci. Total Environ.* **2017**, *586*, 1237–1244.

(36) Baris, D.; Waddell, R.; Beane Freeman, L. E.; Schwenn, M.; Colt, J. S.; Ayotte, J. D.; Ward, M. H.; Nuckols, J.; Schned, A.; Jackson, B.; et al. Elevated Bladder Cancer in Northern New England: The Role of Drinking Water and Arsenic. *J. Natl. Cancer Inst.* **2016**, *108* (9), 1–9.

(37) Kalantzi, I.; Mylona, K.; Sofoulaki, K.; Tsapakis, M.; Pergantis, S. A. Arsenic Speciation in Fish from Greek Coastal Areas. *J. Environ. Sci. (China)* **2017**, *56*, 300–312.

(38) Moreda-Piñeiro, J.; Alonso-Rodríguez, E.; Romarís-Hortas, V.; Moreda-Piñeiro, A.; López-Mahía, P.; Muniategui-Lorenzo, S.; Prada-Rodríguez, D.; Bermejo-Barrera, P. Assessment of the Bioavailability of Toxic and Non-Toxic Arsenic Species in Seafood Samples. *Food Chem.* **2012**, *130* (3), 552–560.

(39) Sattar, A.; Xie, S.; Hafeez, M. A.; Wang, X.; Hussain, H. I.; Iqbal, Z.; Pan, Y.; Iqbal, M.; Shabbir, M. A.; Yuan, Z. Metabolism and Toxicity of Arsenicals in Mammals. *Environ. Toxicol. Pharmacol.* **2016**, *48*, 214–224.

(40) Leffers, L.; Ebert, F.; Taleshi, M. S.; Francesconi, K. A.; Schwerdtle, T. In Vitro Toxicological Characterization of Two Arsenosugars and Their Metabolites. *Mol. Nutr. Food Res.* **2013**, *57*, 1270–1282.

(41) Leffers, L.; Unterberg, M.; Bartel, M.; Hoppe, C.; Pieper, I.; Stertmann, J.; Ebert, F.; Humpf, H. U.; Schwerdtle, T. In Vitro Toxicological Characterisation of the S-

Containing Arsenic Metabolites Thio-Dimethylarsinic Acid and Dimethylarsinic Glutathione. *Toxicology* **2013**, *305*, 109–119.

(42) Meyer, S.; Matissek, M.; Müller, S. M.; Taleshi, M. S.; Ebert, F.; Francesconi, K. A.; Schwerdtle, T. In Vitro Toxicological Characterisation of Three Arsenic-Containing Hydrocarbons. *Metallomics* **2014**, *6* (5), 1023–1033.

(43) Meyer, S.; Raber, G.; Ebert, F.; Leffers, L.; Müller, S. M.; Taleshi, M. S.; Francesconi, K. A.; Schwerdtle, T. In Vitro Toxicological Characterisation of Arsenic-Containing Fatty Acids and Three of Their Metabolites. *Toxicol. Res. (Camb).* **2015**, *4*, 1289–1296.

(44) Meyer, S.; Schulz, J.; Jeibmann, A.; Taleshi, M. S.; Ebert, F.; Francesconi, K. A.; Schwerdtle, T. Arsenic-Containing Hydrocarbons Are Toxic in the in Vivo Model Drosophila Melanogaster. *Metallomics* **2014**, *6* (11), 2010–2014.

(45) Meyer, S.; Raber, G.; Ebert, F.; Taleshi, M. S.; Francesconi, K. A.; Schwerdtle, T. Arsenic-Containing Hydrocarbons and Arsenic-Containing Fatty Acids: Transfer across and Presystemic Metabolism in the Caco-2 Intestinal Barrier Model. *Mol. Nutr. Food Res.* **2015**, *59* (10), 2044–2056.

(46) Caumette, G.; Koch, I.; Reimer, K. J. Arsenobetaine Formation in Plankton: A Review of Studies at the Base of the Aquatic Food Chain. *J. Environ. Monit.* **2012**, *14* (11), 2841–2853.

(47) Ronan, J. M.; Stengel, D. B.; Raab, A.; Feldmann, J.; O'Hea, L.; Bralatei, E.; McGovern, E. High Proportions of Inorganic Arsenic in Laminaria Digitata but Not in Ascophyllum Nodosum Samples from Ireland. *Chemosphere* **2017**, *186*, 17–23.

(48) Miyashita, S. I.; Fujiwara, S.; Tsuzuki, M.; Kaise, T. Cyanobacteria Produce Arsenosugars. *Environ. Chem.* **2012**, *9*, 474–484.

(49) Šlejkovec, Z.; Stajnko, A.; Falnoga, I.; Lipej, L.; Mazej, D.; Horvat, M.; Faganeli, J. Bioaccumulation of Arsenic Species in Rays from the Northern Adriatic Sea. *Int. J. Mol. Sci.* **2014**, *15*, 22073–22091.

(50) Uneyama, C.; Toda, M.; Yamamoto, M.; Morikawa, K. Arsenic in Various Foods: Cumulative Data. *Food Addit. Contam.* **2007**, *24*, 447–534.

(51) Wu, X.; Gao, M.; Wang, L.; Luo, Y.; Bi, R.; Li, L.; Xie, L. The Arsenic Content in Marketed Seafood and Associated Health Risks for the Residents of Shandong, China.

Ecotoxicol. Environ. Saf. **2014**, *102*, 168–173.

(52) Juskelis, R.; Li, W.; Nelson, J.; Cappozzo, J. C. Arsenic Speciation in Rice Cereals for Infants. *J. Agric. Food Chem.* **2013**, *61* (45), 10670–10676.

(53) Lorenzana, R.; Yeow, A.; Colman, J.; Chappell, L.; Choudhury, H. Arsenic in Seafood: Speciation Issues for Human Health Risk Assessment. *Hum. Ecol. Risk Assess.* **2009**, *15*, 185–200.

(54) Whaley-Martin, K. J.; Koch, I.; Moriarty, M.; Reimer, K. J. Arsenic Speciation in Blue Mussels (Mytilus Edulis) along a Highly Contaminated Arsenic Gradient. *Environ. Sci. Technol.* **2012**, *46* (6), 3110–3118.

(55) Sloth, J. J.; Julshamn, K. Survey of Total and Inorganic Arsenic Content in Blue Mussels (Mytilus Edulis L.) from Norwegian Fiords: Revelation of Unusual High Levels of Inorganic Arsenic. *J. Agric. Food Chem.* **2008**, *56*, 1269–1273.

(56) Whaley-Martin, K. J.; Koch, I.; Reimer, K. J. Determination of Arsenic Species in Edible Periwinkles (Littorina Littorea) by HPLC-ICPMS and XAS along a Contamination Gradient. *Sci. Total Environ.* **2013**, *456–457*, 148–153.

(57) OHA. *Technical Report: Soft-Shell Clam Advisory for the Oregon Coast*; 2015.

(58) Julshamn, K.; Nilsen, B. M.; Frantzen, S.; Valdersnes, S.; Maage, A.; Nedreaas, K.; Sloth, J. J. Total and Inorganic Arsenic in Fish Samples from Norwegian Waters. *Food Addit. Contam. Part B Surveill.* **2012**, *5* (4), 229–235.

(59) EFSA. Dietary Exposure to Inorganic Arsenic in the European Population. *EFSA J.* **2014**, *12* (3597), 1–68.

(60) Lynch, H. N.; Greenberg, G. I.; Pollock, M. C.; Lewis, A. S. A Comprehensive Evaluation of Inorganic Arsenic in Food and Considerations for Dietary Intake Analyses. *Sci. Total Environ.* **2014**, *496*, 299–313.

(61) Petursdottir, A. H.; Sloth, J. J.; Feldmann, J. Introduction of Regulations for Arsenic in Feed and Food with Emphasis on Inorganic Arsenic, and Implications for Analytical Chemistry. *Anal. Bioanal. Chem.* **2015**, *407* (28), 8385–8396.

(62) Shibata, Y.; Sekiguchi, M.; Otsuki, A.; Morita, M. Arsenic Compounds in Zoo- and Phyto-Plankton of Marine Origin. *Appl. Organomet. Chem.* **1996**, *10*, 713–719.

(63) Almela, C.; Jesús Clemente, M.; Vélez, D.; Montoro, R. Total Arsenic, Inorganic

Arsenic, Lead and Cadmium Contents in Edible Seaweed Sold in Spain. *Food Chem. Toxicol.* **2006**, *44* (11), 1901–1908.

(64) Hirata, S.; Toshimitsu, H. Determination of Arsenic Species and Arsenosugars in Marine Samples by HPLC-ICP-MS. *Appl. Organomet. Chem.* **2007**, *21* (6), 447–454.

(65) Rose, M.; Lewis, J.; Langford, N.; Baxter, M.; Origgi, S.; Barber, M.; MacBain, H.; Thomas, K. Arsenic in Seaweed-Forms, Concentration and Dietary Exposure. *Food Chem. Toxicol.* **2007**, *45* (7), 1263–1267.

(66) Tukai, R.; Maher, W. A.; McNaught, I. J.; Ellwood, M. J. Measurement of Arsenic Species in Marine Macroalgae by Microwave-Assisted Extraction and High Performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry. *Anal. Chim. Acta* **2002**, *457* (2), 173–185.

(67) Fricke, M. W.; Creed, P. A.; Parks, A. N.; Shoemaker, J. A.; Schwegel, C. A.; Creed, J. T. Extraction and Detection of a New Arsine Sulfide Containing Arsenosugar in Molluscs by IC-ICP-MS and IC-ESI-MS/MS. *J. Anal. At. Spectrom.* **2004**, *19* (11), 1454–1459.

(68) Moreda-Piñeiro, J.; Alonso-Rodríguez, E.; Moreda-Piñeiro, A.; Moscoso-Pérez, C.; Muniategui-Lorenzo, S.; López-Mahía, P.; Prada-Rodríguez, D.; Bermejo-Barrera, P. Simultaneous Pressurized Enzymatic Hydrolysis Extraction and Clean up for Arsenic Speciation in Seafood Samples before High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry Determination. *Anal. Chim. Acta* **2010**, *679*, 63–73.

(69) Francesconi, K. A.; Edmonds, J. S.; Hatcher, B. G. Examination of the Arsenic Constituents of the Herbivorous Marine Gastropod Tectus Pyramis: Isolation of Tetramethylarsonium Ion. *Comp. Biochem. Physiol. Part C, Comp.* **1988**, *90* (2), 313–316.

(70) Cullen, W. R. Chemical Mechanism of Arsenic Biomethylation. *Chem. Res. Toxicol.* **2014**, *27* (4), 457–461.

(71) Hayakawa, T.; Kobayashi, Y.; Cui, X.; Hirano, S. A New Metabolic Pathway of Arsenite : Arsenic – Glutathione Complexes Are Substrates for Human Arsenic Methyltransferase Cyt19. *Arch. Toxicol.* **2005**, *79*, 183–191.

(72) Naranmandura, H.; Suzuki, N.; Suzuki, K. T. Trivalent Arsenicals Are Bound to

Proteins during Reductive Methylation. *Chem. Res. Toxicol.* **2006**, *19* (8), 1010–1018.

(73) Francesconi, K. A.; Edmonds, J. S.; Stick, R. V. Accumulation of Arsenic in Yelloweye Mullet (Aldrichetta Forsteri) Following Oral Administration of Organoarsenic Compounds and Arsenate. *Sci. Total Environ.* **1989**.

(74) Hanaoka, K.; Ohno, H.; Wada, N.; Ueno, S.; Goessler, W.; Kuehnelt, D.; Schlagenhaufen, C.; Kaise, T.; Irgolic, K. J. Occurrence of Organo-Arsenicals in Jellyfishes and Their Mucus. *Chemosphere* **2001**, *44* (4), 743–749.

(75) Challenger, F. Biological Methylation. 1944, pp 315–361.

(76) Nearing, M. M.; Koch, I.; Reimer, K. J. Arsenic Speciation in Edible Mushrooms. *Environ. Sci. Technol.* **2014**, *48* (24), 14203–14210.

(77) Francesconi, K. A.; Hunter, D. A.; Bachmann, B.; Raber, G.; Goessler, W. Uptake and Transformation of Arsenosugars in the Shrimp Crangon Crangon. *Appl. Organomet. Chem.* **1999**, *13*, 669–679.

(78) Thomson, D.; Maher, W.; Foster, S. Arsenic and Selected Elements in Marine Angiosperms, South-East Coast, NSW, Australia. *Appl. Organomet. Chem.* **2007**, *21*, 381–395.

(79) Shibata, Y.; Morita, M. Characterization of Organic Arsenic Compounds in Bivalves. *Appl. Organomet. Chem.* **1992**, *6*, 343–349.

(80) Lischka, S.; Arroyo-Abad, U.; Mattusch, J.; Kühn, A.; Piechotta, C. The High Diversity of Arsenolipids in Herring Fillet (Clupea Harengus). *Talanta* **2013**, *110*, 144–152.

(81) Taleshi, M. S.; Edmonds, J. S.; Goessler, W.; Ruiz-Chancho, M. J.; Raber, G.; Jensen, K. B.; Francesconi, K. A. Arsenic-Containing Lipids Are Natural Constituents of Sashimi Tuna. *Environ. Sci. Technol.* **2010**, *44* (4), 1478–1483.

(82) Edmonds, J. S.; Francesconi, K. A. Arseno-Sugars from Brown Kelp (Ecklonia Radiata) as Intermediates in Cycling of Arsenic in a Marine Ecosystem. *Nature* **1981**, *289* (5798), 602–604.

(83) Edmonds, J. S.; Francesconi, K. A. Arsenic-Containing Ribofuranosides: Isolation from Brown Kelp Ecklonia Radiata and Nuclear Magnetic Resonance Spectra. *J. Chem. Soc. Perkin Trans. 1* **1983**, No. Id, 2375–2382.

(84) Edmonds, J. S.; Francesconi, K. A. Transformations of Arsenic in the Marine Environment. *Experientia* **1987**, *43* (5), 553=557.

(85) De Bettencourt, A. M.; Duarte, M. F.; Florêncio, M. H.; Henriques, F. F.; Madeira, P. A.; Portela, M. I.; Vilas-Boas, L. F. Possible Key Intermediates in Arsenic Biochemistry: Synthesis and Identification by Liquid Chromatography Electrospray Ionization Mass Spectrometry and High Resolution Mass Spectrometry. *Microchem. J.* **2011**, *99* (2), 218–234.

(86) Edmonds, J. S.; Shibata, Y.; Yang, F.; Morita, M. Isolation and Synthesis of 1-Deoxy-1-Dimethylarsinoylribitol-5-Sulfate, a Natural Constituent of Chondria Crassicaulis and Other Red Algae. *Tetrahedron Lett.* **1997**, *38* (33), 5819–5820.

(87) Duncan, E. G.; Maher, W. A.; Foster, S. D.; Mikac, K. M.; Krikowa, F. The Influence of Bacteria on the Arsenic Species Produced by Laboratory Cultures of the Marine Phytoplankton Dunaliella Tertiolecta. *J. Appl. Phycol.* **2014**, *26* (5), 2129–2134.

(88) Duncan, E. G.; Maher, W. A.; Foster, S. D.; Krikowa, F. The Influence of Arsenate and Phosphate Exposure on Arsenic Uptake, Metabolism and Species Formation in the Marine Phytoplankton Dunaliella Tertiolecta. *Mar. Chem.* **2013**, *157*, 78–85.

(89) Duncan, E. G.; Maher, W. A.; Foster, S. D.; Krikowa, F. Influence of Culture Regime on Arsenic Cycling by the Marine Phytoplankton Dunaliella Tertiolecta and Thalassiosira Pseudonana. *Environ. Chem.* **2013**, *10* (2), 91–101.

(90) Larsen, E. H.; Quétel, C. R.; Munoz, R.; Fiala-Medioni, A.; Donard, O. F. X. Arsenic Speciation in Shrimp and Mussel from the Mid-Atlantic Hydrothermal Vents. *Mar. Chem.* **1997**, *57* (3–4), 341–346.

(91) Taylor, V. F.; Jackson, B. P.; Siegfried, M.; Navratilova, J.; Francesconi, K. A.; Kirshtein, J.; Voytek, M. Arsenic Speciation in Food Chains from Mid-Atlantic Hydrothermal Vents. *Environ. Chem.* **2012**, *9* (2), 130–138.

(92) Granchinho, S. C. R.; Franz, C. M.; Polishchuk, E.; Cullen, W. R.; Reimer, K. J. Transformation of Arsenic(V) by the Fungus Fusarium Oxysporum Melonis Isolated from the Alga Fucus Gardneri. *Appl. Organomet. Chem.* **2002**, *16* (12), 721–726.

(93) Feldmann, J.; Krupp, E. M. Critical Review or Scientific Opinion Paper: Arsenosugars-a Class of Benign Arsenic Species or Justification for Developing Partly Speciated Arsenic Fractionation in Foodstuffs? *Anal. Bioanal. Chem.* **2011**, *399*,

1735–1741.

(94) Taylor, V. F.; Jackson, B. P. Concentrations and Speciation of Arsenic in New England Seaweed Species Harvested for Food and Agriculture. *Chemosphere* **2016**, *163*, 6–13.

(95) Ma, Z.; Wu, M.; Yu, H.; Shang, T.; Zhang, T.; Zhao, M.; Lin, L. Total and Inorganic Arsenic Contents in Seaweeds: Absorption, Accumulation, Transformation and Toxicity. *Aquaculture*. 2018, pp 49–55.

(96) Glabonjat, R. A.; Raber, G.; Jensen, K. B.; Ehgartner, J.; Francesconi, K. A. Quantification of Arsenolipids in the Certified Reference Material NMIJ 7405-a (Hijiki) Using HPLC/Mass Spectrometry after Chemical Derivatization. *Anal. Chem.* **2014**, *86*, 10282–10287.

(97) Morita, M.; Shibata, Y. Isolation and Identification of Arseno-Lipid from a Brown Alga, Undaria Pinnatifida (Wakame). *Chemosphere* **1988**, *17* (6), 1147–1152.

(98) Müller, S. M.; Finke, H.; Ebert, F.; Kopp, J. F.; Schumacher, F.; Kleuser, B.; Francesconi, K. A.; Raber, G.; Schwerdtle, T. Arsenic-Containing Hydrocarbons: Effects on Gene Expression, Epigenetics, and Biotransformation in HepG2 Cells. *Arch. Toxicol.* **2018**, *92*, 1751–1765.

(99) Müller, S. M.; Ebert, F.; Raber, G.; Meyer, S.; Bornhorst, J.; Hüwel, S.; Galla, H. J.; Francesconi, K. A.; Schwerdtle, T. Effects of Arsenolipids on in Vitro Blood-Brain Barrier Model. *Arch. Toxicol.* **2018**, *92*, 823–832.

(100) Müller, S. M.; Ebert, F.; Bornhorst, J.; Galla, H.; Francesconi, K. A.; Schwerdtle, T. Arsenic-Containing Hydrocarbons Disrupt a Model in Vitro Blood- Cerebrospinal Fluid Barrier. *J. Trace Elem. Med. Biol.* **2018**, *49*, 171–177.

(101) Niehoff, A. C.; Schulz, J.; Soltwisch, J.; Meyer, S.; Kettling, H.; Sperling, M.; Jeibmann, A.; Dreisewerd, K.; Francesconi, K. A.; Schwerdtle, T.; et al. Imaging by Elemental and Molecular Mass Spectrometry Reveals the Uptake of an Arsenolipid in the Brain of Drosophila Melanogaster. *Anal. Chem.* **2016**, *88*, 5258–5263.

(102) Amayo, K. O.; Petursdottir, A.; Newcombe, C.; Gunnlaugsdottir, H.; Raab, A.; Krupp, E. M.; Feldmann, J. Identification and Quantification of Arsenolipids Using Reversed-Phase HPLC Coupled Simultaneously to High-Resolution ICPMS and High-Resolution Electrospray MS without Species-Specific Standards. *Anal. Chem.* **2011**,

83, 3589–3595.

(103) Amayo, K. O.; Raab, A.; Krupp, E. M.; Marschall, T.; Horsfall, M.; Feldmann, J. Arsenolipids Show Different Profiles in Muscle Tissues of Four Commercial Fish Species. *J. Trace Elem. Med. Biol.* **2014**, *28*, 131–137.

(104) Ruiz-Chancho, M. J.; Taleshi, M. S.; Goessler, W.; Francesconi, K. A. A Method for Screening Arsenolipids in Fish Oils by HPLC-ICPMS. *J. Anal. At. Spectrom.* **2012**, *27*, 501–504.

(105) Sele, V.; Sloth, J. J.; Julshamn, K.; Skov, K.; Amlund, H. A Study of Lipid- and Water-Soluble Arsenic Species in Liver of Northeast Arctic Cod (Gadus Morhua) Containing High Levels of Total Arsenic. *J. Trace Elem. Med. Biol.* **2015**, *30*, 171–179.

(106) Pereira, É. R.; Kopp, J. F.; Raab, A.; Krupp, E. M.; Menoyo, J. D. C.; Carasek, E.; Welz, B.; Feldmann, J. Arsenic Containing Medium and Long Chain Fatty Acids in Marine Fish Oil Identified as Degradation Products Using Reversed-Phase HPLC-ICP-MS/ESI-MS. *J. Anal. At. Spectrom.* **2016**, *31* (9), 1836–1845.

(107) Raab A., Newcombe C., Pitton, D., Ebel R., F. J.; Raab, A.; Newcombe, C.; Pitton, D.; Ebel, R.; Feldmann, J. Comprehensive Analysis of Lipophilic Arsenic Species in a Brown Alga (Saccharina Latissima). *Anal. Chem.* **2013**, *85*, 2817–2824.

(108) Arroyo-Abad, U.; Mattusch, J.; Mothes, S.; Möder, M.; Wennrich, R.; Elizalde-González, M. P.; Matysik, F. M. Detection of Arsenic-Containing Hydrocarbons in Canned Cod Liver Tissue. *Talanta* **2010**, *82*, 38–43.

(109) Raber, G.; Khoomrung, S.; Taleshi, M. S.; Edmonds, J. S.; Francesconi, K. A. Identification of Arsenolipids with GC/MS. *Talanta* **2009**, *78*, 1215–1218.

(110) Sele, V.; Amlund, H.; Berntssen, M. H. G.; Berntsen, J. A.; Skov, K.; Sloth, J. J. Detection of Arsenic-Containing Hydrocarbons in a Range of Commercial Fish Oils by GC-ICPMS Analysis. *Anal. Bioanal. Chem.* **2013**, *405*, 5179–5190.

(111) García-Salgado, S.; Raber, G.; Raml, R.; Magnes, C.; Francesconi, K. A. Arsenosugar Phospholipids and Arsenic Hydrocarbons in Two Species of Brown Macroalgae. *Environ. Chem.* **2012**, *9*, 63–66.

(112) Pétursdóttir, Á. H.; Blagden, J.; Gunnarsson, K.; Raab, A.; Stengel, D. B.; Feldmann, J.; Gunnlaugsdóttir, H. Arsenolipids Are Not Uniformly Distributed within Two

Brown Macroalgal Species Saccharina Latissima and Alaria Esculenta. *Anal. Bioanal. Chem.* **2019**, *411*, 4973–4985.

(113) Amayo, O. K.; Andrea, R.; Krupp, E. M.; Gunnlaugsdottir, H.; Feldmann, J. Novel Identification of Arsenolipids Using Chemical Derivatizations in Conjunction with RP-HPLC-ICPMS/ESMS. *Anal. Chem.* **2013**, *85*, 9321–9327.

(114) Pétursdóttir, Á. H.; Rodrigues De Jesus, J.; Gunnlaugsdóttir, H.; Feldmann, J. Quantification of Labile and Stable Non-Polar Arsenolipids in Commercial Fish Meals and Edible Seaweed Samples. *J. Anal. At. Spectrom.* **2018**, *33*, 102–110.

(115) Viczek, S. A.; Jensen, K. B.; Francesconi, K. A. Arsenic-Containing Phosphatidylcholines: A New Group of Arsenolipids Discovered in Herring Caviar. *Angew. Chemie - Int. Ed.* **2016**, *55*, 5259–5262.

(116) Guttenberger, N.; Glabonjat, R. A.; Tassoti, S.; Francesconi, K. A. Synthetic Access to Arsenic-Containing Phosphatidylcholines. *Tetrahedron Lett.* **2017**, *58*, 2651–2653.

(117) Řezanka, T.; Nedbalová, L.; Barcytė, D.; Vítová, M.; Sigler, K. Arsenolipids in the Green Alga Coccomyxa (Trebouxiophyceae, Chlorophyta). *Phytochemistry* **2019**, *164*, 243–251.

(118) Yu, X.; Xiong, C.; Jensen, K. B.; Glabonjat, R. A.; Stiboller, M.; Raber, G.; Francesconi, K. A. Mono-Acyl Arsenosugar Phospholipids in the Edible Brown Alga Kombu (Saccharina Japonica). *Food Chem.* **2018**, *240*, 817–821.

(119) Glabonjat, R. A.; Raber, G.; Jensen, K. B.; Guttenberger, N.; Zangger, K.; Francesconi, K. A. A 2- O-Methylriboside Unknown Outside the RNA World Contains Arsenic. *Angew. Chemie* **2017**, *129*, 12125–12127.

(120) Glabonjat, R. A.; Raber, G.; Jensen, K. B.; Schubotz, F.; Boyd, E. S.; Francesconi, K. A. Origin of Arsenolipids in Sediments from Great Salt Lake. *Environ. Chem.* **2019**, *16*, 303–311.

(121) Glabonjat, R. A.; Blum, J. S.; Miller, L. G.; Webb, S. M.; Stolz, J. F.; Francesconi, K. A.; Oremland, R. S. Arsenolipids in Cultured Picocystis Strain ML and Their Occurrence in Biota and Sediment from Mono Lake, California. *Life* **2020**, *10* (93), 1–21.

(122) Taleshi, M. S.; Raber, G.; Edmonds, J. S.; Jensen, K. B.; Francesconi, K. A. Arsenolipids in Oil from Blue Whiting Micromesistius Poutassou - Evidence for

Arsenic-Containing Esters. *Sci. Rep.* **2014**, *4*, 1–7.

(123) Petursdottir, A. H.; Fletcher, K.; Gunnlaugsdóttir, H.; Krupp, E.; Kupper, F. C.; Feldmann, J. Environmental Effects on Arsenosugars and Arsenolipids in Ectocarpus (Phaeophyta). *Environ. Chem.* **2016**, *13* (1), 21–33.

(124) Arroyo-Abad, U.; Mattusch, J.; Reemtsma, T.; Piechotta, C. Arsenolipids in Commercial Canned Cod Liver: An Occurrence and Distribution Study. *Eur. J. Lipid Sci. Technol.* **2014**, *116* (10), 1381–1387.

(125) Leufroy, A.; Noël, L.; Beauchemin, D.; Guérin, T. Bioaccessibility of Total Arsenic and Arsenic Species in Seafood as Determined by a Continuous Online Leaching Method. *Anal. Bioanal. Chem.* **2012**, *402* (9), 2849–2859.

(126) Raab, A.; Fecher, P.; Feldmann, J. Determination of Arsenic in Algae - Results of an Interlaboratory Trial: Determination of Arsenic Species in the Water-Soluble Fraction. *Microchim. Acta* **2005**, *151* (3–4), 153–166.

(127) van Elteren, J. T.; Šlejkovec, Z.; Kahn, M.; Goessler, W. A Systematic Study on the Extractability of Arsenic Species from Algal Certified Reference Material IAEA-140/TM (Fucus Sp., Sea Plant Homogenate) Using Methanol/Water Extractant Mixtures. *Anal. Chim. Acta* **2007**, *585*, 24–31.

(128) Lai, V. W. M.; Cullen, W. R.; Harrington, C. F.; Reimer, K. J. Seasonal Changes in Arsenic Speciation in Fucus Species. *Appl. Organomet. Chem.* **1998**, *12* (4), 243–251.

(129) Li, W.; Wei, C.; Zhang, C.; Van Hulle, M.; Cornelis, R.; Zhang, X. A Survey of Arsenic Species in Chinese Seafood. *Food Chem. Toxicol.* **2003**, *41* (8), 1103–1110.

(130) Wolle, M. M.; Conklin, S. D. Speciation Analysis of Arsenic in Seafood and Seaweed : Part I — Evaluation and Optimization of Methods. *Anal. Bioanal. Chem.* **2018**, *410*, 5675–5687.

(131) FAO. *The State of World Fisheries and Aquaculture 2020 - Sustainability in Action*, 2020th ed.; Rome, 2020.

(132) Micha, R.; Khatibzadeh, S.; Shi, P.; Andrews, K. G.; Engell, R. E.; Mozaffarian, D. Global, Regional and National Consumption of Major Food Groups in 1990 and 2010: A Systematic Analysis Including 266 Country-Specific Nutrition Surveys Worldwide. *BMJ Open* **2015**, *5* (9).

(133) Sioen, I.; De Henauw, S.; Van Camp, J.; Volatier, J. L.; Leblanc, J. C. Comparison of the Nutritional-Toxicological Conflict Related to Seafood Consumption in Different Regions Worldwide. *Regul. Toxicol. Pharmacol.* **2009**, *55* (2), 219–228.

(134) Yamauchi, H.; Takahashi, K.; Mashiko, M.; Saitoh, J.; Yamamura, Y. Intake of Different Chemical Species of Dietary Arsenic by the Japanese, and Their Blood and Urinary Arsenic Levels. *Appl. Organomet. Chem.* **1992**, *6* (4), 383–388.

(135) Zava, T. T.; Zava, D. T. Assessment of Japanese Iodine Intake Based on Seaweed Consumption in Japan: A Literature-Based Analysis. *Thyroid Res.* **2011**, *4* (1), 1–7.

(136) Nachman, K. E.; Ginsberg, G. L.; Miller, M. D.; Murray, C. J.; Nigra, A. E.; Pendergrast, C. B. Mitigating Dietary Arsenic Exposure: Current Status in the United States and Recommendations for an Improved Path Forward. *Sci. Total Environ.* **2017**, *581–582*, 221–236.

(137) Francesconi, K. A. Toxic Metal Species and Food Regulations - Making a Healthy Choice. *Analyst* **2007**, *132* (1), 17–20.

(138) Tseng, W. P. Prevalence of Skin Cancer in an Endemic Area of Chronic Arsenicism in Taiwan. *J. Natl. Cancer Inst.* **1968**, *40* (3), 453–463.

(139) Tseng, W. P. Effects and Dose Response Relationships of Skin Cancer and Blackfoot Disease with Arsenic. *Environ. Health Perspect.* **1977**, *Vol.19*, 109–119.

(140) Borak, J.; Hosgood, H. D. Seafood Arsenic: Implications for Human Risk Assessment. *Regul. Toxicol. Pharmacol.* **2007**, *47*, 204–212.

(141) Alexander J., Benford D. J., Boobis A., Ceccatelli S., Cravedi J-.P., Di Domenico A., Doerge D., Dogliotti E., Edler L., F. M. Scientific Opinion on Arsenic in Food : EFSA Panel on Contaminants in the Food Chain (CONTAM). *EFSA J.* **2009**, *7* (10), 1351.

(142) García-Salgado, S.; Quijano, M. A.; Bonilla, M. M. Arsenic Speciation in Edible Alga Samples by Microwave-Assisted Extraction and High Performance Liquid Chromatography Coupled to Atomic Fluorescence Spectrometry. *Anal. Chim. Acta* **2012**, *714*, 38–46.

(143) de la Calle, M. B.; Baer, I.; Robouch, P.; Cordeiro, F.; Emteborg, H.; Baxter, M. J.; Brereton, N.; Raber, G.; Velez, D.; Devesa, V.; et al. Is It Possible to Agree on a Value for Inorganic Arsenic in Food? The Outcome of IMEP-112. *Anal. Bioanal. Chem.* **2012**, *404* (8), 2475–2488.

(144) Niegel, C.; Matysik, F. M. Analytical Methods for the Determination of Arsenosugars-A Review of Recent Trends and Developments. *Anal. Chim. Acta* **2010**, *657* (2), 83–99.

(145) Grotti M., Ardini F., Terol A., Magia E., T. L. Influence of Chemical Species on the Determination of Arsenic Using Inductively Coupled Plasma Mass Spectrometry at a Low Liquid Flow Rate. *J. Anal. At. Spectrom.* **2013**, *28* (11), 1718–1724.

(146) Yu, L. L.; Butler, T. A.; Turk, G. C. Effect of Valence State on ICP-OES Value Assignment of SRM 3103a Arsenic Spectrometric Solution. *Anal. Chem.* **2006**, *78*, 1651–1656.

(147) Yu, L. L.; Wei, C.; Zeisler, R.; Tong, J.; Oflaz, R.; Bao, H.; Wang, J. An Approach for Identification and Determination of Arsenic Species in the Extract of Kelp. *Anal. Bioanal. Chem.* **2015**, *407*, 3517–3524.

(148) Hamed, I.; Özogul, F.; Özogul, Y.; Regenstein, J. M. Marine Bioactive Compounds and Their Health Benefits: A Review. *Compr. Rev. Food Sci. Food Saf.* **2015**, *14* (4), 446–465.

(149) Lund, E. K. Health Benefits of Seafood; Is It Just the Fatty Acids? *Food Chem.* **2013**, *140*, 413–420.

(150) Larsen, R.; Eilertsen, K. E.; Elvevoll, E. O. Health Benefits of Marine Foods and Ingredients. *Biotechnol. Adv.* **2011**, *29*, 508–518.

(151) Larsson, S. C.; Orsini, N. Fish Consumption and the Risk of Stroke a Dose-Response Meta-Analysis. *Stroke* **2011**, *42*, 3621–3623.

(152) Howard, Barbara, L. Js. R.; Deckelbaum, R. J.; Erdman, J. W.; Kris-etherton, P.; Goldberg, I. J.; Kotchen, T. a; Lichtenstein, A. H.; Mitch, W. E.; Mullis, R.; Robinson, K.; et al. Nutrition Committee of the American Heart Association. *Circulation* **2000**, 2296–2311.

(153) Bashandy, S. A. E.; El Awdan, S. A.; Ebaid, H.; Alhazza, I. M. Antioxidant Potential of Spirulina Platensis Mitigates Oxidative Stress and Reprotoxicity Induced by Sodium Arsenite in Male Rats . *Oxid. Med. Cell. Longev.* **2016**, *2016*, 1–8.

(154) Ciferri, O. Spirulina, the Edible Microorganism. *Microbiol. Rev.* **1983**, *47*, 551–578.

(155) Sotiroudis, T. G.; Sotiroudis, G. T. Health Aspects of Spirulina (Arthrospira)

Microalga Food Supplement. *J. Serbian Chem. Soc.* **2013**, *78* (3), 395–405.

(156) Kulshreshtha, A.; J., A.; Jarouliya, U.; Bhadauriya, P.; Prasad, G.; Bisen, P. Spirulina in Health Care Management. *Curr. Pharm. Biotechnol.* **2008**, *9*, 400–405.

(157) De Morais, M. G.; Vaz, B. D. S.; De Morais, E. G.; Costa, J. A. V. Biologically Active Metabolites Synthesized by Microalgae. *Biomed Res. Int.* **2015**, *2015*, 1–15.

(158) Vaz, B. da S.; Moreira, J. B.; De Morais, M. G.; Costa, J. A. V. Microalgae as a New Source of Bioactive Compounds in Food Supplements. *Curr. Opin. Food Sci.* **2016**, *7*, 73–77.

(159) Hamed, I.; Özogul, F.; Özogul, Y.; Regenstein, J. M. Marine Bioactive Compounds and Their Health Benefits: A Review. *Compr. Rev. Food Sci. Food Saf.* **2015**, *14*, 446–465.

(160) Soletto, D.; Binaghi, L.; Lodi, A.; Carvalho, J. C. M.; Converti, A. Batch and Fed-Batch Cultivations of Spirulina Platensis Using Ammonium Sulphate and Urea as Nitrogen Sources. *Aquaculture* **2005**, *243*, 217–224.

(161) Hoseini, S. M.; Khosravi-Darani, K.; Mozafari, M. R. Nutritional and Medical Applications of Spirulina Microalgae. *Mini-Reviews Med. Chem.* **2013**, *13*, 1231–1237.

(162) Coskun, Z. K.; Kerem, M.; Gurbuz, N.; Omeroglu, S.; Pasaoglu, H.; Demirtas, C.; Lortlar, N.; Salman, B.; Pasaoglu, O. T.; Turgut, H. B. The Study of Biochemical and Histopathological Effects of Spirulina in Rats with TNBS-Induced Colitis. *Bratislava Med. J.* **2011**, *112*, 235–243.

(163) Khan, Z.; Bhadouria, P.; Bisen, P. Nutritional and Therapeutic Potential of Spirulina. *Curr. Pharm. Biotechnol.* **2005**, *6*, 373–379.

(164) Belay, A.; Kato, T.; Ota, Y. Spirulina (Arthrospira): Potential Application as an Animal Feed Supplement. *J. Appl. Phycol.* **1996**, *8*, 303–311.

(165) Belay, A.; Ota, Y.; Miyakawa, K.; Shimamatsu, H. Current Knowledge on Potential Health Benefits of Spirulina. *J. Appl. Phycol.* **1993**, *5*, 235–241.

(166) Ali, E. A. I.; Barakat, B. M.; Hassan, R. Antioxidant and Angiostatic Effect of Spirulina Platensis Suspension in Complete Freund's Adjuvant-Induced Arthritis in Rats. *PLoS One* **2015**, *10*, 1–13.

(167) Lordan, S.; Ross, R. P.; Stanton, C. Marine Bioactives as Functional Food Ingredients: Potential to Reduce the Incidence of Chronic Diseases. *Mar. Drugs* **2011**, *9*, 1056–1100.

(168) Khan, M.; Shobha, J. C.; Mohan, I. K.; Naidu, M. U. R.; Sundaram, C.; Singh, S.; Kuppusamy, P.; Kutala, V. K. Protective Effect of Spirulina against Doxorubicin-Induced Cardiotoxicity. *Phyther. Res.* **2005**, *19*, 1030–1037.

(169) Holdt, S. L.; Kraan, S. Bioactive Compounds in Seaweed: Functional Food Applications and Legislation. *J. Appl. Phycol.* **2011**, *23*, 543–597.

(170) Bagchi, D. Nutraceuticals and Functional Foods Regulations in the United States and around the World. *Toxicology* **2006**, *221* (1), 1–3.

(171) Hafting, J. T.; Critchley, A. T.; Cornish, M. L.; Hubley, S. A.; Archibald, A. F. On-Land Cultivation of Functional Seaweed Products for Human Usage. *J. Appl. Phycol.* **2012**, *24*, 385–392.

(172) Bishop, W. M.; Heidi, M. Z. Evaluation of Microalgae for Use as Nutraceuticals and Nutritional Supplements. *J. Nutr. Food Sci.* **2012**, *02*, 1–6.

(173) Romay, C.; Armesto, J.; Remirez, D.; Gonzalez, R.; Ledon, N.; Garcia, I. Antioxidant and Anti-Inflammatory Properties of C-Phycocyanin from Blue-Green Algae. *Inflamm. Res.* **1998**, *47*, 36–41.

(174) Mitra, S.; Siddiqui, W. A.; Khandelwal, S. C-Phycocyanin Protects against Acute Tributyltin Chloride Neurotoxicity by Modulating Glial Cell Activity along with Its Anti-Oxidant and Anti-Inflammatory Property: A Comparative Efficacy Evaluation with N-Acetyl Cysteine in Adult Rat Brain. *Chem. Biol. Interact.* **2015**, *238*, 138–150.

(175) Hussein, M. M. A.; Ali, H. A.; Ahmed, M. M. Ameliorative Effects of Phycocyanin against Gibberellic Acid Induced Hepatotoxicity. *Pestic. Biochem. Physiol.* **2015**, *119*, 28–32.

(176) Nagaoka, S.; Shimizu, K.; Kaneko, H.; Shibayama, F.; Morikawa, K.; Kanamaru, Y.; Otsuka, A.; Hirahashi, T.; Kato, T. A Novel Protein C-Phycocyanin Plays a Crucial Role in the Hypocholesterolemic Action of Spirulina Platensis Concentrate in Rats. *J. Nutr.* **2005**, *135*, 2425–2430.

(177) Bhat, V. B.; Madyastha, K. M. Scavenging of Peroxynitrite by Phycocyanin and Phycocyanobilin from Spirulina Platensis: Protection against Oxidative Damage to

DNA. *Biochem. Biophys. Res. Commun.* **2001**, *285*, 262–266.

(178) Ferreira-Hermosillo, A.; Torres-Duran, P. V.; Juarez-Oropeza, M. A. Hepatoprotective Effects of Spirulina Maxima in Patients with Non-Alcoholic Fatty Liver Disease: A Case Series. *J. Med. Case Rep.* **2010**, *4*, 1–6.

(179) Gantar, M.; Dhandayuthapani, S.; Rathinavelu, A. Phycocyanin Induces Apoptosis and Enhances the Effect of Topotecan on Prostate Cell Line LNCaP. *J. Med. Food* **2012**, *15*, 1091–1095.

(180) Madhyastha, H. K.; Radha, K. S.; Nakajima, Y.; Omura, S.; Maruyama, M. UPA Dependent and Independent Mechanisms of Wound Healing by C-Phycocyanin. *J. Cell. Mol. Med.* **2008**, *12*, 2691–2703.

(181) Madhyastha, H.; Madhyastha, R.; Nakajima, Y.; Omura, S.; Maruyama, M. Regulation of Growth Factors-Associated Cell Migration by C-Phycocyanin Scaffold in Dermal Wound Healing. *Clin. Exp. Pharmacol. Physiol.* **2012**, *39* (1), 13–19.

(182) Farooq, S. M.; Boppana, N. B.; Asokan, D.; Sekaran, S. D.; Shankar, E. M.; Li, C.; Gopal, K.; Bakar, S. A.; Karthik, H. S.; Ebrahim, A. S. C-Phycocyanin Confers Protection against Oxalate-Mediated Oxidative Stress and Mitochondrial Dysfunctions in MDCK Cells. *PLoS One* **2014**, *9*, 1–9.

(183) Li, B.; Gao, M. H.; Chu, X. M.; Teng, L.; Lv, C. Y.; Yang, P.; Yin, Q. F. The Synergistic Antitumor Effects of All-Trans Retinoic Acid and C-Phycocyanin on the Lung Cancer A549 Cells in Vitro and in Vivo. *Eur. J. Pharmacol.* **2015**, *749*, 107–114.

(184) Kapoor, R.; Mehta, U. Supplementary Effect of Spirulina on Hematological Status of Rats during Pregnancy and Lactation. *Plant Foods Hum. Nutr.* **1998**, *52*, 315–324.

(185) Kata, F. S.; Athbi, A. M.; Manwar, E. Q.; Al-Ashoor, A.; Abdel-Daim, M. M.; Aleya, L. Therapeutic Effect of the Alkaloid Extract of the Cyanobacterium Spirulina Platensis on the Lipid Profile of Hypercholesterolemic Male Rabbits. *Environ. Sci. Pollut. Res.* **2018**, *25*, 19635–19642.

(186) Paredes-Carabajal, M. C.; Torres-Duran, P. V.; Diaz-Xagoya, J. C.; Mascher, D.; Juarez-Oropeza, M. A. Effects of Dietary Spirulina Maxima on Endothelium Dependent Vasomotor Responses of Rat Aortic Rings. *Life Sci.* **1997**, *61*, 211–219.

(187) Blé-Castillo, J. L.; Rodríguez-Hernández, A.; Miranda-Zamora, R.; Juárez-Oropeza,

M. A.; Díaz-Zagoya, J. C. Arthrospira Maxima Prevents the Acute Fatty Liver Induced by the Administration of Simvastatin, Ethanol and a Hypercholesterolemic Diet to Mice. *Life Sci.* **2002**, *70*, 2665–2673.

(188) Serban, M. C.; Sahebkar, A.; Dragan, S.; Stoichescu-Hogea, G.; Ursoniu, S.; Andrica, F.; Banach, M. A Systematic Review and Meta-Analysis of the Impact of Spirulina Supplementation on Plasma Lipid Concentrations. *Clin. Nutr.* **2016**, *35*, 842–851.

(189) Ama Moor, V. J.; Nya Biapa, P. C.; Nono Njinkio, B. L.; Moukette Moukette, B.; Sando, Z.; Kenfack, C.; Ateba, B.; Ngo Matip, M. E.; Pieme, C. A.; Ngogang, J. Hypolipidemic Effect and Activation of Lecithin Cholesterol Acyl Transferase (LCAT) by Aqueous Extract of Spirulina Platensis during Toxicological Investigation. *BMC Nutr.* **2017**, *3*, 1–9.

(190) Torres-Duran, P. V.; Ferreira-Hermosillo, A.; Juarez-Oropeza, M. A. Antihyperlipemic and Antihypertensive Effects of Spirulina Maxima in an Open Sample of Mexican Population: A Preliminary Report. *Lipids Health Dis.* **2007**, *6*, 1–8.

(191) Mazokopakis, E. E.; Papadomanolaki, M. G.; Fousteris, A. A.; Kotsiris, D. A.; Lampadakis, I. M.; Ganotakis, E. S. The Hepatoprotective and Hypolipidemic Effects of Spirulina (Arthrospira Platensis) Supplementation in a Cretan Population with Non-Alcoholic Fatty Liver Disease: A Prospective Pilot Study. *Ann. Gastroenterol.* **2014**, *27* (4), 387–394.

(192) Mascher, D.; Paredes-Carbajal, M. C.; Torres-Durán, P. V.; Zamora-González, J.; Díaz-Zagoya, J. C.; Juárez-Oropeza, M. A. Ethanolic Extract of Spirulina Maxima Alters the Vasomotor Reactivity of Aortic Rings from Obese Rats. *Arch. Med. Res.* **2006**, *37*, 50–57.

(193) Deng, R.; Chow, T. J. Hypolipidemic, Antioxidant, and Antiinflammatory Activities of Microalgae Spirulina. *Cardiovasc. Ther.* **2010**, *28*, 33–45.

(194) Teimouri, M.; Yeganeh, S.; Mianji, G. R.; Najafi, M.; Mahjoub, S. The Effect of Spirulina Platensis Meal on Antioxidant Gene Expression, Total Antioxidant Capacity, and Lipid Peroxidation of Rainbow Trout (Oncorhynchus Mykiss). *Fish Physiol. Biochem.* **2019**, *45*, 977–986.

(195) Mazzola, D.; Vigano, G.; Oro, T.; Bertolin, T. E.; Fornari, F.; Costa, J. A. V. Spirulina

Platensis Enhances the Beneficial Effect of Exercise on Oxidative Stress and the Lipid Profile in Rats. *Brazilian Arch. Biol. Technol.* **2015**, *58*, 961–969.

(196) Iwata, K.; Inayama, T.; Kato, T. Effects of Spirulina Platensis on Plasma Lipoprotein Lipase Activity in Fructose-Induced Rats. *J. Nutr. Sci. Vitaminol.* **1990**, *36*, 165–171.

(197) Ismail, M. F.; Ali, D. A.; Fernando, A.; Abdraboh, M. E.; Gaur, R. L.; Ibrahim, W. M.; Raj, M. H. G.; Ouhtit, A. Chemoprevention of Rat Liver Toxicity and Carcinogenesis by Spirulina. *Int. J. Biol. Sci.* **2012**, *5*, 377–387.

(198) Pan, R.; Lu, R.; Zhang, Y.; Zhu, M.; Zhu, W.; Yang, R.; Zhang, E.; Ying, J.; Xu, T.; Yi, H.; et al. Spirulina Phycocyanin Induces Differential Protein Expression and Apoptosis in SKOV-3 Cells. *Int. J. Biol. Macromol.* **2015**, *81*, 951–959.

(199) Deniz, I.; Ozen, M. O.; Yesil-Celiktas, O. Supercritical Fluid Extraction of Phycocyanin and Investigation of Cytotoxicity on Human Lung Cancer Cells. *J. Supercrit. Fluids* **2016**, *108*, 13–18.

(200) Minic, S. L.; Stanic-Vucinic, D.; Mihailovic, J.; Krstic, M.; Nikolic, M. R.; Cirkovic Velickovic, T. Digestion by Pepsin Releases Biologically Active Chromopeptides from C-Phycocyanin, a Blue-Colored Biliprotein of Microalga Spirulina. *J. Proteomics* **2016**, *147*, 132–139.

(201) Karkos, P. D.; Leong, S. C.; Karkos, C. D.; Sivaji, N.; Assimakopoulos, D. A. Spirulina in Clinical Practice: Evidence-Based Human Applications. *Evidence-based Complement. Altern. Med.* **2011**, *2011*, 1–4.

(202) Gao, Y.; Liao, G.; Xiang, C.; Yang, X.; Cheng, X.; Ou, Y. Effects of Phycocyanin on INS-1 Pancreatic β-Cell Mediated by PI3K/Akt/FoxO1 Signaling Pathway. *Int. J. Biol. Macromol.* **2016**, *83*, 185–194.

(203) Ou, Y.; Lin, L.; Pan, Q.; Yang, X.; Cheng, X. Preventive Effect of Phycocyanin from Spirulina Platensis on Alloxan-Injured Mice. *Environ. Toxicol. Pharmacol.* **2012**, *34*, 721–726.

(204) Young, I. C.; Chuang, S. T.; Hsu, C. H.; Sun, Y. J.; Lin, F. H. C-Phycocyanin Alleviates Osteoarthritic Injury in Chondrocytes Stimulated with H2O2 and Compressive Stress. *Int. J. Biol. Macromol.* **2016**, *93*, 852–859.

(205) Ismail, M.; Hossain, M. F.; Tanu, A. R.; Shekhar, H. U. Effect of Spirulina Intervention on Oxidative Stress, Antioxidant Status, and Lipid Profile in Chronic

Obstructive Pulmonary Disease Patients. *Biomed Res. Int.* **2015**, *2015*, 1–8.

(206) Kalafati, M.; Jamurtas, A. Z.; Nikolaidis, M. G.; Paschalis, V.; Theodorou, A. A.; Sakellariou, G. K.; Koutedakis, Y.; Kouretas, D. Ergogenic and Antioxidant Effects of Spirulina Supplementation in Humans. *Medicine and Science in Sports and Exercise*. 2010, pp 142–151.

(207) Koozehchian, M. S.; Kazaj, K. T.; Sarshin, A.; Gaeini, A.; Naderi, A.; Stannard, S. R.; Kaveh, M.; Chandler, J. T. Effects of Short-Term Spirulina Supplementation on Oxidative Stress Markers in Mountaineers at High Altitude. *Medicine & Science in Sports & Exercise*. 2019, p 88.

(208) Wu, Q.; Liu, L.; Miron, A.; Klímová, B.; Wan, D.; Kuča, K. The Antioxidant, Immunomodulatory, and Anti-Inflammatory Activities of Spirulina: An Overview. *Arch. Toxicol.* **2016**, *90*, 1817–1840.

(209) Hafting, J. T.; Craigie, J. S.; Stengel, D. B.; Loureiro, R. R.; Buschmann, A. H.; Yarish, C.; Edwards, M. D.; Critchley, A. T. Prospects and Challenges for Industrial Production of Seaweed Bioactives. *J. Phycol.* **2015**, *51* (5), 821–837.

(210) Warrand, J. Healthy Polysaccharides the next Chapter in Food Products. *Food Technol. Biotechnol.* **2006**, *44*, 355–370.

(211) MacArtain, P.; Gill, C. I. R.; Brooks, M.; Campbell, R.; Rowland, I. R. Special Article Nutritional Value of Edible Seaweeds. *Nutr. Rev.* **2007**, *65*, 535–543.

(212) Bocanegra, A.; Bastida, S.; Benedí, J.; Ródenas, S.; Sánchez-Muniz, F. J. Characteristics and Nutritional and Cardiovascular-Health Properties of Seaweeds. *J. Med. Food* **2009**, *12*, 236–258.

(213) Cottin, S. C.; Sanders, T. A.; Hall, W. L. The Differential Effects of EPA and DHA on Cardiovascular Risk Factors. *Proc. Nutr. Soc.* **2011**, *70*, 215–231.

(214) Harnedy, P. A.; Fitzgerald, R. J. Bioactive Proteins, Peptides, and Amino Acids from Macroalgae. *J. Phycol.* **2011**, *47*, 218–232.

(215) Pangestuti, R.; Kim, S. K. Biological Activities and Health Benefit Effects of Natural Pigments Derived from Marine Algae. *J. Funct. Foods* **2011**, *3*, 255–266.

(216) Stengel, D. B.; Connan, S.; Popper, Z. A. Algal Chemodiversity and Bioactivity: Sources of Natural Variability and Implications for Commercial Application.

Biotechnol. Adv. **2011**, *29* (5), 483–501.

(217) Tanna, B.; Mishra, A. Metabolites Unravel Nutraceutical Potential of Edible Seaweeds: An Emerging Source of Functional Food. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17* (6), 1613–1624.

(218) Andersen, R. A. Diversity of Eukaryotic Algae. *Biodivers. Conserv.* **1992**, *1* (4), 267–292.

(219) Braune, W.; Guiry, M. D. *Seaweeds: A Colour Guide to the Common Benthic Green, Brown and Red Algae of the World's Oceans*, Illustrate.; Guiry, M. D., Ed.; Koeltz Scientific Books: Konigstein, 2011.

(220) A. Minge, M.; Silberman, J. D.; Orr, R. J. S.; Cavalier-Smith, T.; Shalchian-Tabrizi, K.; Burki, F.; Skjæveland, Å.; Jakobsen, K. S. Evolutionary Position of Breviate Amoebae and the Primary Eukaryote Divergence. *Proc. R. Soc. B Biol. Sci.* **2009**, *276* (1657), 597–604.

(221) Mouritsen, O. G. The Emerging Science of Gastrophysics and Its Application to the Algal Cuisine. *Flavour* **2012**, *1* (6), 1–9.

(222) Dawczynski, C.; Schubert, R.; Jahreis, G. Amino Acids, Fatty Acids, and Dietary Fibre in Edible Seaweed Products. *Food Chem.* **2007**, *103* (3), 891–899.

(223) Mouritsen, O. G.; Rhatigan, P.; Pérez-Lloréns, J. L. World Cuisine of Seaweeds: Science Meets Gastronomy. *Int. J. Gastron. Food Sci.* **2018**, *14*, 55–65.

(224) FAO. *The State of the World Fisheries and Aquaculture: Meeting the Sustainable Development Goals*; 2018; Vol. 35.

(225) Kim, J. K.; Stekoll, M.; Yarish, C. Opportunities, Challenges and Future Directions of Open-Water Seaweed Aquaculture in the United States. *Phycologia* **2019**, *58* (5), 446–461.

(226) Mouritsen, O. G.; Dawczynski, C.; Duelund, L.; Jahreis, G.; Vetter, W.; Schröder, M. On the Human Consumption of the Red Seaweed Dulse (Palmaria Palmata (L.) Weber & Mohr). *J. Appl. Phycol.* **2013**, *25* (6), 1777–1791.

(227) Holdt, S. L.; Kraan, S. Bioactive Compounds in Seaweed: Functional Food Applications and Legislation. *J. Appl. Phycol.* **2011**, *23* (3), 543–597.

(228) Rupérez, P. Mineral Content of Edible Marine Seaweeds. *Food Chem.* **2002**, *79* (1),

23–26.

(229) Teas, J.; Pino, S.; Critchley, A.; Braverman, L. E. Variability of Iodine Content in Common Commercially Available Edible Seaweeds. *Thyroid* **2004**, *14* (10), 836–841.

(230) Sánchez-Machado, D. I.; López-Cervantes, J.; López-Hernández, J.; Paseiro-Losada, P. Fatty Acids, Total Lipid, Protein and Ash Contents of Processed Edible Seaweeds. *Food Chem.* **2004**, *85* (3), 439–444.

(231) MacArtain, P.; Gill, C. I. R.; Brooks, M.; Campbell, R.; Rowland, I. R. Nutritional Value of Edible Seaweeds. *Nutr. Rev.* **2007**, *65* (12), 535–543.

(232) Fleurence, J.; Morançais, M.; Dumay, J.; Decottignies, P.; Turpin, V.; Munier, M.; Garcia-Bueno, N.; Jaouen, P. What Are the Prospects for Using Seaweed in Human Nutrition and for Marine Animals Raised through Aquaculture? *Trends Food Sci. Technol.* **2012**, *27* (1), 57–61.

(233) Simopoulos, A. P. The Importance of the Ratio of Omega-6/Omega-3 Essential Fatty Acids. *Biomed. Pharmacother.* **2002**, *56*, 365–379.

(234) Øverland, M.; Mydland, L. T.; Skrede, A. Marine Macroalgae as Sources of Protein and Bioactive Compounds in Feed for Monogastric Animals. *J. Sci. Food Agric.* **2019**, *99* (1), 13–24.

(235) Valentine, R. C.; Valentine, D. L. *Omega-3 Fatty Acids and the DHA Principle*, First.; CRC Press: Boca Raton, 2009.

(236) Poly-Unsaturated Fats , Neural Function & Mental Health. In *Proceedings of an International and Interdisciplinary Symposium. The Royal Danish Academy of Sciences and Letters*; Mouritsen, O. G., Crawford, M. A., Eds.; The Royal Danish Academy of Sciences and Letters: Copenhagen, 2007; p 87.

(237) Cunnane, S. C.; Stewart, K. M. *Human Brain Evolution: The Influence of Freshwater and Marine Food Resources*; Cunnane, S. C., Stewart, K. M., Eds.; Wiley-Blackwell: Hoboken, New Jersey, 2010.

(238) Andrewes, P.; Demarini, D. M.; Funasaka, K.; Wallace, K.; Lai, V. W. M.; Sun, H.; Cullen, W. R.; Kitchin, K. T. Do Arsenosugars Pose a Risk to Human Health? The Comparative Toxicities of a Trivalent and Pentavalent Arsenosugar. *Environ. Sci. Technol.* **2004**, *38*, 4140–4148.

(239) Noguchi, T.; Matsui, T.; Miyazawa, K.; Asakawa, M.; Iijima, N.; Shida, Y.; Fuse, M.; Hosaka, Y.; Kirigaya, C.; Watabe, K.; et al. Poisoning by the Red Alga “ogonori” (Gracilaria Verrucosa) on the Nojima Coast, Yokohama, Kanagawa Prefecture, Japan. *Toxicon* **1994**, *32* (12), 1533–1538.

(240) FAO. *The State of World Fisheries and Aquaculture 2016 - Contributing to Food Security and Nutrition for All.*; Rome, 2016.

(241) Kisten, K.; Moodley, R.; Jonnalagadda, S. B. Elemental Analysis and Nutritional Value of Seaweed from the East Coast of KwaZulu-Natal, South Africa. *Anal. Lett.* **2017**, *50* (3), 580–590.

(242) Food Standards Australia New Zealand. *Imported Food Risk Statement: Hijiki Seaweed and Inorganic Arsenic.*; Food Standards Australia New Zealand: Canberra, Australia, 2016.

(243) Japanese Ministry of Health, L. and W. Arsenic in Hijiki. 2004.

(244) Food Safety Authority of Ireland. *Consumption of Hijiki Seaweed*; 2015.

(245) Committee on Toxicity. *Annual Report of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment*; 2004.

(246) Centre for Food Safety. *Risk in Brief: Hijiki and Arsenic*; Government of Hong Kong Special Administrative Region, 2011.

(247) Superior Health Council of Belgium. Arsenic and Other Elements in Algae and Dietary Supplements Based on Algae. *Super. Heal. Counc.* **2015**, *9149*.

(248) Australia New Zealand Standards. *Australia New Zealand Food Standards Code - Standard 1.4.1 - Contaminants and Natural Toxicants*; Section 92 of the Food Standards Australia New Zealand Act 1991 (Cth): Australia, 2016; pp 1–3.

(249) Food and Health Bureau. *Proposed Amendment to the Food Adulteration (Metallic Contamination) Regulations.*; Food and Environmental Hygiene Department. Centre for Food Safety: Hong Kong, 2017.

(250) CEVA. *Reglementation Algues Alimentaires Synthese CEVA*; France, 2014.

(251) EU Commission. *Commission Regulation (EU) 2015/186 of 6 February 2015 Amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as Regards Maximum Levels for Arsenic, Fluorine, Lead, Mercury,*

Endosulfan and Ambrosia Seeds.; 2015.

(252) Ma, Z.; Lin, L.; Wu, M.; Yu, H.; Shang, T.; Zhang, T.; Zhao, M. Total and Inorganic Arsenic Contents in Seaweeds: Absorption, Accumulation, Transformation and Toxicity. *Aquaculture* **2018**, *497* (July), 49–55.

(253) Llorente-Mirandes, T.; Ruiz-Chancho, M. J.; Barbero, M.; Rubio, R.; López-Sánchez, J. F. Measurement of Arsenic Compounds in Littoral Zone Algae from the Western Mediterranean Sea. Occurrence of Arsenobetaine. *Chemosphere* **2010**, *81*, 867–875.

(254) Ender, E.; Subirana, M. A.; Raab, A.; Krupp, E. M.; Schaumlöffel, D.; Feldmann, J. Why Is NanoSIMS Elemental Imaging of Arsenic in Seaweed (Laminaria Digitata) Important for Understanding of Arsenic Biochemistry in Addition to Speciation Information? *J. Anal. At. Spectrom.* **2019**, *34*, 2295–2302.

(255) Luvonga, C.; Rimmer, C. A.; Yu, L. L.; Lee, S. B. Organoarsenicals in Seafood: Occurrence, Dietary Exposure, Toxicity, and Risk Assessment Considerations - A Review. *J. Agric. Food Chem.* **2020**, *68*, 943–960.

(256) Nybakken, J. W. *Marine Biology: An Ecological Approach*, 4th ed.; Addison-Wessley Publishing: Boston, Massachusetts, 2001.

(257) Uauy, R.; Calderon, F.; Mena, P. Essential Fatty Acids in Somatic Growth and Brain Development. *World Rev. Nutr. Diet.* **2001**, *89* (9), 134–160.

(258) Christenson, J. K.; O'Kane, G. M.; Farmery, A. K.; McManus, A. The Barriers and Drivers of Seafood Consumption in Australia: A Narrative Literature Review. *Int. J. Consum. Stud.* **2017**, *41*, 299–311.

(259) Hu, F. B.; Manson, J. A. E.; Willett, W. C. Types of Dietary Fat and Risk of Coronary Heart Disease: A Critical Review. *J. Am. Coll. Nutr.* **2001**, *20* (1), 5–19.

(260) Agostoni, C.; Trojan, S.; Bellù, R.; Riva, E.; Giovannini, M. Neurodevelopmental Quotient of Healthy Term Infants at 4 Months and Feeding Practice: The Role of Long-Chain Polyunsaturated Fatty Acids. *Pediatr. Res.* **1995**, *38* (2), 262–266.

(261) Makrides, M.; Neumann, M.; Simmer, K.; Gibson, R.; Pater, J. Are Long-Chain Polyunsaturated Fatty Acids Essential Nutrients in Infancy? *Lancet* **1995**, *345* (8963), 1463–1468.

(262) O'Connor, D. L.; Hall, R.; Adamkin, D.; Auestad, N.; Castillo, M.; Connor, W. E.;

Connor, S. L.; Fitzgerald, K.; Groh-Wargo, S.; Hartmann, E. E.; et al. Growth and Development in Preterm Infants Fed Long-Chain Polyunsaturated Fatty Acids: A Prospective, Randomized Controlled Trial. *Pediatrics* **2001**, *108* (2 II), 359–371.

(263) Leaf, A.; Gosbell, A.; McKenzie, L.; Sinclair, A.; Favilla, I. Visual Evoked Potentials and Dietary Long Chain Polyunsaturated Fatty Acids in Preterm Infants. *Early Hum. Dev.* **1996**, *45* (1–2), 35–53.

(264) Cimatti, A. G.; Martini, S.; Munarini, A.; Zioutas, M.; Vitali, F.; Aceti, A.; Mantovani, V.; Faldella, G.; Corvaglia, L. Maternal Supplementation with Krill Oil during Breastfeeding and Long-Chain Polyunsaturated Fatty Acids (LCPUFAs) Composition of Human Milk: A Feasibility Study. *Front. Pediatr.* **2018**, *6* (December), 1–7.

(265) Venugopal, V.; Gopakumar, K. Shellfish: Nutritive Value, Health Benefits, and Consumer Safety. *Compr. Rev. Food Sci. Food Saf.* **2017**, *16*, 1219–1242.

(266) Alves, R. N.; Maulvault, A. L.; Barbosa, V. L.; Fernandez-Tejedor, M.; Tediosi, A.; Kotterman, M.; van den Heuvel, F. H. M.; Robbens, J.; Fernandes, J. O.; Romme Rasmussen, R.; et al. Oral Bioaccessibility of Toxic and Essential Elements in Raw and Cooked Commercial Seafood Species Available in European Markets. *Food Chem.* **2018**, *267*, 15–27.

(267) Tou, J. C.; Jaczynski, J.; Chen, Y.-C. Krill for Human Consumption: *Nutr. Rev.* **2007**, *65* (2), 63–77. https://doi.org/10.1301/nr.2007.feb.63.

(268) FAO, IFAD, UNICEF, WFP, W. *The State of Food Security and Nutrition in the World*; 2020.

(269) UN-DESA. *World Urbanization Prospects: The 2018 Revision*; New York, 2018; Vol. 12.

(270) Szymkowiak, M. Genderizing Fisheries: Assessing over Thirty Years of Women's Participation in Alaska Fisheries. *Mar. Policy* **2020**, *115* (103846), 1–13.

(271) Biswas, N. *Towards Gender-Equitable Small-Scale Fisheries Governance and Development*; Biswas, N., Ed.; FAO: Rome, 2017.

(272) Washington Sea Grant. *Final Report: Geoduck Aquaculture Research Program. Report to the Washington State Legislature. Washington Sea Grant Technical Report WSG-TR 13-03*; 2013.

(273) Hanaoka, K.; Yosida, K.; Tamano, M.; Kuroiwa, T.; Kaise, T.; Maeda, S. Arsenic in the Prepared Edible Brown Alga Hijiki, Hizikia Fusiforme. *Appl. Organomet. Chem.* **2001**, *15*, 561–565.

(274) Jankong, P.; Chalhoub, C.; Kienzl, N.; Goessler, W.; Francesconi, Kevin A Visoottiviseth, P. Arsenic Accumulation and Speciation in Freshwater Fish Living in Arsenic-Contaminated Waters. *Environ. Chem.* **2007**, *4*, 11–17.

(275) Conklin, S. D.; Creed, P. A.; Creed, J. T. Detection and Quantification of a Thio-Arsenosugar in Marine Molluscs by IC-ICP-MS with an Emphasis on the Interaction of Arsenosugars with Sulfide as a Function of PH. *J. Anal. At. Spectrom.* **2006**, *21* (9), 869–875.

(276) Smith, E.; Naidu, R.; Alston, A. M. Arsenic in the Soil Environment - A Review. *Adv. Agron.* **1998**, *64*, 150–195.

(277) Sharma, V. K.; Sohn, M. Aquatic Arsenic: Toxicity, Speciation, Transformations, and Remediation. *Environ. Int.* **2009**, *35* (4), 743–759.

(278) Tamaki, S.; Frankenberger, W. T. Environmental Biochemistry of Arsenic. In *Reviews of Environmental Contamination and Toxicology*; Springer Berlin Heidelberg, 1992; pp 79–110.

(279) Mandal, B. K.; Suzuki, K. T. Arsenic Round the World: A Review. *Talanta* **2002**, *58* (1), 201–235.

(280) Ng, J. C. Environmental Contamination of Arsenic and Its Toxicological Impact on Humans. *Environ. Chem.* **2005**, *2* (3), 146–160.

(281) Amlund, H.; Ingebrigtsen, K.; Hylland, K.; Ruus, A.; Eriksen, D. Ø.; Berntssen, M. H. G. Disposition of Arsenobetaine in Two Marine Fish Species Following Administration of a Single Oral Dose of [14C]Arsenobetaine. *Comp. Biochem. Physiol. - C Toxicol. Pharmacol.* **2006**, *143* (2), 171–178.

(282) Sirot, V.; Guérin, T.; Volatier, J. L.; Leblanc, J. C. Dietary Exposure and Biomarkers of Arsenic in Consumers of Fish and Shellfish from France. *Sci. Total Environ.* **2009**, *407* (6), 1875–1885.

(283) Navas-acien, A.; Francesconi, K. A.; Silbergeld, E. K.; Guallar, E. Seafood Intake and Urine Concentrations of Total Arsenic, Dimethylarsinate and Arsenobetaine in the US Population. *Environ. Res.* **2011**, *111*, 110–118.

(284) Styblo; Del Razo, M. L.; Vega, L.; Germolec, D. R.; Lecluyse, E. L.; Hamilton, G. A.; Reed, W.; Wang, C.; Cullen, W. R.; Thomas, D. J. Comparative Toxicity of Trivalent and Pentavalent Inorganic and Methylated Arsenicals in Rat and Human Cells. *Arch. Toxicol.* **2000**, *74*, 289–299.

(285) Szramek, K.; Walter, L. M.; Mccall, P. Arsenic Mobility in Groundwater / Surface Water Systems in Carbonate-Rich Pleistocene Glacial Drift Aquifers (Michigan). *Appl. Geochemistry* **2004**, *19*, 1137–1155.

(286) Shraim, A.; Sekaran, N. C.; Anuradha, C. D.; Hirano, S. Speciation of Arsenic in Tube-Well Water Samples Collected from West Bengal, India, by High-Performance Liquid Chromatography - Inductively Coupled Plasma Mass. *Appl. Organomet. Chem.* **2002**, *16*, 202–209.

(287) Lin, T.-H.; Huang, Y.-L.; Wang, M.-Y. Arsenic Species in Drinking Water, Hair, Fingernails, and Urine of Patients with Blackfoot Disease. *J. Toxicol. Environ. Heal. Part A* **1998**, *53* (2), 85–93.

(288) Vega, L.; Styblo, M.; Patterson, R.; Cullen, W.; Wang, C.; Germolec, D. Differential Effects of Trivalent and Pentavalent Arsenicals on Cell Proliferation and Cytokine Secretion in Normal Human Epidermal Keratinocytes. *Toxicol. Appl. Pharmacol.* **2001**, *172* (3), 225–232.

(289) Oremland, R. S.; Stoltz, J. F. The Ecology of Arsenic. *Science (80-.).* **2003**, *300*, 939–945.

(290) Rahman, M. A. M.; Hogan, B.; Duncan, E.; Doyle, C.; Krassoi, R.; Rahman, M. A. M.; Naidu, R.; Lim, R. P.; Maher, W.; Hassler, C. Toxicity of Arsenic Species to Three Freshwater Organisms and Biotransformation of Inorganic Arsenic by Freshwater Phytoplankton (Chlorella Sp. CE-35). *Ecotoxicol. Environ. Saf.* **2014**, *106*, 126–135.

(291) Stolz, J. F.; Basu, P.; Santini, J. M.; Oremland, R. S. Arsenic and Selenium in Microbial Metabolism. *Annu. Rev. Microbiol.* **2006**, *60*, 107–130.

(292) Murray, L. A.; Raab, A.; Marr, I. L.; Feldmann, J. Biotransformation of Arsenate to Arsenosugars by Chlorella Vulgaris. *Appl. Organomet. Chem.* **2003**, *17*, 669–674.

(293) Jiang, Y.; Purchase, D.; Jones, H.; Garelick, H. Technical Note: Effects of Arsenate (As5+) on Growth and Production of Glutathione (GSH) and Phytochelatins (PCS) in

Chlorella Vulgaris. *Int. J. Phytoremediation* **2011**, *13* (8), 834–844.

(294) Levy, J. L.; Stauber, J. L.; Adams, M. S.; Maher, W. A.; Kirby, J. K.; Jolley, D. F. Toxicity , Biotransformation, and Mode of Action of Arsenic in Two Freshwater Microalgae (Chlorella Sp. and Monoraphidium Arcuatum). *Environ. Toxicol. Chem.* **2005**, *24*, 2630–2639.

(295) Button, M.; Moriarty, M. M.; Watts, M. J.; Zhang, J.; Koch, I.; Reimer, K. J. Arsenic Speciation in Field-Collected and Laboratory-Exposed Earthworms Lumbricus Terrestris. *Chemosphere* **2011**, *85* (8), 1277–1283.

(296) Vahter, M. Species Differences in the Metabolism of Arsenic Compounds. *Appl. Organomet. Chem.* **1994**, *8* (3), 175–182.

(297) Concha, G.; Vogler, G.; Nermell, B.; Vahter, M. Intra-Individual Variation in the Metabolism of Inorganic Arsenic. *Int. Arch. Occup. Environ. Health* **2002**, *75* (8), 576–580.

(298) Sakurai, T. Biomethylation of Arsenic Is Essentially Detoxicating Event. *J. Heal. Sci.* **2003**, *49* (3), 171–178.

(299) Kenyon, E. M.; Hughes, M. F. A Concise Review of the Toxicity and Carcinogenicity of Dimethylarsinic Acid. *Toxicology* **2001**, *160*, 227–236.

(300) Wei, M.; Wanibuchi, H.; Morimura, K.; Iwai, S.; Yoshida, K.; Endo, G.; Nakae, D.; Fukushima, S. Carcinogenicity of Dimethylarsinic Acid in Male F344 Rats and Genetic Alterations in Induced Urinary Bladder Tumors. *Carcinogenesis* **2002**, *23*, 1387–1397.

(301) Hunt, K. M.; Srivastava, R. K.; Elmets, C. A.; Athar, M. The Mechanistic Basis of Arsenicosis: Pathogenesis of Skin Cancer. *Cancer Lett.* **2014**, *354* (2), 211–219

(302) Jomova, K.; Jenisova, Z.; Feszterova, M.; Baros, S.; Liska, J.; Hudecova, D.; Rhodes, C. J.; Valko, M. Bacteria Degradation of Arsenobetaine via Dimethylarsinoylacetate. *J. Appl. Toxicol.* **2011**, *31* (February), 95–107.

(303) Rosen, B. P.; Ajees, A. A.; Mcdermott, T. R. Life and Death with Arsenic. *BioEssays* **2011**, *33* (5), 350–357.

(304) Harkins, P. C.; Petersson, G.; Haake, P. Distortion of O - P - O Bond Angles in Phosphorus Monoanions : Ab Initio Studies. *J. Inorg. Biochem.* **1996**, *61*, 25–41.

(305) Long, J. W.; Ray Jr., W. J. Kinetics and Thermodynamics of the Formation of Glucose Arsenate. Reaction of Glucose Arsenate with Phosphoglucomutaset. *Biochemistry* **1973**, *12* (20), 3932–3937.

(306) Moore, S. A.; Moennich, D. M.; Gresser, M. J. Synthesis and Hydrolysis of ADP-Arsenate by Beef Heart Submitochondrial Particles. *J. Biol. Chem.* **1983**, *258* (10), 6266–6271.

(307) Gresser, M. ADP-Arsenate: Formation by Submitochondrial Particles under Phosphorylating Conditions. *J. Biol. Chem.* **1981**, *256* (12), 5981–5983.

(308) Chan, T. L.; Thomas, B. R.; Wadkins C. L. The Formation and Isolation of an Arsenylated Component of Rat Liver Mitochondria. J. *J. Biol. Chem.* **1969**, *244* (11), 2883–2390.

(309) Lloyd, J. R.; Oremland, R. S. Microbial Transformations of Arsenic in the Environment: From Soda Lakes to Aquifers. *Elements* **2006**, *2* (2), 85–90.

(310) Sun, Y.; Liu, G.; Cai, Y. Thiolated Arsenicals in Arsenic Metabolism: Occurrence, Formation, and Biological Implications. *J. Environ. Sci. (China)* **2016**, *49*, 59–73.

(311) Finnegan, P. M.; Chen, W. Arsenic Toxicity: The Effects on Plant Metabolism. *Front. Physiol.* **2012**, *3*, 1–18.

(312) Hughes, M. F. Arsenic Toxicity and Potential Mechanisms of Action. *Toxicol. Lett.* **2002**, *133*, 1–16.

(313) Styblo, M.; Drobná, Z.; Jaspers, I.; Lin, S.; Thomas, D. J. The Role of Biomethylation in Toxicity and Carcinogenicity of Arsenic : A Research Update. *Environ. Health Perspect.* **2002**, *110* (5), 767–771.

(314) Reichl, F.-X.; Szinicz, L.; Kreppel, H.; Forth, W. Effect of Arsenic on Carbohydrate Metabolism after Single or Repeated Injection in Guinea Pigs. *Arch. Toxicol.* **1988**, *62*, 473–475.

(315) Szinicz L., F. W. Effect of As2O3 on Gluconeogenesis. *Arch. Toxicol.* **1988**, *61* (6), 444–449.

(316) Rodriguez, V. M.; Del Razo, L. M.; Limon-Pacheco, J. H.; Giordano, M.; Sanchez-Penna, L. C.; Uribe-Querol, E.; Gutierrez-Ospina, G.; Gonsebatt, M. E. Glutathione Reductase Inhibition and Methylated Arsenic Distribution in Cd1 Mice Brain and

Liver. *Toxicological* **2005**, *84* (1), 157–166.

(317) Su, C.-K.; Yang, C.-H.; Lin, C.-H.; Sun, Y.-C. In-Vivo Evaluation of the Permeability of the Blood – Brain Barrier to Arsenicals, Molybdate, and Methylmercury by Use of Online Microdialysis – Packed Minicolumn – Inductively Coupled Plasma Mass Spectrometry. *Anal. Bioanal. Chem.* **2014**, *406*, 239–247.

(318) Xi, S.; Sun, W.; Wang, F.; Jin, Y.; Sun, G. Transplacental and Early Life Exposure to Inorganic Arsenic Affected Development and Behavior in Offspring Rats. *Arch. Toxicol.* **2009**, *83*, 549–556.

(319) Leffers, L.; Wehe, C. A.; Hu, S.; Bartel, M.; Ebert, F.; Taleshi, M. S.; Galla, H.-J.; Karst, U.; Francesconi, K. A.; Schwerdtle, T. Metallomics Metabolites and Presystemic Metabolism of Thio-Dimethylarsinic Acid in Caco-2 Cells. *Metallomics* **2013**, *5*, 1031–1042.

(320) Chen, B. C.; Chou, W. C.; Chen, W. Y.; Liao, C. M. Assessing the Cancer Risk Associated with Arsenic-Contaminated Seafood. *J. Hazard. Mater.* **2010**, *181*, 161–169.

(321) Arnold, L. L.; Cano, M.; St John, M.; Eldan, M.; van Gemert, M.; Cohen, S. M. Effects of Dietary Dimethylarsinic Acid on the Urine and Urothelium of Rats. *Carcinogenesis* **1999**, *20* (11), 2171–2179.

(322) Cohen, S. M.; Arnold, L. L.; Eldan, M. .; Lewis, A. S. .; Beck, B. D. *Methylated Arsenicals: The Implications of Metabolism and Carcinogenicity Studies in Rodents to Human Risk Assessment*; 2006; Vol. 36, pp 99–133.

(323) Donohue, Joyce Morrissey Abernathy, C. O. Exposure to Inorganic Arsenic from Fish and Shellfish. In *Arsenic Exposure and Health Effects*; 1999; pp 89–98.

(324) Witt, B.; Meyer, S.; Ebert, F.; Francesconi, K. A.; Schwerdtle, T. Toxicity of Two Classes of Arsenolipids and Their Water-Soluble Metabolites in Human Differentiated Neurons. *Arch. Toxicol.* **2017**, *91* (9), 3121–3134.

(325) Suhendrayatna; Ohki, A.; Kuroiwa, T.; Maeda, S. Arsenic Compounds in the Freshwater Green Microalga Chlorella Vulgaris after Exposure to Arsenite. *Appl. Organomet. Chem.* **1999**, *13* (2), 127–133.

(326) Hasegawa, H.; Sohrin, Y.; Seki, K.; Sato, M.; Norisuye, K.; Naito, K.; Matsui, M. Biosynthesis and Release of Methylarsenic Compounds during the Growth of

Freshwater Algae. *Chemosphere* **2001**, *43*, 265–272.

(327) Howard, A. G.; Comber, S. D. W.; Kifle, D.; Antai, E. E.; Purdie, D. A. Arsenic Speciation and Seasonal Changes in Nutrient Availability and Micro-Plankton Abundance in Southampton Water , U . K . *Estaurine Coast. Shelf Sci.* **1995**, *40*, 435–450.

(328) Hasegawa, H.; Rahman, M. A.; Matsuda, T.; Kitahara, T.; Maki, T.; Ueda, K. Effect of Eutrophication on the Distribution of Arsenic Species in Eutrophic and Mesotrophic Lakes. *Sci. Total Environ.* **2009**, *407*, 1418–1425.

(329) Hasegawa, H.; Matsui, M.; Okamura, S.; Hojo, M.; Iwasaki, N.; Sohrin, Y. Arsenic Speciation Including ` Hidden ' Arsenic in Natural Waters. *Appl. Organomet. Chem.* **1999**, *13*, 113–119.

(330) Sohrin, Y.; Matsui, M.; Kawashima, M.; Hojo, M.; Hasegawa, H. Arsenic Biogeochemistry Affected by Eutrophication in Lake Biwa , Japan. *Environ. Sci. Technol.* **1997**, *31* (10), 2712–2720.

(331) Meyer, J.; Schmidt, A.; Michalke, K.; Reinhard, H. Volatilisation of Metals and Metalloids by the Microbial Population of an Alluvial Soil. *Syst. Appl. Microbiol.* **2007**, *30*, 229–238.

(332) Bentley, R.; Chasteen, T. G. Microbial Methylation of Metalloids : Arsenic , Antimony , and Bismuth. *Microbiol. Mol. Biol. Rev.* **2002**, *66* (2), 250–271.

(333) Meyer, J.; Michalke, K.; Kouril, T.; Reinhard, H. Volatilisation of Metals and Metalloids : An Inherent Feature of Methanoarchaea ? *Syst. Appl. Microbiol.* **2008**, *31*, 81–87.

(334) Rehman, K.; Naranmandura, H. Arsenic Metabolism and Thioarsenicals. *Metallomics* **2012**, *4* (9), 881–892.

(335) Suzuki, K. T.; Mandal, B. K.; Katagiri, A.; Sakuma, Y.; Kawakami, A.; Ogra, Y.; Yamaguchi, K.; Sei, Y.; Yamanaka, K.; Anzai, K.; et al. Dimethylthioarsenicals as Arsenic Metabolites and Their Chemical Preparations. *Chem. Res. Toxicol.* **2004**, *17*, 914–921.

(336) Le, X. C.; Lu, X.; Ma, M.; Cullen, W. R.; Aposhian, H. V.; Zheng, B. Speciation of Key Arsenic Metabolic Intermediates in Human Urine. *Anal. Chem.* **2000**, *72*, 5172–5177.

(337) Cohen, S. M.; Arnold, L. L.; Beck, B. D.; Lewis, A. S.; Eldan, M. Evaluation of the Carcinogenicity of Inorganic Arsenic. *Crit. Rev. Toxicol.* **2013**, *43* (9), 711–752.

(338) Cohen, S. M.; Chowdhury, A.; Arnold, L. L. Inorganic Arsenic: A Non-Genotoxic Carcinogen. *J. Environ. Sci. (China)* **2016**, *49*, 28–37.

(339) Aposhian, H. V. Enzymatic Methylation of Arsenic Spicies and Other New Approaches to Arsenic Toxicity. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37* (1), 397–419.

(340) Vahter, M. Mechanisms of Arsenic Biotransformation. *Toxicology* **2002**, *181*, 211.

(341) Vahter, M. Methylation of Inorganic Arsenic in Different Mammalian Species and Population Groups. *Sci. Prog.* **1999**, *82* (1), 69–88.

(342) Raml, R.; Rumpler, A.; Goessler, W.; Vahter, M.; Li, L.; Ochi, T.; Francesconi, K. A. Thio-Dimethylarsinate Is a Common Metabolite in Urine Samples from Arsenic-Exposed Women in Bangladesh. *Toxicol. Appl. Pharmacol.* **2007**, *222* (3), 374–380.

(343) Petrick, J. S.; Ayala-fierro, F.; Cullen, W. R.; Carter, D. E.; Aposhian, H. V. Monomethylarsonous Acid (MMA III) Is More Toxic Than Arsenite in Chang Human Hepatocytes. *Toxicol. Appl. Pharmacol.* **2000**, *163*, 203–207.

(344) Dopp, E.; Kligerman, A. D.; Diaz-Bone, R. A. Organoarsenicals. Uptake, Metabolism and Toxicity. In *Organometallics in Environment and Toxicology: Metal Ions in Life Sciences*; 2010; Vol. 7, pp 231–265.

(345) Moe, B.; Peng, H.; Lu, X.; Chen, B.; Chen, L. W. L.; Gabos, S.; Li, X. F.; Le, X. C. Comparative Cytotoxicity of Fourteen Trivalent and Pentavalent Arsenic Species Determined Using Real-Time Cell Sensing. *J. Environ. Sci. (China)* **2016**, *49*, 113–124.

(346) Hippler, J.; Zdrenka, R.; Reichel, R. A. D.; Weber, D. G.; Rozynek, P.; Johnen, G.; Dopp, E.; Hirner, A. V. Intracellular, Time-Resolved Speciation and Quantification of Arsenic Compounds in Human Urothelial and Hepatoma Cells. *J. Anal. At. Spectrom.* **2011**, *26* (12), 2396–2403.

(347) Naranmandura, H.; Suzuki, N.; Iwata, K.; Hirano, S.; Suzuki, K. T. Arsenic Metabolism and Thioarsenicals in Hamsters and Rats. *Chem. Res. Toxicol.* **2007**, *20*, 616–624.

(348) Shaw, J. R.; Glaholt, S. P.; Greenberg, N. S.; Sierra-Alvarez, R.; Folt, C. L. Acute Toxicity of Arsenic to Daphnia Pulex: Influence of Organic Functional Groups and Oxidation State. *Environ. Toxicol. Chem.* **2007**, *26* (7), 1532–1537.

(349) Hellweger, F. L.; Lall, U. Modeling the Effect of Algal Dynamics on Arsenic Speciation in Lake Biwa. *Environ. Sci. Technol.* **2004**, *38* (24), 6716–6723.

(350) Arbouine, M. W., Wilson, H. K. The Effect of Seafood Consumption on the Assessment of Occupational Exposure to Arsenic by Urinary Arsenic Speciation Measurements. *J. Trace Elem. Electrolytes Health Dis.* **1992**, *6* (3), 153–160.

(351) Heinrich-ramm, R.; Mindt-Prüfert, S.; Szadkowski, D.; Mindt-prufert, S.; Szadkowski, D. Arsenic Species Excretion after Controlled Seafood Consumption. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2002**, *778*, 263–273.

(352) Branch, S.; Ebdont, L.; O'neill, P. Determination of Arsenic Species in Fish by Directly Coupled High- Performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry. *J. O F Anal. At. Spectrom.* **1994**, *9*, 33–37.

(353) Jin, Y.; Xi, S.; Li, X.; Lu, C.; Li, G.; Xu, Y.; Qu, C.; Niu, Y.; Sun, G. Arsenic Speciation Transported through the Placenta from Mother Mice to Their Newborn Pups. *Environ. Res.* **2006**, *101*, 349–355.

(354) Bredfeldt, T. G.; Jagadish, B.; Eblin, K. E.; Mash, E. A.; Gandolfi, A. J. Monomethylarsonous Acid Induces Transformation of Human Bladder Cells. *Toxicol. Appl. Pharmacol.* **2006**, *216*, 69–79.

(355) Dopp, E.; Hartmann, L. M.; Recklinghausen, U. Von; Florea, A. M.; Rabieh, S.; Zimmermann, U.; Shokouhi, B.; Yadav, S.; Hirner, A. V; Rettenmeier, A. W. Forced Uptake of Trivalent and Pentavalent Methylated and Inorganic Arsenic and Its Cyto- / Genotoxicity in Fibroblasts and Hepatoma Cells. *Toxicol. Sci.* **2005**, *87* (1), 46–56.

(356) Tseng, C. H. A Review on Environmental Factors Regulating Arsenic Methylation in Humans. *Toxicol. Appl. Pharmacol.* **2009**, *235* (3), 338–350.

(357) Francesconi, K. A.; Edmonds, J. S.; Morita, M. Biotransformation of Arsenic in Marine Environment. *Adv. Environ. Sci. Technol.* **1994**, *26*, 221–261.

(358) Yamauchi, H.; Fowler, B. A. Toxicity and Metabolism or Inorganic and Methylated Arsenicals. *Adv. Environ. Sci. Technol.* **1994**, *27* (35), 33–53.

(359) Kaise, T.; Yamauchi, H.; Horiguchi, Y.; Tani, T.; Watanabe, S.; Hirayama, T.; Fukui, S. A Comparative Study on Acute Toxicity of Methylarsonic Acid, Dimethylarsinic Acid and Trimethylarsine Oxide in Mice. *Appl. Organomet. Chem.* **1989**, *3*, 273–277.

(360) Marafante, E.; Vahter, M.; Dencker, L. Metabolism of Arsenocholine in Mice, Rats and Rabbits. *Sci. Total Environ.* **1984**, *34* (3), 223–240.

(361) Kaise, T.; Horiguchi, Y.; Fukui, S.; Shiomi, K.; Chino, M.; Kikuchi, T. Acute Toxicity and Metabolism of Arsenocholine in Mice. *Appl. Organomet. Chem.* **1992**, *6*, 369–373.

(362) Petrick, J. S.; Jagadish, B.; Mash, E. A.; Aposhian, H. V. Monomethylarsonous Acid (MMA III) and Arsenite: LD50 in Hamsters and In Vitro Inhibition of Pyruvate Dehydrogenase. *Chem. Res. Toxicol.* **2001**, *14* (6), 651–656.

(363) Shiomi, K. Arsenic in Marine Organisms: Chemical Forms and Toxico- Logical Aspects. In *Arsenic in the Environment. Part II: Human Health and Ecosystem Effects*; Nriagu, J. O., Ed.; John Wiley: New York, 1994; pp 261–282.

(364) Sakurai, T. Biological Effects of Organic Arsenic Compounds in Seafood. *Appl. Organomet. Chem.* **2002**, *16*, 401–405.

(365) Ciardullo, S.; Aureli, F.; Raggi, A.; Cubadda, F. Arsenic Speciation in Freshwater Fish: Focus on Extraction and Mass Balance. *Talanta* **2010**, *81* (1–2), 213–221.

(366) Harrington, C. F.; Brima, E. I.; Jenkins, R. O. Biotransformation of Arsenobetaine by Microorganisms from the Human Gastrointestinal Tract. *Chem. Speciat. Bioavailab.* **2008**, *20*, 173–180.

(367) Devesa, V.; Súñer, M. A.; Algora, S.; Vélez, D.; Montoro, R.; Jalón, M.; Urieta, I.; Macho, M. L. Organoarsenical Species Contents in Cooked Seafood. *J. Agric. Food Chem.* **2005**, *53* (22), 8813–8819.

(368) Devesa, V.; Martínez, A.; Súñer, M. A.; Vélez, D.; Almela, C.; Montoro, R. Effect of Cooking Temperatures on Chemical Changes in Species of Organic Arsenic in Seafood. *J. Agric. Food Chem.* **2001**, *49* (5), 2272–2276.

(369) Fricke, M. W.; Zeller, M.; Sun, H.; Lai, V. W. M.; Cullen, W. R.; Shoemaker, J. A.; Witkowski, M. R.; Creed, J. T. Chromatographic Separation and Identification of Products from the Reaction of Dimethylarsinic Acid with Hydrogen Sulfide. *Chem. Res. Toxicol.* **2005**, *18* (12), 1821–1829.

(370) Jeong, S.; Lee, H.; Kim, Y. T.; Yoon, H. O. Development of a Simultaneous Analytical Method to Determine Arsenic Speciation Using HPLC-ICP-MS: Arsenate, Arsenite, Monomethylarsonic Acid, Dimethylarsinic Acid, Dimethyldithioarsinic Acid, and Dimethylmonothioarsinic Acid. *Microchem. J.* **2017**, *134*, 295–300.

(371) Hansen, H. R.; Pickford, R.; Thomas-Oates, J.; Jaspars, M.; Feldmann, J. 2-Dimethylarsinothioyl Acetic Acid Identified in a Biological Sample: The First Occurrence of a Mammalian Arsinothioyl Metabolite. *Angew. Chemie - Int. Ed.* **2004**, *116*, 341–344.

(372) Raml, R.; Goessler, W.; Traar, P.; Ochi, T.; Francesconi, K. A. Novel Thioarsenic Metabolites in Human Urine after Ingestion of an Arsenosugar, 2′,3′-Dihydroxypropyl 5-Deoxy-5-Dimethylarsinoyl-β-D-Riboside. *Chem. Res. Toxicol.* **2005**, *18*, 1444–1450.

(373) Raml, R.; Goessler, W.; Francesconi, K. A. Improved Chromatographic Separation of Thio-Arsenic Compounds by Reversed-Phase High Performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry. *J. Chromatogr. A* **2006**, *1128*, 164–170.

(374) Raml, R.; Raber, G.; Rumpler, A.; Bauernhofer, T.; Goessler, W.; Francesconi, K. A. Individual Variability in the Human Metabolism of an 5-Deoxy-5-Dimethylarsinoyl-β-D-Riboside, a Naturally Occurring Arsenical in Seafood. *Chem. Res. Toxicol.* **2009**, *22*, 1534–1540.

(375) Wang, D.; Shimoda, Y.; Kurosawa, H.; Liu, J.; Xu, X.; Liu, X. Excretion Patterns of Arsenic and Its Metabolites Inhuman Saliva and Urine after Ingestion of Chinese Seaweed. *Int. J. Environ. Anal. Chem.* **2015**, *95* (5), 378–389.

(376) Styblo, M.; Serves, S. V; Cullen, W. R.; Thomas, D. J. Comparative Inhibition of Yeast Glutathione Reductase by Arsenicals and Arsenothiols. *Chem. Res. Toxicol.* **1997**, *10*, 27–33.

(377) Wang, Q. Q.; Thomas, D. J.; Naranmandura, H. Importance of Being Thiomethylated: Formation, Fate, and Effects of Methylated Thioarsenicals. *Chem. Res. Toxicol.* **2015**, *28* (3), 281–289.

(378) Chen, R.; Smith, B. W.; Winefordner, J. D.; Tu, M. S.; Kertulis, G.; Ma, L. Q. Arsenic Speciation in Chinese Brake Fern by Ion-Pair High-Performance Liquid

Chromatography-Inductively Coupled Plasma Mass Spectroscopy. *Anal. Chim. Acta* **2004**, *504* (2), 199–207.

(379) Naranmandura, H.; Ibata, K.; Suzuki, K. T. Toxicity of Dimethylmonothioarsinic Acid toward Human Epidermoid Carcinoma A431 Cells. *Chem. Res. Toxicol.* **2007**, *20*, 1120–1125.

(380) Naranmandura, H.; Ogra, Y.; Iwata, K.; Lee, J.; Suzuki, K. T.; Weinfeld, M.; Le, X. C. Evidence for Toxicity Differences between Inorganic Arsenite and Thioarsenicals in Human Bladder Cancer Cells. *Toxicol. Appl. Pharmacol.* **2009**, *238* (2), 133–140.

(381) Naranmandura, H.; Carew, M. W.; Xu, S.; Lee, J.; Leslie, E. M.; Weinfeld, M.; Le, X. C. Comparative Toxicity of Arsenic Metabolites in Human Bladder Cancer EJ-1 Cells. *Chem. Res. Toxicol.* **2011**, *24*, 1586–1596.

(382) Bartel, M.; Ebert, F.; Leffers, L.; Karst, U.; Schwerdtle, T. Toxicological Characterization of the Inorganic and Organic Arsenic Metabolite Thio-DMAV in Cultured Human Lung Cells. *J. Toxicol.* **2011**, *2011*, 1–9.

(383) Ebert, F.; Leffers, L.; Weber, T.; Berndt, S.; Mangerich, A.; Beneke, S.; Bürkle, A.; Schwerdtle, T. Toxicological Properties of the Thiolated Inorganic Arsenic and Arsenosugar Metabolite Thio-Dimethylarsinic Acid in Human Bladder Cells. *J. Trace Elem. Med. Biol.* **2014**, *28*, 138–146.

(384) Ochi, T.; Kita, K.; Suzuki, T.; Rumpler, A.; Goessler, W.; Francesconi, K. A. Cytotoxic, Genotoxic and Cell-Cycle Disruptive Effects of Thio-Dimethylarsinate in Cultured Human Cells and the Role of Glutathione. *Toxicol. Appl. Pharmacol.* **2008**, *228* (1), 59–67.

(385) Unterberg, M.; Leffers, L.; Hübner, F.; Humpf, H. U.; Lepikhov, K.; Walter, J.; Ebert, F.; Schwerdtle, T. Toxicity of Arsenite and Thio-DMAVafter Long-Term (21 Days) Incubation of Human Urothelial Cells: Cytotoxicity, Genotoxicity and Epigenetics. *Toxicol. Res. (Camb).* **2014**, *3*, 456–464.

(386) McSheehy, S.; Szpunar, J. Speciation of Arsenic in Edible Algae by Bi-Dimensional Size-Exclusion Anion Exchange HPLC with Dual ICP-MS and Electrospray MS/MS Detection. *J. Anal. At. Spectrom.* **2000**, *15*, 79–87.

(387) McSheehy, S.; Marcinek, M.; Chassaigne, H.; Szpunar, J. Identification of Dimethylarsinoyl-Riboside Derivatives in Seaweed by Pneumatically Assisted

Electrospray Tandem Mass Spectrometry. *Anal. Chim. Acta* **2000**, *410*, 71–84.

(388) McSheehy, S.; Pohl, P.; Łobiński, R.; Szpunar, J. Complementarity of Multidimensional HPLC-ICP-MS and Electrospray MS-MS for Speciation Analysis of Arsenic in Algae. *Anal. Chim. Acta* **2001**, *440* (1), 3–16.

(389) McSheehy, S.; Pohl, P.; Vélez, D.; Szpunar, J. Multidimensional Liquid Chromatography with Parallel ICP MS and Electrospray MS/MS Detection as a Tool for the Characterization of Arsenic Species in Algae. *Anal. Bioanal. Chem.* **2002**, *372* (3), 457–466.

(390) Miguens-Rodriguez, M.; Pickford, R.; Thomas-Oates, J. E.; Pergantis, S. A. Arsenosugar Identification in Seaweed Extracts Using High-Performance Liquid Chromatography/Electrospray Ion Trap Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2002**, *16* (5), 323–331.

(391) Almela, C.; Laparra, J. M.; Vélez, D.; Barberá, R.; Farré, R.; Montoro, R. Arsenosugars in Raw and Cooked Edible Seaweed: Characterization and Bioaccessibility. *J. Agric. Food Chem.* **2005**, *53* (18), 7344–7351.

(392) Francesconi, K. A.; Edmonds, J. S.; Stick, R. V.; Skelton, B. W.; White, A. H. Arsenic-Containing Ribosides from the Brown Alga Sargassum Lacerifolium: X-Ray Molecular Structure of 2-Amino-3- [5'-Deoxy-5'-(Dimethylarsinoyl) - Ri Bosyloxy] Propane-I -Sulphonic Acid. *J. Chem. Soc., Perkin Trans.* **1991**, *1*, 2707–2716.

(393) Foster, S.; Maher, W. Arsenobetaine and Thio-Arsenic Species in Marine Macroalgae and Herbivorous Animals: Accumulated through Trophic Transfer or Produced in Situ? *J. Environ. Sci. (China)* **2016**, *49*, 131–139.

(394) Shibata Y., Jin K., M. M. Arsenic Compounds in the Edible Red Alga, Porphyra Tenera, and in Nori and Yakinori, Food Items Produced from Red Algae. *Appl. Organomet. Chem.* **1990**, *4* (3), 255–260.

(395) Shibata, Y.; Morita, M. A Novel, Trimethylated Arseno-Sugar Isolated from the Brown Alga Sargassum Thunbergii. *Agric. Biol. Chem.* **1988**, *52* (4), 1087–1089.

(396) Shibata, Y.; Morita, M.; Edmonds, J. S.; Shibata, Y. Purification and Identification of Arsenic-Containing Ribofuranosides from the Edible Brown Seaweed, Laminaria Japonica (Makonbu). *Agric. Biol. Chem.* **1987**, *51* (2), 391–398.

(397) Francesconi, K. A.; Edmondsa, J. S.; Stick, R. V.; Edmonds, J. S.; V, S. R.; Stick, R.

V. Arsenic Compounds from the Kidney of the Giant Clam Tridacna Maxima: Isolation and Identification of an Arsenic-Containing Nucleoside. *J. Chem. Soc. perkins trans.* **1992**, *1*, 1349–1357.

(398) McSheehy, S.; Szpunar, J.; Lobinski, R.; Haldys, V.; Tortajada, J.; Edmonds, J. S. Characterization of Arsenic Species in Kidney of the Clam Tridacna Derasa by Multidimensional Liquid Chromatography-ICPMS and Electrospray Time-of-Flight Tandem Mass Spectrometry. *Anal. Chem.* **2002**, *74* (10), 2370–2378.

(399) Francesconi, K. A.; Goessler, W.; Panutrakul, S.; Irgolic, K. J. A Novel Arsenic Containing Riboside (Arsenosugar) in Three Species of Gastropod. *Sci. Total Environ.* **1998**, *221* (2–3), 139–148.

(400) McSheehy, S.; Pohl, P.; Lobiński, R.; Szpunar, J. Investigation of Arsenic Speciation in Oyster Test Reference Material by Multidimensional HPLC-ICP-MS and Electrospray Tandem Mass Spectrometry (ES-MS-MS). *Analyst* **2001**, *126* (7), 1055–1062.

(401) Madsen, A. D.; Goessler, W.; Pedersen, S. N.; Francesconi, K. A. Characterization of an Algal Extract by HPLC-ICP-MS and LC-Electrospray MS for Use in Arsenosugar Speciation Studies. *J. Anal. At. Spectrom.* **2000**, *15* (6), 657–662.

(402) Morita, M.; Shibata, Y. Chemical Forms of Arsenic in Marine Macroalgae. *Appl. Organomet. Chem.* **1990**, *4* (3), 181–190.

(403) Le, X. C.; Cullen, W. R.; Reimer, K. J. Human Urinary Arsenic Excretion after One-Time Ingestion of Seaweed, Crab and Shrimp. *Clin. Chem.* **1994**, *40* (4), 617–624.

(404) Ma, M. S.; Le, X. C. Effect of Arsenosugar Ingestion on Urinary Arsenic Speciation. *Clin. Chem.* **1998**, *44*, 539–550.

(405) Gamble, B. M.; Gallagher, P. A.; Shoemaker, J. A.; Wei, X.; Schwegel, C. A.; Creed, J. T. An Investigation of the Chemical Stability of Arsenosugars in Simulated Gastric Juice and Acidic Environments Using IC-ICP-MS and IC-ESI-MS/MS. *Analyst* **2002**, *127*, 781–785.

(406) García Sartal, C.; Barciela-Alonso, M. del C.; Bermejo-Barrera, P. Effect of the Cooking Procedure on the Arsenic Speciation in the Bioavailable (Dialyzable) Fraction from Seaweed. *Microchem. J.* **2012**, *105*, 65–71.

(407) Edmonds, J. S.; Francesconi, K. A. Arsenic in Seafoods: Human Health Aspects and

Regulations. *Mar. Pollut. Bull.* **1993**, *26* (12), 665–674.

(408) Edmonds, J. S., Francesconi K. A., Hansen J. A. Dimethyloxarsylethanol from Anaerobic Decomposition of Brown Kelp (Ecklonia Radiata) - a Likely Precursor of Arsenobetaine in Marine Fauna. *Experientia* **1982**, *38*, 643–644.

(409) Francesconi, K. A.; Tanggaar, R.; McKenzie, C. J.; Goessler, W. Arsenic Metabolites in Human Urine after Ingestion of an Arsenosugar. *Clin. Chem.* **2002**, *48*, 92–101.

(410) Lai V. W.-M., Cullen W. R., Harrington C. F., R. K. J.; Available, C.; Products, A. The Characterization of Arsenosugars in Commercially Available Algal Products Including A. *Appl. Organomet. Chem.* **1997**, *11*, 797–803.

(411) Nischwitz, V.; Kanaki, K.; Pergantis, S. A. Mass Spectrometric Identification of Novel Arsinothioyl-Sugars in Marine Bivalves and Algae. *J. Anal. At. Spectrom.* **2006**, *21*, 33–40.

(412) Pergantis, S. A.; Francesconi, K. A.; Goessler, W.; Thomas-Oates, J. E. Characterization of Arsenosugars of Biological Origin Using Fast Atom Bombardment Tandem Mass Spectrometry. *Anal. Chem.* **1997**, *69*, 4931–4937.

(413) Sakurai, T.; Kaise, T.; Ochi, T.; Saitoh, T.; Matsubara, C. Study of in Vitro Cytotoxicity of a Water Soluble Organic Arsenic Compound, Arsenosugar, in Seaweed. *Toxicology* **1997**, *122*, 205–212.

(414) Feldmann, J.; John, K.; Pengprecha, P. Arsenic Metabolism in Seaweed-Eating Sheep from Northern Scotland. *Fresenius J Anal Chem* **2000**, *368*, 116–121.

(415) Hansen, H. R.; Raab, A.; Francesconi, K. A.; Feldmann, J. Metabolism of Arsenic by Sheep Chronically Exposed to Arsenosugars as a Normal Part of Their Diet. 1. Quantitative Intake, Uptake, and Excretion. *Environ. Sci. Technol.* **2003**, *37* (5), 845–851.

(416) Schmeisser, E.; Rumpler, A.; Kollroser, M.; Rechberger, G.; Goessler, W.; Francesconi, K. A. Arsenic Fatty Acids Are Human Urinary Metabolites of Arsenolipids Present in Cod Liver. *Angew. Chemie Int. Ed.* **2006**, *45*, 150–154.

(417) Schmeisser, E.; Goessler, W.; Francesconi, K. A. Human Metabolism of Arsenolipids Present in Cod Liver. *Anal. Bioanal. Chem.* **2006**, *385* (2), 367–376.

(418) Van Hulle, M.; Zhang, C.; Schotte, B.; Mees, L.; Vanhaecke, F.; Vanholder, R.;

Zhang, X. R.; Cornelis, R. Identification of Some Arsenic Species in Human Urine and Blood after Ingestion of Chinese Seaweed Laminaria. *J. Anal. At. Spectrom.* **2004**, *19* (1), 58–64.

(419) Taylor, V. F.; Li, Z.; Sayarath, V.; Palys, T. J.; Morse, K. R.; Scholz-Bright, R. A.; Karagas, M. R. Distinct Arsenic Metabolites Following Seaweed Consumption in Humans. *Sci. Rep.* **2017**, *7*, 1–9.

(420) Taleshi, M. S.; Seidler-Egdal, R. K.; Jensen, K. B.; Schwerdtle, T.; Francesconi, K. A. Synthesis and Characterization of Arsenolipids: Naturally Occurring Arsenic Compounds in Fish and Algae. *Organometallics* **2014**, *33*, 1397–1403.

(421) Arroyo-Abad, U.; Hu, Z.; Findeisen, M. M.; Pfeifer, D.; Mattusch, J.; Reemtsma, T.; Piechotta, C. Synthesis of Two New Arsenolipids and Their Identification in Fish. *Eur. J. Lipid Sci. Technol.* **2016**, *118*, 445–452.

(422) Arroyo-Abad, U.; Pfeifer, M.; Mothes, S.; Stärk, H. J.; Piechotta, C.; Mattusch, J.; Reemtsma, T. Determination of Moderately Polar Arsenolipids and Mercury Speciation in Freshwater Fish of the River Elbe (Saxony, Germany). *Environ. Pollut.* **2016**, *208*, 458–466.

(423) Arroyo-Abad, U.; Lischka, S.; Piechotta, C.; Mattusch, J.; Reemtsma, T. Determination and Identification of Hydrophilic and Hydrophobic Arsenic Species in Methanol Extract of Fresh Cod Liver by RP-HPLC with Simultaneous ICP-MS and ESI-Q-TOF-MS Detection. *Food Chem.* **2013**, *141*, 3093–3102.

(424) Francesconi, K. A.; Schwerdtle, T. Fat-Soluble Arsenic – New Lipids with a Sting in Their Tail. *Lipid Technol.* **2016**, *28*, 96–98.

(425) Fukuda, S.; Terasawa, M.; Shiomi, K. Phosphatidylarsenocholine, One of the Major Arsenolipids in Marine Organisms: Synthesis and Metabolism in Mice. *Food Chem. Toxicol.* **2011**, *49*, 1598–1603.

(426) Cooney, R. V; Mumma, R. O.; Benson, A. A. Arsoniumphospholipid in Algae. *Proc. Natl. Acad. Sci.* **1978**, *75* (9), 4262–4264.

(427) Benson, A. A.; Summons, R. E. The American Association for the Advancement of Science. *Science (80-.).* **1981**, *211* (4481), 482–483.

(428) Guttenberger, N.; Glabonjat, R. A.; Jensen, K. B.; Zangger, K.; Francesconi, K. A. Synthesis of Two Arsenic-Containing Cyclic Ethers: Model Compounds for a Novel

Group of Naturally-Occurring Arsenolipids. *Tetrahedron Lett.* **2016**, *57* (41), 4578–4580.

(429) Guttenberger, N.; Sagmeister, P.; Glabonjat, R. A.; Hirner, S.; Francesconi, K. A. Facile Access to Arsenic-Containing Triacylglycerides. *Tetrahedron Lett.* **2017**, *58* (4), 362–364.

(430) Witt, B.; Ebert, F.; Meyer, S.; Francesconi, K. A.; Schwerdtle, T. Assessing Neurodevelopmental Effects of Arsenolipids in Pre-Differentiated Human Neurons. *Mol. Nutr. Food Res.* **2017**, *61* (11), 1–10.

(431) Witt, B.; Bornhorst, J.; Mitze, H.; Ebert, F.; Meyer, S.; Francesconi, K. A.; Schwerdtle, T. Arsenolipids Exert Less Toxicity in a Human Neuron Astrocyte Co-Culture as Compared to the Respective Monocultures. *Metallomics* **2017**, *9* (5), 442–446.

(432) Al Amin, M. H.; Xiong, C.; Glabonjat, R. A.; Francesconi, K. A.; Oguri, T.; Yoshinaga, J. Estimation of Daily Intake of Arsenolipids in Japan Based on a Market Basket Survey. *Food Chem. Toxicol.* **2018**, *118*, 245–251.

(433) Amayo, K. O.; Raab, A.; Krupp, E. M.; Feldmann, J. Identification of Arsenolipids and Their Degradation Products in Cod-Liver Oil. *Talanta* **2014**, *118*, 217–223.

(434) Gamble, B. M.; Gallagher, P. A.; Shoemaker, J. A.; Parks, A. N.; Freeman, D. M.; Schwegel, C. A.; Creed, J. T. An Investigation of the Chemical Stability of Arsenosugars in Basic Environments Using IC-ICP-MS and IC-ESI-MS/MS. *Analyst* **2003**, *128* (12), 1458–1461.

(435) Nischwitz, V.; Pergantis, S. A. Identification of the Novel Thio-Arsenosugars DMThioAsSugarCarboxyl, DMThioAsSugarCarbamate and DMThioAsSugarAdenine in Extracts of Giant Clam Tissues by High-Performance Liquid Chromatography Online with Electrospray Tandem Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 3579–3585.

(436) Easterling, M. R.; Styblo, M.; Evans, M. V.; Kenyon, E. M. Pharmacokinetic Modeling of Arsenite Uptake and Metabolism in Hepatocytes - Mechanistic Insights and Implications for Further Experiments. *J. Pharmacokinet. Pharmacodyn.* **2002**, *29* (3), 207–234.

(437) Soeroes, C.; Goessler, W.; Francesconi, K. A.; Schmeisser, E.; Raml, R.; Kienzl, N.;

Kahn, M.; Fodor, P.; Kuehnelt, D.; Naito, S.; et al. Thio Arsenosugars in Freshwater Mussels from the Danube in Hungary. *J. Environ. Monit.* **2005**, *7*, 688–692.

(438) Krupp, E.; Feldmann, J.; Pétursdóttir, Á. H.; Küpper, F. C.; Gunnlaugsdóttir, H.; Fletcher, K. Environmental Effects on Arsenosugars and Arsenolipids in Ectocarpus (Phaeophyta). *Environ. Chem.* **2015**, *13* (1), 21.

(439) Xue, X. M.; Ye, J.; Raber, G.; Rosen, B. P.; Francesconi, K.; Xiong, C.; Zhu, Z.; Rensing, C.; Zhu, Y. G. Identification of Steps in the Pathway of Arsenosugar Biosynthesis. *Environ. Sci. Technol.* **2019**, *53* (2).

(440) Chen, M. L.; Ma, L. Y.; Chen, X. W. New Procedures for Arsenic Speciation: A Review. *Talanta* **2014**, *125*, 78–86.

(441) García-Salgado, S.; Quijano, M. Á. Stability of Toxic Arsenic Species and Arsenosugars Found in the Dry Alga Hijiki and Its Water Extracts. *Talanta* **2014**, *128*, 83–91.

(442) Naranmandura, H.; Xu, S.; Koike, S.; Pan, L. Q.; Chen, B.; Wang, Y. W.; Rehman, K.; Wu, B.; Chen, Z.; Suzuki, N. The Endoplasmic Reticulum Is a Target Organelle for Trivalent Dimethylarsinic Acid (DMAIII)-Induced Cytotoxicity. *Toxicol. Appl. Pharmacol.* **2012**, *260* (3), 241–249.

(443) Naranmandura, H.; Iwata, K.; Suzuki, K. T.; Ogra, Y. Distribution and Metabolism of Four Different Dimethylated Arsenicals in Hamsters. *Toxicol. Appl. Pharmacol.* **2010**, *245* (1), 67–75.

(444) Ciminelli, V. S. T. T.; Gasparon, M.; Ng, J. C.; Silva, G. C.; Caldeira, C. L. Dietary Arsenic Exposure in Brazil: The Contribution of Rice and Beans. *Chemosphere* **2017**, *168*, 996–1003.

(445) Kumari, B.; Kumar, V.; Sinha, A. K.; Ahsan, J.; Ghosh, A. K.; Wang, H.; DeBoeck, G. Toxicology of Arsenic in Fish and Aquatic Systems. *Environ. Chem. Lett.* **2017**, *15* (1), 43–64.

(446) Thomas, K. W.; Sheldon, L. S.; Pellizzari, E. D.; Handy, R. W.; Roberds, J. M.; Berry, M. R. Testing Duplicate Diet Sample Collection Methods for Measuring Personal Dietary Exposures to Chemical Contaminants. *J. Expo. Anal. Environ. Epidemiol.* **1997**, *7* (1), 17–36.

(447) Ruttens, A.; Blanpain, A. C.; De Temmerman, L.; Waegeneers, N. Arsenic Speciation

in Food in Belgium. Part 1: Fish, Molluscs and Crustaceans. *J. Geochemical Explor.* **2012**, *121*, 55–61.

(448) Rasheed, H.; Kay, P.; Slack, R.; Gong, Y. Y.; Carter, A. Human Exposure Assessment of Different Arsenic Species in Household Water Sources in a High Risk Arsenic Area. *Sci. Total Environ.* **2017**, *584–585*, 631–641.

(449) Mcsheehy, S.; Mester, Z. Arsenic Speciation in Marine Certified Reference Materials Part 1. Identification of Water-Soluble Arsenic Species Using Multidimensional Liquid Chromatography Combined with Inductively Coupled Plasma, Electrospray and Electrospray High-Field Asymmetric W. *J. Anal. At. Spectrom.* **2004**, *19* (3), 373–380.

(450) Pickford, R.; Miguens-Rodriguez, M.; Afzaal, S.; Speir, P.; Pergantis, S. A.; Thomas-Oates, J. E. Application of the High Mass Accuracy Capabilities of FT-ICR-MS and Q-ToF-MS to the Characterisation of Arsenic Compounds in Complex Biological Matrices. *J. Anal. At. Spectrom.* **2002**, *17* (3), 173–176.

(451) Pergantis, S. A.; Wangkarn, S.; Francesconi, K. A.; Thomas-Oates, J. E. Identification of Arsenosugars at the Picogram Level Using Nanoelectrospray Quadrupole Time-of-Flight Mass Spectrometry. *Anal. Chem.* **2000**, *72* (2), 357–366.

(452) Nearing, M. M.; Koch, I.; Reimer, K. J. Complementary Arsenic Speciation Methods : A Review. *Spectrochim. Acta Part B* **2014**, *99*, 150–162.

(453) Wells, M. L.; Potin, P.; Craigie, J. S.; Raven, J. A.; Merchant, S. S.; Helliwell, K. E.; Smith, A. G.; Camire, M. E.; Brawley, S. H. Algae as Nutritional and Functional Food Sources : Revisiting Our Understanding. *J. Appl. Phycol.* **2017**, *29*, 949–982.

(454) Soeroes, C.; Goessler, W.; Francesconi, K. A.; Kienzl, N.; Schaeffer, R.; Fodor, P.; Kuehnelt, D. Arsenic Speciation in Farmed Hungarian Freshwater Fish. *J. Agric. Food Chem.* **2005**, *53* (23), 9238–9243.

(455) Schaeffer, R.; Francesconi, K. A.; Kienzl, N.; Soeroes, C.; Fodor, P.; Váradi, L.; Raml, R.; Goessler, W.; Kuehnelt, D. Arsenic Speciation in Freshwater Organisms from the River Danube in Hungary. *Talanta* **2006**, *69* (4), 856–865.

(456) Miyashita, S.; Shimoya, M.; Kamidate, Y.; Kuroiwa, T.; Shikino, O.; Fujiwara, S.; Francesconi, K. A.; Kaise, T. Rapid Determination of Arsenic Species in Freshwater Organisms from the Arsenic-Rich Hayakawa River in Japan Using HPLC-ICP-MS.

Chemosphere **2009**, *75* (8), 1065–1073.

(457) Khan, M.; Francesconi, K. A. Preliminary Studies on the Stability of Arsenolipids: Implications for Sample Handling and Analysis. *J. Environ. Sci. (China)* **2016**, *49*, 97–103.

(458) Le, X. C. Speciation of Arsenic Compounds Using High-Performance Liquid Chromatography at Elevated Temperature and Selective Hydride Generation Atomic Fluorescence Detection. *Anal. Chem.* **1996**, *68* (24), 4501–4506.

(459) Wolle, M. M.; Conklin, S. D. Speciation Analysis of Arsenic in Seafood and Seaweed: Part II—Single Laboratory Validation of Method. *Anal. Bioanal. Chem.* **2018**.

(460) Gómez-Ariza, J. L.; Sánchez-Rodas, D.; Giráldez, I.; Morales, E. A Comparison between ICP-MS and AFS Detection for Arsenic Speciation in Environmental Samples. *Talanta* **2000**, *51*, 257–268.

(461) Šlejkovec, Z.; Kápolna, E.; Ipolyi, I.; van Elteren, J. T. Arsenosugars and Other Arsenic Compounds in Littoral Zone Algae from the Adriatic Sea. *Chemosphere* **2006**, *63*, 1098–1105.

(462) Maher, W. A.; Foster, S. D.; Taylor, A. M.; Krikowa, F.; Duncan, E. G.; Chariton, A. A. Arsenic Distribution and Species in Two Zostera Capricorni Seagrass Ecosystems, New South Wales, Australia. *Environ. Chem.* **2011**, *8*, 9–18.

(463) McSheehy, S.; Szpunar, J.; Morabito, R.; Quevauviller, P. The Speciation of Arsenic in Biological Tissues and the Certification of Reference Materials for Quality Control. *TrAC - Trends Anal. Chem.* **2003**, *22* (4), 191–209.

(464) Lewis, J.; Stokes, P.; Brereton, N.; Baxter, M.; Macarthur, R. Stability of Arsenic Speciation in Fish Muscle Samples, under Different Storage and Sample Preparation Conditions. *Microchem. J.* **2012**, *105*, 56–59.

(465) Dahl, L.; Molin, M.; Amlund, H.; Meltzer, H. M.; Julshamn, K.; Alexander, J.; Sloth, J. J.; Margrete, H.; Julshamn, K.; Alexander, J.; et al. Stability of Arsenic Compounds in Seafood Samples during Processing and Storage by Freezing. *Food Chem.* **2010**, *123* (3), 720–727.

(466) García Salgado, S.; Quijano Nieto, M. A.; Simón, M. M. B. Assessment of Total Arsenic and Arsenic Species Stability in Alga Samples and Their Aqueous Extracts.

Talanta **2008**, *75*, 897–903.

(467) Whitfield, F. B.; Freeman, D. J.; Shaw, K. J. Trimethylarsine: An Important off-Flavour Component in Some Prawn Species. *Chem. Ind* **1983**, *20*, 786–787.

(468) Narukawa, T.; Chiba, K. Heat-Assisted Aqueous Extraction of Rice Flour for Arsenic Speciation Analysis. *J. Agric. Food Chem.* **2010**, *58*, 8183–8188.

(469) Alava, P.; Van De Wiele, T.; Tack, F.; Du Laing, G. Extensive Grinding and Pressurized Extraction with Water Are Key Points for Effective and Species Preserving Extraction of Arsenic from Rice. *Anal. Methods* **2012**, *4*, 1237–1243.

(470) Majors, R. E. Sample Preparation Perspectives. *LCGC North Am.* **1991**, *9* (1), 16–20.

(471) Van Hulle, M.; Zhang, C.; Zhang, X.; Cornelis, R. Arsenic Speciation in Chinese Seaweeds Using HPLC-ICP-MS and HPLC-ES-MS. *Analyst* **2002**, *127* (5), 634–640.

(472) Kohlmeyer, U.; Jantzen, E.; Kuballa, J.; Jakubik, S. Benefits of High Resolution IC-ICP-MS for the Routine Analysis of Inorganic and Organic Arsenic Species in Food Products of Marine and Terrestrial Origin. *Anal. Bioanal. Chem.* **2003**, *377*, 6–13.

(473) Beauchemin, D.; Bednas, M. E.; Berman, S. S.; McLaren, J. W.; Siu, K. W. M.; Sturgeon, R. E. Exchange of Comments on Identification and Quantitation of Arsenic Species in a Dogfish Muscle Reference Material for Trace Elements. Reply to Comments. *Anal. Chem.* **1988**, *60*, 2209–2212.

(474) Kuehnelt, D.; Irgolic, K. J.; Goessler, W. Comparison of Three Methods for the Extraction of Arsenic Compounds from the NRCC Standard Reference Material DORM-2 and the Brown Alga Hijiki Fuziforme. *Appl. Organomet. Chem.* **2001**, *15*, 445–456.

(475) Goessler, W.; Kuehnelt, D.; Schlagenhaufen, C.; Slejltovec, Z.; Irgolic, K. J. Arsenobetaine and Other Arsenic Compounds in the National Research Council of Canada Certified Reference Materials DORM 1 and DORM 2. *J. Anal. At. Spectrom.* **1998**, *13*, 183–187.

(476) Villa-Lojo, M. C.; Alonso-Rodríguez, E.; López-Mahía, P.; Muniategui-Lorenzo, S.; Prada-Rodríguez, D. Coupled High Performance Liquid Chromatography-Microwave Digestion-Hydride Generation-Atomic Absorption Spectrometry for Inorganic and Organic Arsenic Speciation in Fish Tissue. *Talanta* **2002**, *57*, 741–750.

(477) Thomas, D. J.; Styblo, M.; Lin, S. The Cellular Metabolism and Systemic Toxicity of Arsenic. *Toxicol. Appl. Pharmacol.* **2001**, *176* (2), 127–144.

(478) Ackely, K. L.; B'Hymer, C.; Sutton, K. L.; Caruso, J. A. Speciation of Arsenic in Fish Tissue Using Microwave-Assisted Extraction Followed by HPLC-ICP-MS. *J. Anal. At. Spectrom.* **1999**, *14*, 845–850.

(479) Akter, K.; Naidu, R. Analytical Tools for Assessing the Dynamics of Arsenic in Environmental Samples. *Manag. Arsen. Environ. - From Soil to Hum. Heal.* **2006**, 58–74.

(480) Moreda-Pineiro, A.; Pena-Vazquez, E.; Hermelo-Herbello, P.; Bermejo-Barrera, P.; Moreda-Pineiro, J.; Alonso-Rodriguez, E.; Muniategui-Lorenzo, S.; Lopez-Mahia, P.; Prada-Rodriguez, D.; Moreda-Piñeiro, A.; et al. Matrix Solid-Phase Dispersion as a Sample Pretreatment for the Speciation of Arsenic in Seafood Products. *Anal. Chem.* **2008**, *80* (23), 9272–9278.

(481) Bermejo, P.; Capelo, J. L.; Mota, A.; Madrid, Y.; Cámara, C. Enzymatic Digestion and Ultrasonication: A Powerful Combination in Analytical Chemistry. *TrAC - Trends Anal. Chem.* **2004**, *23* (9), 654–663.

(482) Sakai, T. Separation and Analysis of Toxic Arsenic Species, Using LC-ICP-MS. *Agilent Technologies Application Note*. 2000, pp 5980-0262E.

(483) Sarzanini, C. Liquid Chromatography: A Tool for the Analysis of Metal Species. *J. Chromatogr. A* **1999**, *850*, 213–228.

(484) Sarzanini, C.; Bruzzoniti, M. C. Metal Species Determination by Ion Chromatography. *TrAC - Trends Anal. Chem.* **2001**, *20*, 304–310.

(485) Montes-Bayón, M.; DeNicola, K.; Caruso, J. A. Liquid Chromatography –Inductively Coupled Plasma Mass Spectrometry. *J. Chromatogr. A* **2003**, *1000*, 457–476.

(486) Santos, C. M. M.; Nunes, M. A. G.; Barbosa, I. S.; Santos, G. L.; Peso-Aguiar, M. C.; Korn, M. G. A.; Flores, E. M. M.; Dressler, V. L. Evaluation of Microwave and Ultrasound Extraction Procedures for Arsenic Speciation in Bivalve Mollusks by Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry. *Spectrochim. Acta - Part B At. Spectrosc.* **2013**, *86*, 108–114.

(487) Simon, S.; Lobos, G.; Pannier, F.; De Gregori, I.; Pinochet, H.; Potin-Gautier, M. Speciation Analysis of Organoarsenical Compounds in Biological Matrices by

Coupling Ion Chromatography to Atomic Fluorescence Spectrometry with On-Line Photooxidation and Hydride Generation. *Anal. Chim. Acta* **2004**, *521*, 99–108.

(488) Braeuer, S.; Borovička, J.; Goessler, W. A Unique Arsenic Speciation Profile in Elaphomyces Spp. ("Deer Truffles")—Trimethylarsine Oxide and Methylarsonous Acid as Significant Arsenic Compounds. *Anal. Bioanal. Chem.* **2018**, *410* (9), 2283–2290.

(489) Braeuer, S.; Goessler, W. Arsenic Species in Mushrooms, with a Focus on Analytical Methods for Their Determination – A Critical Review. *Anal. Chim. Acta* **2019**, *1073*, 1–21.

(490) Gao, Y.; Baisch, P.; Mirlean, N.; Manoel, F.; Júnior, S.; Larebeke, N. Van; Baeyens, W.; Leermakers, M. Arsenic Speciation in Fish and Shellfish from the North Sea (Southern Bight) and Açu Port Area (Brazil) and Health Risks Related to Seafood Consumption. *Chemosphere* **2018**, *191*, 89–96.

(491) Geng, W.; Komine, R.; Ohta, T.; Nakajima, T.; Takanashi, H.; Ohki, A. Arsenic Speciation in Marine Product Samples : Comparison of Extraction – HPLC Method and Digestion – Cryogenic Trap Method. *Talanta* **2009**, *79*, 369–375.

(492) Dietz, C.; Sanz, J.; Sanz, E.; Muñoz-Olivas, R.; Cámara, C. Current Perspectives in Analyte Extraction Strategies for Tin and Arsenic Speciation. *J. Chromatogr. A* **2007**, *1153*, 114–129.

(493) Bayona, J. M. Supercritical Fluid Extraction in Speciation Studies. *TrAC - Trends Anal. Chem.* **2000**, *19*, 107–112.

(494) Wang, Z. F.; Cui, Z. J. Supercritical Fluid Extraction and Gas Chromatography Analysis of Arsenic Species from Solid Matrices. *Chinese Chem. Lett.* **2016**, *27*, 241–246.

(495) Gallagher, P. A.; Shoemaker, J. A.; Wei, X.; Brockhoff-Schwegel, C. A.; Creed, J. T. Extraction and Detection of Arsenicals in Seaweed via Accelerated Solvent Extraction with Ion Chromatographic Separation and ICP-MS Detection. *Fresenius. J. Anal. Chem.* **2001**, *369* (1), 71–80.

(496) Wahlen, R. Fast and Accurate Determination of Arsenobetaine in Fish Tissues Using Accelerated Solvent Extraction and HPLC-ICP-MS Determination. *J. Chromatogr. Sci.* **2004**, *42* (4), 217–222.

(497) Carabias-Martínez, R.; Rodríguez-Gonzalo, E.; Revilla-Ruiz, P.; Hernández-Méndez, J. Pressurized Liquid Extraction in the Analysis of Food and Biological Samples. *J. Chromatogr. A* **2005**, *1089*, 1–17.

(498) Leufroy, A.; Noël, L.; Dufailly, V.; Beauchemin, D.; Guérin, T. Determination of Seven Arsenic Species in Seafood by Ion Exchange Chromatography Coupled to Inductively Coupled Plasma-Mass Spectrometry Following Microwave Assisted Extraction: Method Validation and Occurrence Data. *Talanta* **2011**, *83* (3), 770–779.

(499) Kirby, J.; Maher, W.; Ellwood, M.; Krikowa, F. Arsenic Species Determination in Biological Tissues by HPLC-ICP-MS and HPLC-HG-ICP-MS. *Aust. J. Chem.* **2004**, *57*, 957–966.

(500) Han, C.; Cao, X.; Yu, J. J.; Wang, X. R.; Shen, Y. Arsenic Speciation in Sargassum Fusiforme by Microwave-Assisted Extraction and LC-ICP-MS. *Chromatographia* **2009**, *69* (5–6), 587–591.

(501) Wu, J.; Mester, Z.; Pawliszyn, J. Speciation of Organoarsenic Compounds by Polypyrrole-Coated Capillary in-Tube Solid Phase Microextraction Coupled with Liquid Chromatography/Electrospray Ionization Mass Spectrometry. *Anal. Chim. Acta* **2000**, *424*, 211–222.

(502) Luque-García, J. L.; Luque De Castro, M. D. Ultrasound: A Powerful Tool for Leaching. *TrAC - Trends Anal. Chem.* **2003**, *22*, 41–47.

(503) Šlejkovec, Z.; Bajc, Z.; Doganoc, D. Z. Arsenic Speciation Patterns in Freshwater Fish. *Talanta* **2004**, *62*, 931–936.

(504) Schaeffer, R.; Soeroes, C.; Ipolyi, I.; Fodor, P.; Thomaidis, N. S. Determination of Arsenic Species in Seafood Samples from the Aegean Sea by Liquid Chromatography-(Photo-Oxidation)-Hydride Generation-Atomic Fluorescence Spectrometry. *Anal. Chim. Acta* **2005**, *547*, 109–118.

(505) Ochsenkühn-Petropulu, M.; Varsamis, J.; Parissakis, G. Speciation of Arsenobetaine in Marine Organisms Using a Selective Leaching/Digestion Procedure and Hydride Generation Atomic Absorption Spectrometry. *Anal. Chim. Acta* **1997**, *337*, 323–327.

(506) Pizarro, I.; Gómez, M.; Cámara, C.; Palacios, M. A. Arsenic Speciation in Environmental and Biological Samples: Extraction and Stability Studies. *Anal. Chim. Acta* **2003**, *495*, 85–98.

(507) Amran, M. B.; Lagarde, F.; Leroy, M. J. F. Determination of Arsenic Species in Marine Organisms by HPLC-ICP-OES and HPLC-HG-QFAAS. *Mikrochim. Acta* **1997**, *127* (3--4), 195–202.

(508) Nischwitz, V.; Pergantis, S. A. Improved Arsenic Speciation Analysis for Extracts of Commercially Available Edible Marine Algae Using HPLC-ES-MS/MS. *J. Agric. Food Chem.* **2006**, *54* (18), 6507–6519.

(509) Pedersen, S. N.; Francesconi, K. A. Liquid Chromatography Electrospray Mass Spectrometry with Variable Fragmentor Voltages Gives Simultaneous Elemental and Molecular Detection of Arsenic Compounds. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 641–645.

(510) Francesconi, K. A.; Edmonds, J. S. A Novel Arsenical in Clam Kidney Identified by Liquid Chromatography/Electrospray Ionisation Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2001**, *15* (17), 1641–1646.

(511) Mandal, B. K.; Ogra, Y.; Anzai, K.; Suzuki, K. T. Speciation of Arsenic in Biological Samples. *Toxicol. Appl. Pharmacol.* **2004**, *198* (3), 307–318.

(512) Wangkarn, S.; Pergantis, S. A. High-Speed Separation of Arsenic Compounds Using Narrow-Bore High-Performance Liquid Chromatography on-Line with Inductively Coupled Plasma Mass Spectrometry. *J. Anal. At. Spectrom.* **2000**, *15*, 627–633.

(513) Gallagher, P. A.; Wei, X.; A. Shoemaker, J.; A. Brockhoff, C.; Creed, J. T. Detection of Arsenosugars from Kelp Extracts via IC-Electrospray Ionization-MS-MS and IC Membrane Hydride Generation ICP-MS. *J. Anal. At. Spectrom.* **1999**, *14* (12), 1829–1834.

(514) Reis, V. A. T.; Duarte, A. C. Analytical Methodologies for Arsenic Speciation in Macroalgae: A Critical Review. *TrAC - Trends Anal. Chem.* **2018**, *102*, 170–184.

(515) Alberti, J.; Rubio, R.; Rauret, G. Extraction Method for Arsenic Speciation in Marine Organisms. *Fresenius J Anal Chem* **1995**, *351*, 420–425.

(516) Devalla, S.; Feldmann, J. Determination of Lipid-Soluble Arsenic Species in Seaweed-Eating Sheep from Orkney. *Appl. Organomet. Chem.* **2003**, *17*, 906–912.

(517) Francesconi, K. A.; Kuehnelt, D. Determination of Arsenic Species: A Critical Review of Methods and Applications. *Analyst* **2004**, *129*, 373–395.

(518) Cao, X.; Hao, C.; Wang, G.; Yang, H.; Chen, D.; Wang, X. Sequential Extraction Combined with HPLC-ICP-MS for As Speciation in Dry Seafood Products. *Food Chem.* **2009**, *113* (2), 720–726.

(519) Kahn, M.; Raml, R.; Schmeisser, E.; Vallant, B.; Francesconi, K. A.; Goessler, W. Two Novel Thio-Arsenosugars in Scallops Identified with HPLC-ICPMS and HPLC-ESMS. *Environ. Chem.* **2005**, *2* (3), 171–176.

(520) Foster, S.; Maher, W.; Krikowa, F.; Apte, S. A Microwave-Assisted Sequential Extraction of Water and Dilute Acid Soluble Arsenic Species from Marine Plant and Animal Tissues. *Talanta* **2007**, *71* (2), 537–549.

(521) Sadee, B. A.; Foulkes, M. E.; Hill, S. J. An Evaluation of Extraction Techniques for Arsenic in Staple Diets (Fish and Rice) Utilising Both Classical and Enzymatic Extraction Methods. *Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess.* **2016**, *33* (3), 433–441.

(522) Maher, W. A.; Foster, S.; Krikowa, F.; Duncan, E.; St John, A.; Hug, K.; Moreau, J. W. Thio Arsenic Species Measurements in Marine Organisms and Geothermal Waters. *Microchem. J.* **2013**, *111*, 82–90.

(523) Moreda-Piñeiro, A.; Moreda-Piñeiro, J.; Herbello-Hermelo, P.; Bermejo-Barrera, P.; Muniategui-Lorenzo, S.; López-Mahía, P.; Prada-Rodríguez, D. Application of Fast Ultrasound Water-Bath Assisted Enzymatic Hydrolysis - High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry Procedures for Arsenic Speciation in Seafood Materials. *J. Chromatogr. A* **2011**, *1218* (39), 6970–6980.

(524) Zou, H.; Zhou, C.; Li, Y.; Yang, X.; Wen, J.; Hu, X.; Sun, C. Occurrence, Toxicity, and Speciation Analysis of Arsenic in Edible Mushrooms. *Food Chem.* **2019**, *281*, 269–284.

(525) Pardo-Martínez, M.; Viñas, P.; Fisher, A.; Hill, S. J. Comparison of Enzymatic Extraction Procedures for Use with Directly Coupled High Performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry for the Speciation of Arsenic in Baby Foods. *Anal. Chim. Acta* **2001**, *441*, 29–36.

(526) Fitzpatrick, S.; Ebdon, L.; Foulkes, M. E. Separation and Detection of Arsenic and Selenium Species in Environmental Samples by HPLC-ICP-MS. *Int. J. Environ. Anal.*

Chem. **2002**, *82*, 835–841.

(527) Chen, S.; Guo, Q.; Liu, L. Determination of Arsenic Species in Edible Mushrooms by High-Performance Liquid Chromatography Coupled to Inductively Coupled Plasma Mass Spectrometry. *Food Anal. Methods* **2017**, *10* (3), 740–748.

(528) Reyes, L. H.; Mar, J. L. G.; Rahman, G. M. M.; Seybert, B.; Fahrenholz, T.; Kingston, H. M. S. Simultaneous Determination of Arsenic and Selenium Species in Fish Tissues Using Microwave-Assisted Enzymatic Extraction and Ion Chromatography-Inductively Coupled Plasma Mass Spectrometry. *Talanta* **2009**, *78*, 983–990.

(529) Sanz, E.; Muñoz-Olivas, R.; Cámara, C. Evaluation of a Focused Sonication Probe for Arsenic Speciation in Environmental and Biological Samples. *J. Chromatogr. A* **2005**, *1097*, 1–8.

(530) Sanz, E.; Muñoz-Olivas, R.; Dietz, C.; Sanz, J.; Cámara, C. Alternative Extraction Methods for Arsenic Speciation in Hair Using Ultrasound Probe Sonication and Pressurised Liquid Extraction. *J. Anal. At. Spectrom.* **2007**, *22*, 131–139.

(531) Peña-Farfal, C.; Moreda-Piñeiro, A.; Bermejo-Barrera, A.; Bermejo-Barrera, P.; Pinochet-Cancino, H.; De Gregori-Henríquez, I. Ultrasound Bath-Assisted Enzymatic Hydrolysis Procedures as Sample Pretreatment for the Multielement Determination in Mussels by Inductively Coupled Plasma Atomic Emission Spectrometry. *Anal. Chem.* **2004**, *76*, 3541–3547.

(532) Bonomi, F.; Fiocchi, A.; Frøkiær, H.; Gaiaschi, A.; Iametti, S.; Poiesi, C.; Rasmussen, P.; Restani, P.; Rovere, P. Reduction of Immunoreactivity of Bovine β-Lactoglobulin upon Combined Physical and Proteolytic Treatment. *J. Dairy Res.* **2003**, *70*, 51–59.

(533) Zoorob, G. K.; McKiernan, J. W.; Caruso, J. A. ICP-MS for Elemental Speciation Studies. *Mikrochim. Acta* **1998**, *128*, 145–168.

(534) Wenclawiak, B. W.; Krah, M. Reactive Supercritical Fluid Extraction and Chromatography of Arsenic Species. *Fresenius. J. Anal. Chem.* **1995**, *351*, 134–138.

(535) Kingston, H. M.; Haswell, S. J. *Microwave-Enhanced Chemistry: Fundamentals, Sample Preparation, and Applications*; Kingston, H. M., Haswell, S. J., Eds.; American Chemical Society: Washington DC, 1997.

(536) Vilanoand, M.; Rubio, R. Determination of Arsenic Species in Oyster Tissue by Microwave-Assisted Extraction and Liquid Chromatography - Atomic Fluorescence

Detection. *Appl. Organomet. Chem.* **2001**, *15*, 658–666.

(537) Dagnac, T.; Padró, A.; Rubio, R.; Rauret, G. Speciation of Arsenic in Mussels by the Coupled System Liquid Chromatography - UV Irradiation - Hydride Generation-Inductively Coupled Plasma Mass Spectrometry. *Talanta* **1999**, *48*, 763–772.

(538) Dagnac, T.; Padró, A.; Rubio, R.; Rauret, G. Optimisation of the Extraction of Arsenic Species from Mussels with Low Power Focused Microwaves by Applying a Doehlert Design. *Anal. Chim. Acta* **1998**, *364*, 19–30.

(539) Nobrega, J. A.; Trevizan, L. C.; Araujo, G. C. L.; Nogueira, A. R. A. Focused-Microwave-Assisted Strategies for Sample Preparation. *Spectrochim. Acta Part B* **2002**, *57*, 1855–1876.

(540) Chemat, F.; Zill-E-Huma; Khan, M. K. Applications of Ultrasound in Food Technology: Processing, Preservation and Extraction. *Ultrason. Sonochem.* **2011**, *18* (4), 813–835.

(541) Chemat, F.; Rombaut, N.; Sicaire, A.-G.; Meullemiestre, A.; Fabiano-Tixier, A.-S.; Abert-Vian, M. Ultrasound Assisted Extraction of Food and Natural Products. Mechanisms, Techniques, Combinations, Protocols and Applications. A Review. *Ultrason. Sonochem.* **2017**, *34*, 540–560.

(542) Letellier, M.; Budzinski, H. Microwave Assisted Extraction of Organic Compounds. *Analusis* **1999**, *27*, 259–271.

(543) Dietz, C.; Sanz, J.; Camara, C. Recent Developments in Solid-Phase Microextractioncoatings and Related Techniques. *J. Chromatogr. A* **2006**, *1103*, 183.192.

(544) Mester, Z.; Sturgeon, R. Solid Phase Microextraction as a Tool for Trace Element Determination. *Compr. Anal. Chem.* **2003**, *41*, 371–391.

(545) Barker, S. A.; Long, A. R.; Short, C. R. Isolation of Drug Residues from Tissues by Solid Phase Dispersion. *J. Chromatogr. A* **1989**, *475*, 353–361.

(546) Kristenson, E. M.; Ramos, L.; Brinkman, U. A. T. Recent Advances in Matrix Solid-Phase Dispersion. *TrAC - Trends Anal. Chem.* **2006**, *25*, 96–111.

(547) Ramos, L.; Ramos, J. J.; Brinkman, U. A. T. Miniaturization in Sample Treatment for Environmental Analysis. *Anal. Bioanal. Chem.* **2005**, *381*, 119–140.

(548) Moreda-Piñeiro, J.; Alonso-Rodríguez, E.; López-Mahía, P.; Muniategui-Lorenzo, S.; Prada-Rodríguez, D.; López-Mahía, P.; Prada-Rodríguez, D.; Romarís-Hortas, V.; Míguez-Framil, M.; Moreda-Piñeiro, A.; et al. Matrix Solid-Phase Dispersion of Organic Compounds and Its Feasibility for Extracting Inorganic and Organometallic Compounds. *TrAC - Trends Anal. Chem.* **2009**, *28* (1), 110–116.

(549) Barker, S. A. Matrix Solid-Phase Dispersion. *J. Chromatogr. A* **2000**, *885*, 115–127.

(550) Barker, S. A. Matrix Solid Phase Dispersion (MSPD). *J. Biochem. Biophys. Methods* **2007**, *70*, 151–162.

(551) Duncan, E. G.; Maher, W. A.; Foster, S. D. Contribution of Arsenic Species in Unicellular Algae to the Cycling of Arsenic in Marine Ecosystems. *Environ. Sci. Technol.* **2015**, *49*, 33–50.

(552) Kuehnelt, D.; Irgolic, K. J.; Goessler, W. Comparison of Three Methods for the Extraction of Arsenic Compounds from the NRCC Standard Reference Material DORM-2 and the Brown Alga Hijiki Fuziforme. *Appl. Organomet. Chem.* **2001**, *15* (6), 445–456.

(553) Francesconi, K. A. Complete Extraction of Arsenic Species: A Worthwhile Goal? *Appl. Organomet. Chem.* **2003**, *17* (9), 682–683.

(554) Leermakers, M.; Baeyens, W.; De Gieter, M.; Smedts, B.; Meert, C.; De Bisschop, H. C.; Morabito, R.; Quevauviller, P. Toxic Arsenic Compounds in Environmental Samples: Speciation and Validation. *TrAC - Trends in Analytical Chemistry*. 2006, pp 1–10.

(555) Sanz, E.; Muñoz-Olivas, R.; Cámara, C. A Rapid and Novel Alternative to Conventional Sample Treatment for Arsenic Speciation in Rice Using Enzymatic Ultrasonic Probe. *Anal. Chim. Acta* **2005**, *535*, 227–235.

(556) Harrington, C. F.; Ojo, A. A.; Lai, V. W. M.; Reimer, K. J.; Cullen, W. R. The Identification of Some Water-Soluble Arsenic Species in the Marine Brown Algae Fucus Distichus. *Appl. Organomet. Chem.* **1997**, *11* (12), 931–940.

(557) Camel, V. Solid Phase Extraction of Trace Elements. *Spectrochim. Acta - Part B At. Spectrosc.* **2003**, *58*, 1177–1233.

(558) López-Gonzálvez, M. A.; Gómez, M. M.; Cámara, C.; Palacios, M. A. On-Line Microwave Oxidation for the Determination of Organoarsenic Compounds by High-

Performance Liquid Chromatography-Hydride Generation Atomic Absorption Spectrometry. *J. Anal. At. Spectrom.* **1994**, *9*, 291–295.

(559) Gomez, M.; Camara, C.; Palacios, M. A.; Lopez-Gonzalez, A. Anionic Cartridge Preconcentrators for Inorganic Arsenic , Monomethylarsonate and Dimethylarsinate Determination by on-Line HPLC-HG-AAS. *Fresenius. J. Anal. Chem.* **1997**, *357*, 844–849.

(560) Modolon, S. D. M.; Felippe, A. C.; Fizon, T. E.; Da Silva, L.; Da Silva Paula, M. M.; Dal-Bó, A. G. Self-Association of Sodium Deoxycholate with EHEC Cellulose Cooperatively Induced by Sodium Dodecanoate. *Carbohydr. Polym.* **2014**, *111*, 425–432.

(561) Schramel, O.; Michalke, B.; Kettrup, A. Application of Capillary Electrophoresis-Electrospray Ionisation Mass Spectrometry to Arsenic Speciation. *J. Anal. At. Spectrom.* **1999**, *14*, 1339–1342.

(562) Schramel, O.; Michalke, B.; Kettrup, A. Analysis of Metal Species by Using Electrospray Ionization Mass Spectrometry and Capillary Electrophoresis-Electrospray Ionization Mass Spectrometry. *J. Chromatogr. A* **1998**, *819*, 231–242.

(563) Corr, J. J.; Anacleto, J. F. Analysis of Inorganic Species by Capillary Electrophoresis-Mass Spectrometry and Ion Exchange Chromatography-Mass Spectrometry Using an Ion Spray Source. *Anal. Chem.* **1996**, *68*, 2155–2163.

(564) B'Hymer, C.; Caruso, J. A. Arsenic and Its Speciation Analysis Using High-Performance Liquid Chromatography and Inductively Coupled Plasma Mass Spectrometry. *J. Chromatogr. A* **2004**, *1045*, 1–13.

(565) Gong, Z.; Lu, X.; Ma, M.; Watt, C.; Le, X. C. Arsenic Speciation Analysis. *Talanta* **2002**, *58* (1), 77–96.

(566) Michalke, B. The Coupling of LC to ICP-MS in Element Speciation - Part II: Recent Trends in Application. *TrAC - Trends Anal. Chem.* **2002**, *21*, 154–165.

(567) Arslan, Y.; Yildirim, E.; Gholami, M.; Bakirdere, S. Lower Limits of Detection in Speciation Analysis by Coupling High-Performance Liquid Chromatography and Chemical-Vapor Generation. *TrAC - Trends Anal. Chem.* **2011**, *30*, 569–585.

(568) Debusschere, L.; Demesmay, C.; Rocca, J. L. Arsenic Speciation by Coupling Capillary Zone Electrophoresis with Mass Spectrometry. *Chromatographia* **2000**, *51*,

262–268.

(569) Abian, J. The Coupling of Gas and Liquid Chromatography with Mass Spectrometry. *J. Mass Spectrom.* **1999**, *34*, 157–168.

(570) Fenn, J. B. Ion Formation from Charged Droplets: Roles of Geometry, Energy, and Time. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 524–535.

(571) Gale, D. C.; Smith, R. D. Small Volume and Low Flow-rate Electrospray Lonization Mass Spectrometry of Aqueous Samples. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 1017–1021.

(572) Moseley, M. A.; Jorgenson, J. W.; Shabanowitz, J.; Hunt, D. F.; Tomer, K. B. Optimization of Capillary Zone Electrophoresis/Electrospray Ionization Parameters for the Mass Spectrometry and Tandem Mass Spectrometry Analysis of Peptides. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 289–300.

(573) Takada, Y.; Sakairi, M.; Koizumi, H. Atmospheric Pressure Chemical Ionization Interface for Capillary Electrophoresis/Mass Spectrometry. *Anal. Chem.* **1995**, *67*, 1474–1476.

(574) Cai, J.; Henion, J. Capillary Electrophoresis - Mass Spectrometry - Review. *J. Chromatogr. A* **1995**, *703*, 667–692.

(575) Kovačevič, M.; Leber, R.; Kohlwein, S. D.; Goessler, W. Application of Inductively Coupled Plasma Mass Spectrometry to Phospholipid Analysis. *J. Anal. At. Spectrom.* **2004**, *19*, 80–84.

(576) Truei, Y.; Gu, T.; Tsai, G.; Tsao, G. T. Large-Scale Gradient Elution Chromatography. *Adv. Biochem. Eng. / Biotechnol.* **1992**, *47*, 1–44.

(577) Raber, G.; Francesconi, K. A.; Irgolic, K. J.; Goessler, W. Determination of "arsenosugars" in Algae with Anion-Exchange Chromatography and an Inductively Coupled Plasma Mass Spectrometer as Element-Specific Detector. *Fresenius. J. Anal. Chem.* **2000**, *367* (2), 181–188.

(578) Sloth, J. J.; Larsen, E. H.; Julshamn, K. Determination of Organoarsenic Species in Marine Samples Using Gradient Elution Cation Exchange HPLC-ICP-MS. *J. Anal. At. Spectrom.* **2003**, *18*, 452–459.

(579) Guerin, T.; Astruc, A.; Astruc, M. Speciation of Arsenic and Selenium Compounds by

HPLC Hyphenated to Specific Detectors: A Review of the Main Separation Techniques. *Talanta* **1999**, *50* (1), 1–24.

(580) Do, B.; Robinet, S.; Pradeau, D.; Guyon, F. Speciation of Arsenic and Selenium Compounds by Ion-Pair Reversed-Phase Chromatography with Electrothermic Atomic Absorption Spectrometry - Application of Experimental Design for Chromatographic Optimisation. *J. Chromatogr. A* **2001**, *918*, 87–98.

(581) Chen, Z.; Akter, K. F.; Rahman, M. M.; Naidu, R. Speciation of Arsenic by Ion Chromatography Inductively Coupled Plasma Mass Spectrometry Using Ammonium Eluents. *J. Sep. Sci.* **2006**, *29*, 2671–2676.

(582) Falk, K.; Emons, H. Speciation of Arsenic Compounds by Ion-Exchange HPLC-ICP-MS with Different Nebulizers. *J. Anal. At. Spectrom.* **2000**, *15*, 643–649.

(583) Guérin, T.; Astruc, A.; Astruc, M.; Batel, A.; Borsier, M. Chromatographic Ion-Exchange Simultaneous Separation of Arsenic and Selenium Species with Inductively Coupled Plasma-Mass Spectrometry On-Line Detection. *J. Chromatogr. Sci.* **1997**, *35*, 213–220.

(584) Nakazato, T.; Tao, H.; Taniguchi, T.; Isshiki, K. Determination of Arsenite, Arsenate, and Monomethylarsonic Acid in Seawater by Ion-Exclusion Chromatography Combined with Inductively Coupled Plasma Mass Spectrometry Using Reaction Cell and Hydride Generation Techniques. *Talanta* **2002**, *58*, 121–132.

(585) Morin, P.; Amran, M. B.; Favier, S.; Heimburger, R.; Leroy, M. Speciation of Arsenical Species by Anion-Exchange and Ion-Pair Reversed-Phase Liquid Chromatography. *Fresenius. J. Anal. Chem.* **1991**, *339*, 504–509.

(586) Ammann, A. A. Determination of Strong Binding Chelators and Their Metal Complexes by Anion-Exchange Chromatography and Inductively Coupled Plasma Mass Spectrometry. *J. Chromatogr. A* **2002**, *947*, 205–216.

(587) Horváth, C. G.; Lipsky, S. R. Peak Capacity in Chromatography. *Anal. Chem.* **1967**, *39* (14), 1893.

(588) Kanaki, K.; Pergantis, S. A. Precursor Ion Scanning for the Non-Targeted Detection of Individual Arsenosugars in Extracts of Marine Organisms. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1925–1931.

(589) Schellinger, A. P.; Carr, P. W. Isocratic and Gradient Elution Chromatography: A

Comparison in Terms of Speed, Retention Reproducibility and Quantitation. *J. Chromatogr. A* **2006**, *1109* (2), 253–266.

(590) Ritsema, R.; Dukan, L.; Roig I Navarro, T.; Van Leeuwen, W.; Oliveira, N.; Wolfs, P.; Lebret, E. Speciation of Arsenic Compounds in Urine by LC-ICP MS. *Appl. Organomet. Chem.* **1998**, *12*, 591–599.

(591) Saverwyns, S.; Zhang, X.; Vanhaecke, F.; Cornelis, R.; Moens, L.; Dams, R. Speciation of Six Arsenic Compounds Using High-Performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry with Sample Introduction by Thermospray Nebulization. *J. Anal. At. Spectrom.* **1997**, *12*, 1047–1052.

(592) Caruso, J. A.; Heitkemper, D. T.; B'Hymer, C. An Evaluation of Extraction Techniques for Arsenic Species from Freeze-Dried Apple Samples. *Analyst* **2001**, *126*, 136–140.

(593) Heitkemper, D.; Creed, J.; Caruso, J.; Fricke, F. L. Speciation of Arsenic in Urine Using High-Performance Liquid Chromatography with Inductively Coupled Plasma Mass Spectrometric Detection. *J. Anal. At. Spectrom.* **1989**, *4*, 279–284.

(594) Zheng, J.; Hintelmann, H.; Dimock, B.; Dzurko, M. S. Speciation of Arsenic in Water, Sediment, and Plants of the Moira Watershed, Canada, Using HPLC Coupled to High Resolution ICP-MS. *Anal. Bioanal. Chem.* **2003**, *377*, 14–24.

(595) Day, J. A.; Montes-Bayón, M.; Vonderheide, A. P.; Caruso, J. A. A Study of Method Robustness for Arsenic Speciation in Drinking Water Samples by Anion Exchange HPLC-ICP-MS. *Anal. Bioanal. Chem.* **2002**, *373*, 664–668.

(596) Yu, L. L.; Stanoyevitch, R. C.; Zeisler, R. SI Traceable Determination of Arsenic Species in Kelp (Thallus Laminariae). *Anal. Methods* **2017**, *9*, 4267–4274.

(597) B'Hymer, C.; Caruso, J. A. Evaluation of HPLC Systems for the Separation and Quantification of Arsenic Compounds from Apple Extracts. *J. Liq. Chromatogr. Relat. Technol.* **2002**, *25*, 639–653.

(598) Sheppard, B. S.; Caruso, J. A.; Heitkemper, D. T.; Wolnik, K. A. Arsenic Speciation by Ion Chromatography with Inductively Coupled Plasma Mass Spectrometric Detection. *Analyst* **1992**, *117*, 971–975.

(599) Vassileva, E.; Becker, A.; Broekaert, J. A. C. Determination of Arsenic and Selenium

Species in Groundwater and Soil Extracts by Ion Chromatography Coupled to Inductively Coupled Plasma Mass Spectrometry. *Anal. Chim. Acta* **2001**, *441*, 135–146.

(600) Mattusch, J.; Wennrich, R.; Schmidt, A. C.; Reisser, W. Determination of Arsenic Species in Water, Soils and Plants. *Fresenius. J. Anal. Chem.* **2000**, *366*, 200–203.

(601) Lintschinger, J.; Schramel, P.; Hatalak-Rauscher, A.; Wendler, I.; Michalke, B. A New Method for the Analysis of Arsenic Species in Urine by Using HPLC-ICP-MS. *Fresenius. J. Anal. Chem.* **1998**, *362*, 313–318.

(602) Bissen, M.; Frimmel, F. H. Speciation of As(III), As(V), MMA and DMA in Contaminated Soil Extracts by HPLC-ICP/MS. *Fresenius. J. Anal. Chem.* **2000**, *367*, 51–55.

(603) Chen, Z. L.; Khan, N. I.; Owens, G.; Naidu, R. Elimination of Chloride Interference on Arsenic Speciation in Ion Chromatography Inductively Coupled Mass Spectrometry Using an Octopole Collision/Reaction System. *Microchem. J.* **2007**, *87*, 87–90.

(604) Suzuki, K. T.; Tomita, T.; Ogra, Y.; Ohmichi, M. Glutathione-Conjugated Arsenics in the Potential Hepato-Enteric Circulation in Rats. *Chem. Res. Toxicol.* **2001**, *14*, 1604–1611.

(605) Beauchemin, D.; Siu, K. W. M.; McLaren, J. W.; Berman, S. S. Determination of Arsenic Species by High-Performance Liquid Chromatography - Inductively Coupled Plasma Mass Spectrometry. *J. Anal. At. Spectrom.* **1989**, *4* (3), 285–289.

(606) Terlecka, E. Arsenic Speciation Analysis in Water Samples: A Review of the Hyphenated Techniques. *Environ. Monit. Assess.* **2005**, *107* (1–3), 259–284.

(607) Ding, H.; Wang, J.; Dorsey, J. G.; Caruso, J. A. Arsenic Speciation by Micellar Liquid Chromatogrpahy with Inductively Coupled Plasma Mass Spectrometric Detection. *J. Chromatogr. A* **1995**, *694*, 425–431.

(608) Armstrong, D. W.; Nome, F. Partitioning Behavior of Solutes Eluted with Micellar Mobile Phases in Liquid Chromatography. *Anal. Chem.* **1981**, *53*, 1662–1666.

(609) Sakai, T.; Inoue, Y.; Date, Y.; Aoyama, T.; Yoshida, K.; Endo, G. Simultaneous Determination of Neutral, Anionic and Cationic Compounds within One Chromatographic Run Using an Inductively Coupled Plasma Mass Spectrometer as

Element-Specific Detector. *Appl. Organomet. Chem.* **2001**, *15*, 285–290.

(610) Wuilloud, R. G.; Altamirano, J. C.; Smichowski, P. N.; Heitkemper, D. T. Investigation of Arsenic Speciation in Algae of the Antarctic Region by HPLC-ICP-MS and HPLC-ESI-Ion Trap MS. *J. Anal. At. Spectrom.* **2006**, *21*, 1214–1223.

(611) Schaeffer R., Fodor P., S. C. Development of a Liquid Chromatography/Electrospray Selected Reaction Monitoring Method for the Determination of Organoarsenic Species in Marine and Freshwater Samples. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2979–2989.

(612) Lu, X.; Nguyen, N.; Gabos, S.; Le, X. C. Arsenic Speciation in Cattail (Typha Latifolia) Using Chromatography and Mass Spectrometry. *Mol. Nutr. Food Res.* **2009**, *53*, 566–571.

(613) Mandal Y. AU3 - Suzuki, K.T., B. K. A.-O.; Mandal, B. K.; Ogra, Y.; Suzuki, K. T. Identification of Dimethylarsinous and Monomethylarsonous Acids in Human Urine of the Arsenic-Affected Areas in West Bengal, India RN - Chem. Res. Toxicol., v. 14, p. 371-378. *Chem. Res. Toxicol.* **2001**, *14* (4), 371–378.

(614) Xie, R.; Johnson, W.; Spayd, S.; Hall, G. S.; Buckley, B. Arsenic Speciation Analysis of Human Urine Using Ion Exchange Chromatography Coupled to Inductively Coupled Plasma Mass Spectrometry. *Anal. Chim. Acta* **2006**, *578*, 186–194.

(615) Šlejkovec, Z.; Van Elteren, J. T.; Byrne, A. R. Determination of Arsenic Compounds in Reference Materials by HPLC-(UV)- HG-AFS. *Talanta*. 1999, pp 619–627.

(616) Burger, M.; Schwarz, G.; Gundlach-Graham, A.; Käser, D.; Hattendorf, B.; Günther, D. Capabilities of Laser Ablation Inductively Coupled Plasma Time-of-Flight Mass Spectrometry. *J. Anal. At. Spectrom.* **2017**, *32* (10), 1946–1959.

(617) Pröfrock, D.; Prange, A. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for Quantitative Analysis in Environmental and Life Sciences: A Review of Challenges, Solutions, and Trends. *Appl. Spectrosc.* **2012**, *66* (8), 843–868.

(618) Zubarev, R. A.; Makarov, A. Orbitrap Mass Spectrometry. *Anal. Chem.* **2013**, *85* (11), 5288–5296.

(619) Maher, W.; Krikowa, F.; Kirby, J.; Townsend, A. T.; Snitch, P. Measurement of Trace Elements in Marine Environmental Samples Using Solution ICPMS. Current and Future Application. *Aust. J. Chem.* **2003**, *56*, 103–116.

(620) Chiba, K.; Narukawa, T. The Effect of Plasma Reactions on Arsenic Measurement by ICP Spectrometry. *Anal. Sci.* **2014**, *30* (1), 175–181.

(621) Wallschläger, D.; Stadey, C. J. Determination of (Oxy) Thioarsenates in Sulfidic Waters. *Anal. Chem.* **2007**, *79* (10), 3873–3880.

(622) Nam, S. H.; Oh, H. J.; Min, H. S.; Lee, J. H. A Study on the Extraction and Quantitation of Total Arsenic and Arsenic Species in Seafood by HPLC-ICP-MS. *Microchem. J.* **2010**, *95* (1), 20–24.

(623) Axelsson, B. O.; Jörnten-Karlsson, M.; Michelsen, P.; Abou-Shakra, F. The Potential of Inductively Coupled Plasma Mass Spectrometry Detection for High-Performance Liquid Chromatography Combined with Accurate Mass Measurement of Organic Pharmaceutical Compounds. *Rapid Commun. Mass Spectrom.* **2001**, *15* (6), 375–385.

(624) Leber, R.; Kohlwein, S. D.; Goessler, W.; Kovac, M.; Kovačevič, M.; Leber, R.; Kohlwein, S. D.; Goessler, W.; Kovacevic, M.; Leber, R.; et al. Application of Inductively Coupled Plasma Mass Spectrometry to Phospholipid Analysis ˇ Evic. *J. Anal. At. Spectrom.* **2004**, *19*, 80–84.

(625) Meermann, B.; Kießhauer, M. Development of an Oxygen-Gradient System to Overcome Plasma Instabilities during HPLC/ICP-MS Measurements Using Gradient Elution. *J. Anal. At. Spectrom.* **2011**, *26* (10), 2069–2075.

(626) Grotti, M.; Terol, A.; Todolí, J. L. Speciation Analysis by Small-Bore HPLC Coupled to ICP-MS. *TrAC - Trends Anal. Chem.* **2014**, *61*, 92–106.

(627) Kirby, J.; Maher, D. W.; Ellwood, A. M.; A, F. K.; Maher, W.; Ellwood, M.; Krikowa, F. Arsenic Species Determination in Biological Tissues by HPLC–ICP–MS and HPLC–HG–ICP–MS. *Aust. J. Chem.* **2004**, *57*, 957–966.

(628) Schmeisser, E.; Raml, R.; Francesconi, K. A.; Kuehnelt, D.; Lindberg, A. L.; Sörös, C.; Goessler, W. Thio Arsenosugars Identified as Natural Constituents of Mussels by Liquid Chromatography-Mass Spectrometry. *Chem. Commun.* **2004**, *16*, 1824–1825.

(629) Pétursdóttir, Á. H.; Gunnlaugsdóttir, H.; Jörundsdóttir, H.; Mestrot, A.; Krupp, E. M.; Feldmann, J. HPLC-HG-ICP-MS: A Sensitive and Selective Method for Inorganic Arsenic in Seafood. *Anal. Bioanal. Chem.* **2012**, *404* (8), 2185–2191.

(630) Sánchez-Rodas, D.; Geiszinger, A.; Gómez-Ariza, J. L.; Francesconi, K. A. Determination of an Arsenosugar in Oyster Extracts by Liquid Chromatography-

Electrospray Mass Spectrometry and Liquid Chromatography-Ultraviolet Photo-Oxidation-Hydride Generation Atomic Fluorescence Spectrometry. *Analyst* **2002**, *127* (1), 60–65.

(631) Howard, A. G.; Salou, C. Cysteine Enhancement of the Cryogenic Trap Hydride AAS Determination of Dissolved Arsenic Species. *Anal. Chim. Acta* **1996**, *333*, 89–96.

(632) Kumar, R. A.; Riyazuddin, P.; Ramesh Kumar, A.; Riyazuddin, P. Mechanism of Volatile Hydride Formation and Their Atomization in Hydride Generation Atomic Absorption Spectrometry. *Anal. Sci.* **2005**, *21* (12), 1401–1410.

(633) Carrero, P.; Malavé, A.; Burguera, J. L.; Burguera, M.; Rondón, C. Determination of Various Arsenic Species by Flow Injection Hydride Generation Atomic Absorption Spectrometry: Investigation of the Effects of the Acid Concentration of Different Reaction Media on the Generation of Arsines. *Anal. Chim. Acta* **2001**, *438* (1–2), 195–204.

(634) Pohl, P.; Prusisz, B. Ion-Exchange Column Chromatography - An Attempt to Speciate Arsenic. *TrAC - Trends Anal. Chem.* **2004**, *23* (1), 63–69.

(635) Karadjova, I. B.; Lampugnani, L.; Onor, M.; D'Ulivo, A.; Tsalev, D. L. Continuous Flow Hydride Generation-Atomic Fluorescence Spectrometric Determination and Speciation of Arsenic in Wine. *Spectrochim. Acta - Part B At. Spectrosc.* **2005**, *60* (6), 816–823.

(636) Gomez-Ariza, J. L.; Sanchez-Rodas, D.; Beltran, R.; Corns, W.; Stockwel, P. Evaluation of Atomic Fluorescence Spectrometry as a Sensitive Detection Technique for Arsenic Speciation. *Appl. Organomet. Chem.* **1998**, *12* (6), 439–447.

(637) Lindberg, A. L.; Goessler, W.; Grandér, M.; Nermell, B.; Vahter, M. Evaluation of the Three Most Commonly Used Analytical Methods for Determination of Inorganic Arsenic and Its Metabolites in Urine. *Toxicol. Lett.* **2007**, *168* (3), 310–318.

(638) Schmeisser, E.; Goessler, W.; Kienzl, N.; Francesconi, K. A. Volatile Analytes Formed from Arsenosugars: Determination by HPLC-HG-ICPMS and Implications for Arsenic Speciation Analyses. *Anal. Chem.* **2004**, *76* (2), 418–423.

(639) Hummer, A. A.; Rompel, A. The Use of X-Ray Absorption and Synchrotron Based Micro-X-Ray Fluorescence Spectroscopy to Investigate Anti-Cancer Metal Compounds in Vivo and in Vitro. *Metallomics* **2013**, *5* (6), 597–614.

(640) Ortega, R. Direct Speciation Analysis of Inorganic Elements in Single Cells Using X-Ray Absorption Spectroscopy. *J. Anal. At. Spectrom.* **2011**, *26* (1), 23–29.

(641) Larsen, E. H. Method Optimization and Quality Assurance in Speciation Analysis Using High Performance Liquid Chromatography with Detection by Inductively Coupled Plasma Mass Spectrometry. *Spectrochimica acta, Part B: Atomic spectroscopy*. 1998, pp 253–265.

(642) Kohlmeyer, U.; Kuballa, J.; Jantzen, E. Simultaneous Separation of 17 Inorganic and Organic Arsenic Compounds in Marine Biota by Means of High-Performance Liquid Chromatography/Inductively Coupled Plasma Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2002**, *16* (10), 965–974.

(643) Khan, M.; Jensen, K. B.; Francesconi, K. A. A Method for Determining Arsenolipids in Seawater by HPLC-High Resolution Mass Spectrometry. *Talanta* **2016**, *153*, 301–305.

(644) Serpe, F. P.; Russo, R.; Gallo, P.; Severino, L. Method for Speciation of Organoarsenic in Mussels by Liquid Chromatography Coupled to Electrospray Ionization and QTRAP Tandem Mass Spectrometry. *J. Food Prot.* **2013**, *76* (7), 1293–1299.

(645) Sadee, B.; Foulkes, M. E.; Hill, S. J. Coupled Techniques for Arsenic Speciation in Food and Drinking Water: A Review. *J. Anal. At. Spectrom.* **2015**, *30* (1), 102–118.

(646) Denisov, E.; Damoc, E.; Lange, O.; Makarov, A. Orbitrap Mass Spectrometry with Resolving Powers above 1,000,000. *Int. J. Mass Spectrom.* **2012**, *325–327*, 80–85.

(647) Marshall, A. G. Milestones in Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Technique Development. *Int. J. Mass Spectrom.* **2000**, *200* (1–3), 331–356.

(648) Marshall, A. G.; Verdun, F. R. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. In *Fourier Transforms in NMR, Optical, and Mass Spectrometry: A User's Handbook*; Elsevier Science Publishers B.V.: Amsterdam, 1990; pp 225–278.

(649) Hsieh, Y. J.; Jiang, S. J. Application of HPLC-ICP-MS and HPLC-ESI-MS Procedures for Arsenic Speciation in Seaweeds. *J. Agric. Food Chem.* **2012**, *60*, 2083–2089.

(650) Corr, J. J.; Larsen, E. H. Arsenic Speciation by Liquid Chromatography Coupled with

Ionspray Tandem Mass Spectrometry. *J. Anal. At. Spectrom.* **1996**, *11* (12), 1215–1224.

(651) Kuehnelt, D.; Goessler, W.; Francesconi, K. A. Nitrogen Purity Influences the Occurrence of As+ions in High-Performance Liquid Chromatography/Electrospray Ionization Mass Spectrometric Analysis of Four Common Arsenosugars. *Rapid Commun. Mass Spectrom.* **2003**, *17* (7), 654–659.

(652) Nischwitz, V.; Pergantis, S. A. Liquid Chromatography Online with Selected Reaction Monitoring Electrospray Mass Spectrometry for the Determination of Organoarsenic Species in Crude Extracts of Marine Reference Materials. *Analytical Chemistry*. 2005, pp 5551–5563.

(653) Nischwitz, V.; Pergantis, S. A. Optimisation of an HPLC Selected Reaction Monitoring Electrospray Tandem Mass Spectrometry Method for the Detection of 50 Arsenic Species. *J. Anal. At. Spectrom.* **2006**, *21* (11), 1277–1286.

(654) Nischwitz, V.; Pergantis, S. A. Mapping of Arsenic Species and Identification of a Novel Arsenosugar in Giant Clams Tridacna Maxima and Tridacna Derasa Using Advanced Mass Spectrometric Techniques. *Environ. Chem.* **2007**, *4* (3), 187–196.

(655) Kanaki, K.; Pergantis, S. A. Development of Mass Spectrometric Methods for Detecting Arsenic-Glutathione Complexes. *J. Am. Soc. Mass Spectrom.* **2008**, *19* (10), 1559–1567.

(656) McAdam D. P., Perera A. M. A., S. R. V. The Synthesis of (R)-2',3'-Dihydroxypropyl 5-Deoxy-5-Dimethylarsinyl-β-D-Riboside, a Naturally Occurring Arsenic-Containing Carbohydrate. *Aust. J. Chem.* **1987**, *40*, 1901–1908.

(657) Edmonds, J. S.; Francesconi, K. A.; Stick, R. V. Arsenic Compounds from Marine Organisms. *Nat. Prod. Rep.* **1993**, *10* (4), 421–428.

(658) Traar P., Rumpler A., T. M.; Gerald S., F. K. A. Synthesis of Naturally Occurring Arsenic-Containing Carbohydrates. *Aust. J. Chem.* **2009**, *62*, 538–545.

(659) Traar, P.; Francesconi, K. A. Synthetic Routes for Naturally-Occurring Arsenic-Containing Ribosides. *Tetrahedron Lett.* **2006**, *47* (30), 5293–5296.

(660) McAdam D. P., S. R. V. The Synthesis of (R)-2′,3′-Dihydroxypropyl 5-Deoxy-5-Dimethylarsinoyl-β-D-Riboside, a Naturally-Occurring, Arsenic-Containing Carbohydrate. *Tetrahedron Lett.* **1986**, *27* (2), 251–254.

(661) Liu, J.; O'Brien, D. H.; Irgolics, K. J. Synthesis of 1-0-(2',3'-Dihydroxypropyl)5-Deoxy-β-D-Ribofuranosides with (CH3)2As, (CH3)2As=S or (CH3)3As+ Groups as Substituents at the 5-Position. *Appl. Organomet. Chem.* **1996**, *10*, 13–22.

(662) Stick, R. V.; Stubbs, K. A.; Tilbrook, D. M. G. M. G. An Improved Synthesis of (R)-2,3-Dihydroxypropyl 5-Deoxy-5-Dimethylarsinyl-β-D-Riboside, a Common Marine Arsenical. *Aust. J. Chem.* **2001**, *54* (54), 181–183.

(663) Yu, L. L.; Browning, J. F.; Burdette, C. Q.; C., C. G.; Chieh, K. D.; Davis, W. C.; Kassim, B. L.; Long, S. E.; Murphy, K. E.; Oflaz, R.; et al. Development of a Kelp Powder (Thallus Laminariae) Standard Reference Material. *Anal. Bioanal. Chem.* **2018**, *410*, 1265–1278.

(664) Larsen, E. H.; Stürup, S. Carbon-Enhanced Inductively Coupled Plasma Mass Spectrometric Detection of Arsenic and Selenium and Its Application to Arsenic Speciation. *J. Anal. At. Spectrom.* **1994**, *9*, 1099–1105.

(665) Chatterjee, A. Behaviour of Cationic Arsenic Compounds in a Microwave System with Nitric Acid and Hydrogen Peroxide. *J. Anal. At. Spectrom.* **2000**, *15* (6), 753–758.

(666) Oliveira, A. C. M.; Bechtel, P. J.; Nguyen, D. X.; Gurer, L.; Crapo, C. A.; Fong, Q.; Ralonde, R. Chemical Composition and Texture of Commercial Geoduck Clams (*Panopea Abrupta*) Harvested in Southeast Alaska. *J. Shellfish Res.* **2011**, *30* (3), 761–769.

(667) Yoshinaga, J.; Shibata, Y.; Horiguchi, T.; Morita, M. NIES Certified Reference Materials for Arsenic Speciation. *Accredit. Qual. Assur.* **1997**, *2* (3), 154–156.

(668) Pingret, D.; Fabiano-Tixier, A. S.; Bourvellec, C. Le; Renard, C. M. G. C.; Chemat, F. Lab and Pilot-Scale Ultrasound-Assisted Water Extraction of Polyphenols from Apple Pomace. *J. Food Eng.* **2012**, *111* (1), 73–81.

(669) Sališová, M.; Toma, Š.; Mason, T. J. Comparison of Conventional and Ultrasonically Assisted Extractions of Pharmaceutically Active Compounds from Salvia Officinalis. *Ultrason. Sonochem.* **1997**, *4* (2), 131–134.

(670) Toma, M.; Vinatoru, M.; Paniwnyk, L.; Mason, T. J. Investigation of the Effects of Ultrasound on Vegetal Tissues during Solvent Extraction. *Ultrason. Sonochem.* **2001**, *8* (2), 137–142.

(671) Glisic, S. B.; Ristic, M.; Skala, D. U. The Combined Extraction of Sage (Salvia Officinalis L.): Ultrasound Followed by Supercritical CO2 Extraction. *Ultrason. Sonochem.* **2011**, *18* (1), 318–326.

(672) McKiernan, J. W.; Creed, J. T.; Brockhoff, C. A.; Caruso, J. A.; Lorenzana, R. M. A Comparison of Automated and Traditional Methods for the Extraction of Arsenicals from Fish. *J. Anal. At. Spectrom.* **2002**, *14* (4), 607–613.

(673) Barber, C. A.; Burdette, C. Q.; Hayes, H. V; Luvonga, C.; Phillips, M. M.; Rimmer, C. A.; Wood, L. J.; Yu, L. *Health Assessment Measurements Quality Assurance Program : Exercise 5 Final Report Health Assessment Measurements Quality Assurance Program : Exercise 5 Final Report*; Gaithersburg, 2021.

(674) Almela, C.; Algora, S.; Benito, V.; Clemente, M. J.; Devesa, V.; Súñer, M. A.; Vélez, D.; Montoro, R. Heavy Metal, Total Arsenic, and Inorganic Arsenic Contents of Algae Food Products. *J. Agric. Food Chem.* **2002**, *50*, 918–923.

(675) Heussner, A. H.; Mazija, L.; Fastner, J.; Dietrich, D. R. Toxin Content and Cytotoxicity of Algal Dietary Supplements. *Toxicol. Appl. Pharmacol.* **2012**, *265*, 263–271.

(676) Rabb, S. A.; Le, M. D.; Yu, L. L. A Novel Approach to Converting Alkylated Arsenic to Arsenic Acid for Accurate ICP-OES Determination of Total Arsenic in Candidate Speciation Standards. *Microchem. J.* **2018**, *143*, 133–139.

(677) Gibbons, G. Top 10 List Shows Significant Increase in Seafood Consumption https://aboutseafood.com/press_release/top-10-list-shows-significant-increase-in-seafood-consumption/ (accessed Sep 11, 2020).

(678) Ellisor, D. L.; Phillips, M.; Place, B.; Rimmer, C.; Wood, L. A Reference Material Suite for Evaluating Seafood Authenticity and Safety. 2015, p 670.

(679) Krishnakumar, P. K.; Qurban, M. A.; Stiboller, M.; Nachman, K. E.; Joydas, T. V.; Manikandan, K. P.; Mushir, S. A.; Francesconi, K. A. Arsenic and Arsenic Species in Shellfish and Finfish from the Western Arabian Gulf and Consumer Health Risk Assessment. *Science of the Total Environment*. 2016, pp 1235–1244.

(680) Jenkins, R. O.; Ritchie, A. W.; Edmonds, J. S.; Goessler, W.; Molenat, N.; Kuehnelt, D.; Harrington, C. F.; Sutton, P. G. Bacterial Degradation of Arsenobetaine via Dimethylarsinoylacetate. *Arch. Microbiol.* **2003**, *180*, 142–150.

(681) Kaise, T.; Sakurai, T.; Saitoh, T.; Matsubara, C. Biotransformation of Arsenobetaine to Trimethylarsine Oxide by Marine Microorganisms in a Gill of Clam Meretrix Lusoria. *Chemosphere* **1998**, *37*, 443–449.

(682) Lai, V. W. M.; Sun, Y.; Ting, E.; Cullen, W. R.; Reimer, K. J. Arsenic Speciation in Human Urine: Are We All the Same? *Toxicol. Appl. Pharmacol.* **2004**, *198*, 297–306.

(683) Shimbo, S.; Hayase, A.; Murakami, M.; Hatai, I.; Higashikawa, K.; Moon, C. S.; Zhang, Z. W.; Watanabe, T.; Iguchi, H.; Ikeda, M. Use of a Food Composition Database to Estimate Daily Dietary Intake of Nutrient or Trace Elements in Japan, With Reference to Its Limitation. *Food Addit. Contam.* **1996**, *7*, 775–786.

(684) Finke, H.; Wandt, V. K.; Ebert, F.; Guttenberger, N.; Glabonjat, R. A.; Stiboller, M.; Francesconi, K. A.; Raber, G.; Schwerdtle, T. Toxicological Assessment of Arsenic-Containing Phosphatidylcholines in HepG2 Cells. *Metallomics* **2020**, *12* (7), 1159–1170.

(685) Stiboller, M.; Raber, G.; Lenters, V.; Gjengedal, E. L. F.; Eggesbø, M.; Francesconi, K. A. Arsenolipids Detected in the Milk of Nursing Mothers. *Environ. Sci. Technol. Lett.* **2017**, *4* (7), 273–279.

(686) Stiboller, M.; Freitas, F. P.; Francesconi, K. A.; Schwerdtle, T.; Nogueira, A. J. A.; Raber, G. Lipid-Soluble Arsenic Species Identified in the Brain of the Marine Fish Skipjack Tuna (Katsuwonus Pelamis) Using a Sequential Extraction and HPLC/Mass Spectrometry. *J. Anal. At. Spectrom.* **2019**, *34* (12), 2440–2450.

(687) Luvonga, C.; Rimmer, C. A.; Yu, L. L.; Lee, S. B. Analytical Methodologies for the Determination of Organoarsenicals in Edible Marine Species: A Review. *J. Agric. Food Chem.* **2020**, *68*, 1910–1934.

(688) Allen, F.; Pon, A.; Wilson, M.; Greiner, R.; Wishart, D. CFM-ID: A Web Server for Annotation, Spectrum Prediction and Metabolite Identification from Tandem Mass Spectra. *Nucleic Acids Res.* **2014**, *12*, 94–99.

(689) Tautenhahn, R.; Cho, K.; Uritboonthai, W.; Zhu, Z.; Patti, G. J.; Siuzdak, G. An Accelerated Workflow for Untargeted Metabolomics Using the METLIN Database. *Nat. Biotechnol.* **2012**, *30* (9), 826–828.

(690) Colby, S. M.; Nuñez, J. R.; Hodas, N. O.; Corley, C. D.; Renslow, R. R. Deep Learning to Generate in Silico Chemical Property Libraries and Candidate Molecules

for Small Molecule Identification in Complex Samples. *Anal. Chem.* **2020**, *92* (2), 1720–1729.

(691) Creek, D. J.; Dunn, W. B.; Fiehn, O.; Griffin, J. L.; Hall, R. D.; Lei, Z.; Mistrik, R.; Neumann, S.; Schymanski, E. L.; Sumner, L. W.; et al. Metabolite Identification: Are You Sure? And How Do Your Peers Gauge Your Confidence? *Metabolomics* **2014**, *10* (3), 350–353.

(692) Sumner, L. W.; Amberg, A.; Barrett, D.; Beale, M. H.; Beger, R.; Daykin, C. A.; Fan, T. W. M.; Fiehn, O.; Goodacre, R.; Griffin, J. L.; et al. Proposed Minimum Reporting Standards for Chemical Analysis: Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics* **2007**, *3* (3), 211–221.

(693) Beisken, S.; Eiden, M.; Salek, R. M. Getting the Right Answers: Understanding Metabolomics Challenges. *Expert Rev. Mol. Diagnostics* **2015**, *15* (1), 97–109.

(694) Fiehn, O. Metabolomics - The Link between Genotypes and Phenotypes. *Plant Mol. Biol.* **2002**, *48* (1–2), 155–171.

(695) Coniglio, D.; Calvano, C. D.; Ventura, G.; Losito, I.; Cataldi, T. R. I. Arsenosugar Phospholipids (As-PL) in Edible Marine Algae: An Interplay between Liquid Chromatography with Electrospray Ionization Multistage Mass Spectrometry and Phospholipases A1 and A2 for Regiochemical Assignment. *J. Am. Soc. Mass Spectrom.* **2020**, *31* (6), 1260–1270.

(696) Cubadda, F.; Jackson, B. P.; Cottingham, K. L.; Van Horne, Y. O.; Kurzius-Spencer, M. Human Exposure to Dietary Inorganic Arsenic and Other Arsenic Species: State of Knowledge, Gaps and Uncertainties. *Sci. Total Environ.* **2017**, *579*, 1228–1239.

(697) Niehoff, A.-C.; Kettling, H.; Pirkl, A.; Chiang, Y. N.; Dreisewerd, K.; Yew, J. Y. Analysis of Drosophila Lipids by Matrix-Assisted Laser Desorption/Ionization Mass Spectrometric Imaging. *Anal. Chem.* **2014**, *86* (22), 11086–11092.

(698) Chávez-Capilla, T.; Beshai, M.; Maher, W.; Kelly, T.; Foster, S. Bioaccessibility and Degradation of Naturally Occurring Arsenic Species from Food in the Human Gastrointestinal Tract. *Food Chem.* **2016**, *212*, 189–197.