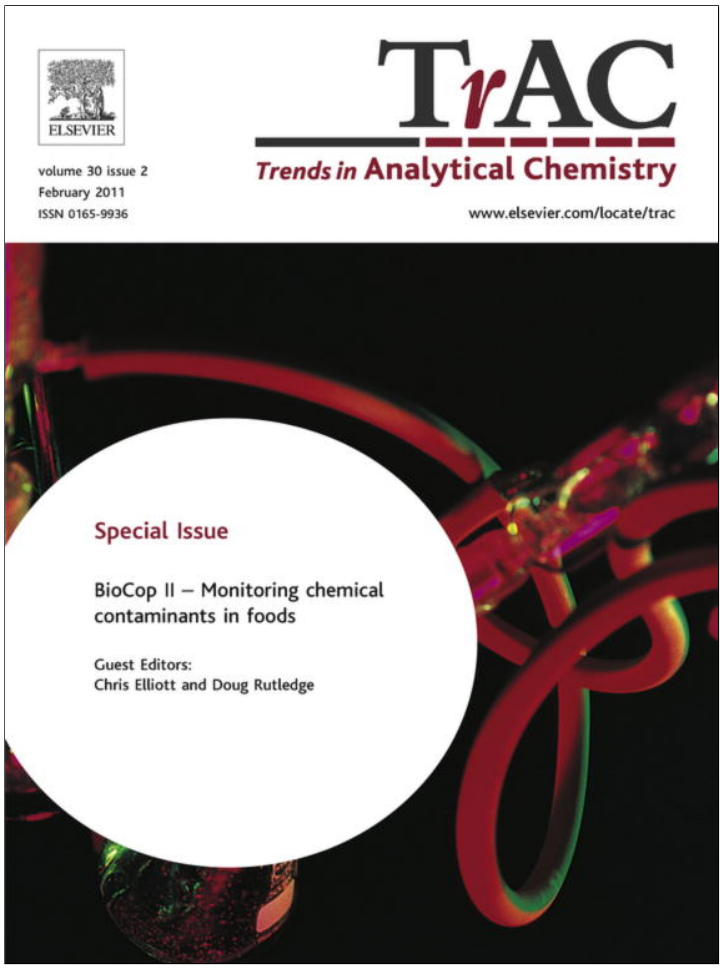

In-vivo and *in-vitro* testing to assess the bioaccessibility and the bioavailability of arsenic, selenium and mercury species in food samples

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***In-vivo* and *in-vitro* gastrointestinal (GI) extractions, also known as oral bioaccessibility and bioavailability, are important approaches to assess chemical risk to humans. We give an overview of *in-vivo* and *in-vitro* bioaccessibility and bioavailability assays for testing arsenic, selenium and mercury (As, Se and Hg) species from food samples. We critically evaluate the parameters affecting *in-vivo* and *in-vitro* processes. In addition, we consider the effect of cooking food on bioaccessibility and bioavailability, and stability and transformation, of species during *in-vivo* or *in-vitro* processes. The bioaccessibility and bioavailability of As, Se and Hg species are affected by the sample matrix, cooking food and the experimental conditions applied (gastric and intestinal pH, incubation temperature and residence time). Regarding species degradation and transformation during *in-vitro* procedures, good stability has been observed for most As species, except for certain arsenosugars. Important transformations during *in-vitro* processes have been reported for Se species [e.g., conversion of γ-glu-Se-MeSeCys to Se-MeSeCys, and organic Se species (MeSeCys, SeCys2 and SeMet) degradation to inorganic Se]. Finally, we summarize speciation and detection conditions for As, Se and Hg speciation, and quality control to assure reliable measurements.**

Keywords: Arsenic species; Bioaccessibility; Bioavailability; Food; *In-vitro* assay; *In-vivo* assay; Mercury species; Selenium species; Simulated dialysis; Simulated gastric and intestinal digestion

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1. Introduction

Human intake of arsenic, selenium and mercury (As, Se and Hg) species occurs mainly via food ingestion [1–4], as a consequence of bio-accumulation and biotransformation processes in biota. Because of the wide-ranging levels of toxicity exhibited by different organometallic species, mainly As and Hg, to assess dietary risk in humans, assessment of the chemical forms of these elements is more important than knowledge of their total contents [5]. As inorganic forms [As(III) and As(V)] exhibit high toxicity [6], but their methylated metabolites [monomethylarsonic acid

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(MA) and dimethylarsinic acid (DMA)] are less toxic, while other major organoarsenicals in seafood {arsenobetaine (AB) [7,8], trimethylarsine oxide (TMAO), and arsenocholine (AC)} have low or negligible toxicity [9]. Organomercury species (e.g., MeHg, a major Hg species in seafood) [10] exhibit higher toxicity than the inorganic form [Hg(II)]. Se, mainly as selenomethionine (SeMet) which is the most absorbable Se form in humans [11], has been recognized as an integral component of different enzymes, which participate in the antioxidant protection of cells.

The determination of trace elements in food products and bioaccessibility and bioavailability studies of these minerals is of special concern because some of them are essential elements for humans but also because some are highly toxic. In particular, toxic elements (e.g., heavy metals) tend to accumulate in animals and humans and, as commented above, some species (e.g., MeHg or inorganic As) are extremely toxic.

Because some elements are essential minerals for humans and because other trace elements are toxic, there have been different bioaccessibility and bioavailability studies of foodstuffs, as reviewed by Intawongse and Dean for total-mineral contents [12]. However, the bioaccessibility and bioavailability of the different species of essential or toxic elements are also important because the benefit (if the mineral is essential) or the damage (if the mineral is toxic) depends on the chemical form of the element that is absorbed.

In the context of human health-risk assessment, bioavailability refers to the fraction of substance that reaches the systemic circulation (blood) from the gastrointestinal (GI) tract (bioavailable fraction) and that is available to promote its action in the exposed organism [13]. A first step in assessment of bioavailability is the study of the bioaccessibility, which indicates the maximum fraction of a trace element or other substance in food that is theoretically released from its matrix in the GI tract (bioaccessible fraction), and thus becomes available for intestinal absorption (i.e. enters the blood stream) [14]. Bioavailability and bioaccessibility are affected by the type and the composition of food, and the GI conditions. Special physiological conditions of the consumer (e.g., age or the state of health) and the distribution of compound species in the food product are also important factors affecting bioavailability. Ziegler et al. [15] discussed these considerations for several trace minerals (e.g., lead).

Estimating element bioavailability in food can be performed by *in-vivo* (dosing experimental humans or animals with several concentrations of the target compound) or *in-vitro* methods. While the ultimate test for human health-risk assessment is human experimentation (using stable isotopes [16]) that provides better results for estimating bioavailability than *in-vivo* animal and *in-vitro* methods, this sort of test is unethical. As a result, *in-vivo* studies using animal models (e.g., primates, swine, dog, rabbit, and rodents) have been developed to predict contaminant bioavailability. Due to their close relatedness to man, monkeys are the first choice for *in-vivo* bioavailability studies, but there have been few studies with primates due to the prohibitive cost associated with their use [17]. Rodents have been used as animal models for a wide variety of medical and environmental applications. *In-vivo* assessments utilizing swine (with jugular catheters inserted into female Large White swine for routine blood sampling) [18] are considered appropriate for human health-risk assessment because swine are remarkably similar to humans in terms of physiology, digestive tract, bone development, nutritional requirement (swine are monogastric omnivores) and mineral metabolism [17]. Furthermore, young swine are considered to be a good physiological model for GI absorption of a contaminant. They possess a gall bladder that excretes bile into the small intestines when food is present, and coprophagia is not required to maintain nutritional status [17]. Swine can be trained to eat contaminated food or the contaminant can be easily delivered through a gastric tube. Repeat blood sampling in the swine is relatively easy compared to smaller animals, and cross-over studies are possible thereby reducing variability and the number of animals required [17]. However, testing with animals is expensive, difficult to perform, and ethically controversial, and it provides limited data in each experiment [19]. As an alternative, the bioavailability of trace minerals has also been estimated through various *in-vitro* methods [12].

In-vitro methods involve conditions (temperature, agitation, pH, and enzyme and chemical composition) similar to those found in the human body during digestion. There are several *in-vitro* approaches to measurement:

(1) the maximum soluble concentration of the target compound in the simulated GI solution after filtration or centrifugation (bioaccessible fraction);
(2) the soluble fraction of the compound (bioaccessible fraction) achieved by using human GI microbiota (Simulator of the Human Intestinal Microbial Ecosystem, SHIME) [20,21];
(3) the dialyzable fraction of the compound, which can dialyze through a semi-permeable membrane with a specified pore size (dialysate or bioavailable fraction) at equilibrium [22] or non-equilibrium [23–25] conditions; and,
(4) the fraction of the compound capable of being retained or transported through a solid or microporous supports (bioavailable fraction) in which human Caco-2 cells grown are incorporated (intestinal epithelial model) [26].

These methods provide effective approximations to *in-vivo* situations and offer the advantages of simplicity, rapidity, ease of control, low cost, high precision and good reproducibility. Some *in-vitro* procedures also

simulate digestion with saliva. In the mouth, samples are ground mechanically in the presence of saliva, which allows larger sample components to be broken down into smaller fragments, thereby increasing the surface area. Saliva contains a lubricating substance, known as mucus, buffers maintaining the acid–base balance, and salivary amylase (an enzyme that initiates hydrolysis of carbohydrates). Because the entire process in the mouth will last from a few seconds to minutes, and because the pH of saliva is close to neutral (6.5), significant compound dissolution from food samples is not expected in this stage [12]. It is therefore usually assumed that saliva has only a negligible effect on the level of mobilization of compounds [14], and, in most cases, only simulated gastric and intestinal extractions are considered.

In-vitro testing methods have been used most for assessing oral bioaccessibility of total trace metals in soil and food samples. These methods were reviewed by Intawongse and Dean [12]. In this article, our aim is to discuss the current status of oral bioaccessibility and bioavailability in terms of the release of As, Se and Hg species from food samples, focusing on the parameters that influence GI extraction, including gastric and intestinal conditions, residence time and food constituents. We also consider the effect of cooking food on species bioaccessibility and bioavailability and on species stability and transformation during *in-vivo* and *in-vitro* processes,. In addition, we discuss speciation and detection conditions for As, Se and Hg species and quality control (QC) to assure measurements.

2. *In-vivo* and *in-vitro* testing

Table 1 summarizes the applications of *in-vivo* and *in-vitro* GI extraction methods for assessing the bioaccessibility and bioavailability of As, Se and Hg species in food samples. Foodstuffs considered in these studies include:

(1) cereals and vegetables (e.g., rice, chard, radish, lettuce, beans, onion, chives, garlic);
(2) seafood [e.g., edible seaweeds, and fish and shellfish (sole, Greenland halibut, crayfish, swordfish, sardine, tuna, cod, narwhal, Arctic char, and clams)]; and,
(3) other products s(e.g., Brazil nuts, cow milk, and yeast).

As can be seen in Table 1, although GI conditions (composition of gastric and intestinal juices, and incubation temperature and time) are similar for most of the *in-vitro* procedures, several GI models were used, mainly SHIME and intestinal epithelial models. As we comment below, metal speciation plays a major role in determining the amount of metal bioaccessible and bioavailable to humans. In addition, some of these foods have been fortified by several metallic species (As and Se species, mainly) in order to:

(1) obtain the metal species more suitable for food fortification and hence improve the bioavailable content of some essential species for human life in the diet of several countries (i.e. Northern Europe is Se deficient); and,
(2) study the influence of the exposure of certain plants and animals to certain organometallic species of an element (toxic or essential) on the bioavailability of this element in the organism grown under this exposure.

Table 2 summarizes relevant analytical data on measurements of these compounds showing separation and detection conditions for As, Se and Hg species.

2.1. As species in food samples

For As species, studies are focused on assessing bioaccessible and bioavailable As species in raw and cooked cereals and vegetables (rice grain, chard, radish, lettuce, mung bean) [18,27–29], edible seaweed [30–33] and seafood [33–36].

Juhasz et al. assessed As-species bioavailability in rice bought in the supermarket (samples in which all As was present as inorganic forms), and rice grown in the greenhouse (irrigated with As-contaminated water where around 86% of the As was present as DMA) [18]. These authors also studied As bioavailability in the presence of several As species in the cooking water for the rice. Results demonstrated that DMA was poorly absorbed by oral administration, and low absolute bioavailability values were assessed (only 33 ± 3% of the total rice-bound As was bioavailable). Conversely, in supermarket-bought rice cooked in water contaminated with sodium arsenate, As was present entirely in the inorganic form, and bioavailability was high (89 ± 9%). Also, these authors assessed the bioavailability of the different forms of As in chard (*Beta vulgaris L.*), radish (*Rhapanus sativus L.*), lettuce (*Lactuca sativa L.*) and mung beans (*Vigna radiata L.*) irrigated using arsenate-contaminated water under greenhouse conditions [28]. Results showed total-As bioavailability of 52 ± 18% and 50 ± 13%, for chard and lettuce, respectively. Higher As-bioavailability values were observed for radish (77 ± 20%), while all As present in mung beans was bioavailable (98 ± 23%). In addition, As bioavailability may be influenced by the non-digestible polysaccharide component of the vegetable. Thus, As bioavailability decreased in the order mung beans > radish > lettuce = chard.

Laparra et al. [27] studied the effect of cooking (with As-contaminated water) on inorganic As contents in rice, and bioavailability of As(III) and As(V) after simulated GI digestion was established through the extent of As retention and transport by Caco-2 cells used as a model of intestinal epithelia. After cooking, inorganic As contents increased significantly. The bioavailability of

Table 1. Application of *in-vivo* and *in-vitro* gastrointestinal (GI) extraction methods to bioaccessibility and bioavailability from foodstuff samples containing arsenic, selenium and mercury (As, Se and Hg) species

	Sample matrix	*In-vivo*/*In-vitro* assay	Results	Ref.
As species				
As(III), As(V), MA, DMA	Rice, chard, radish, lettuce and mung beans	Swine were fed twice daily (500 g low-As swine pellets), 2 and 10 h after: – intravenous As dosages (MMA, DMA, As(III), As(V): 20 μg/kg; – oral As dosage (MMA, DMA, As(III), As(V): 80–100 μg/kg; – animal feeding with As-contaminated rice (170–270 g) Blood samples were taken over 26 h after dosage, and plasma was separated from red blood cells by centrifugation (4000 rpm for 10 min) and then stored at –20°C before As analysis	As species determining the amount of As bioavailable *Rice:* – DMA was poorly absorbed (only 33 ± 3% of the total rice-bound arsenic was bioavailable), while – inorganic As bioavailability was high (89 ± 9%) – *Chard, radish, lettuce and mung beans:* – MMA and DMA were poorly absorbed (0%); while – As(III) bioavailability (6.7 ± 1.7% for radish to 90.2 ± 0.2% for mung beans); and. – As(V) bioavailability was high (10.2 ± 0.3% for mung beans to 84.6 ± 21.8% for radish)	[18,28]
Inorganic As, As(III) and As(V)	Rice	Gastric juice: 1.0 g pepsin in 0.1 M HCl (10 mL) Intestinal juice: 0.2 g pancreatin and 1.25 g bile extract in 0.1 M $NaHCO_3$ (50 mL) Gastric digestion: sample (10 g) plus 90 mL of DIW and at pH 2.0 (with 6.0 M HCl), plus gastric juice. The mixture was made up to 100 g with DIW and incubated in a shaking water bath (120 strokes/min at 37°C, 2 h) Intestinal digestion: gastric digest at pH 5.0 (1.0 M $NaHCO_3$) plus intestinal juice was incubated at 37°C for 2 h. Finally, pH was adjusted to 7.2 (0.5 M NaOH) Arsenic-uptake assays (Caco-2 cells): after heating (100°C, 4 min) and cooling (by immersion in an ice bath) of the bioaccesssible fraction, 40 g of the inactivated digests were centrifuged (26891 × g, 30 min, at 4°C). Glucose and HEPES (5 mM and 50 mM final concentration, respectively) were added (to facilitate cell viability), and water or NaCl was added (to adjust the osmolarity to 310 ± 10 mOsm/kg). Caco-2 cells were seeded onto polycarbonate membrane filters (Transwell inserts of 24-mm diameter and 0.4-μm pore size at a density of 5×10^4 cells/cm^2). 15–18 days after seeding, the culture medium was aspirated and cell monolayers were washed three times with PBS. Afterwards, bioaccessible fraction (1.5 mL) was added to the apical chamber, and fresh MEM (2 mL) was added to the basal side. Cell cultures were incubated (37°C, 4 h, 5% CO_2, and 95% relative humidity). Both apical and basal media of the inserts were recovered by aspiration, and total arsenic was analyzed to evaluate transepithelial transport	Inorganic As bioaccessible in cooked rice varied (63–100%). As(III) and As(V) bioaccessibility varied within 3.0–20% and 60–90%, respectively Arsenic-uptake percentages obtained by Caco-2 cells varied (0.6–6.4% for As retention, 3.3–11.4% for As transport, and 3.9–17.8% for total cellular uptake)	[27]

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Table 1 (*continued*)

	Sample matrix	*In-vivo*/*In-vitro* assay	Results	Ref.
Total As, inorganic As (As(III) + As(V)), As(III) and As(V)	Edible seaweeds	Gastric juice: 1.0 g pepsin in 0.1 M HCl (10 mL) Intestinal juice: 0.2 g pancreatin and 1.25 g bile extract in 0.1 M $NaHCO_3$ (50 mL) Gastric digestion: 5.0 g of sample was added to 90–160 mL of DIW and pH was adjusted to 2.0 (6.0 M HCl), and gastric juice (0.01 g of pepsin/5 g seaweed) was added. The mixture was made up to 100–170 g with DIW and incubated in a shaking water bath (120 strokes/min at 37°C, 2 h) Intestinal digestion: gastric digest adjusted to pH 5.0 (1.0 M $NaHCO_3$), plus intestinal juice (0.0025 g of pancreatin/5 g seaweed, plus 0.015 g of bile extracts/5 g of seaweed) was incubated (37°C, 2 h). Finally, pH was adjusted to 7.2 (0.5 M NaOH) 40 g of the digest was centrifuged (15 000 rpm, 30 min at 4°C) to separate bioaccessible fraction and precipitate	Total As and inorganic As: – bioaccessibility is a function of seaweed composition: 67.2% and 48.6%, respectively, for Porphyra sp; 62.3% and 74.7%, respectively, for Hizikia fusiforme; and, 32.0% and 77.2%, respectively, for Enteromorpha sp. –bioaccessibility increased significantly after cooking, attaining 79.9% and 72.6%, respectively, in Porphyra sp. and 65.7% and 87.9%, respectively, in Hizikia fusiforme – As(III) and As(V): – As(III) and As(V) bioaccessible fractions were within 36–94% and 5.0–51%, respectively – As(III) and As(V) bioaccessible fractions in cooked seaweed were 7.1–25.4% and 7.5–23.8%, respectively	[30,31]
Glycerol ribose, phosphate ribose, sulfonate ribose, and sulfate ribose	Edible seaweeds	*Idem* to [30,31]	Total As bioaccessible in raw and cooked seaweed varied (38–87% and 57–106%, respectively. Glycerol ribose, phosphate ribose, sulfonate ribose and sulfate ribose bioaccessible fractions in raw seaweeds varied (98–112%, 89–120%, 81–120% and 84–124%, respectively). Similar percentages were obtained for cooked seaweeds	[32]
As(III), As(V), MA, DMA, glycerol ribose, phosphate ribose, sulfonate ribose, and sulfate ribose	Seaweed and clams	Gastric juice: 30.03 g glycine in 800 ml of DIW at pH 1.5 (1.0 M HCl) and diluted to 1.0 L Intestinal juice: 0.0175 g bile salts and 0.005 g pancreatin Gastric digestion: 0.25 g (clam) or 1.0 g (seaweed) of sample was added to 10 mL of gastric juice. After mixture shaking on a platform shaker (150 rpm at 37°C, 1 h) and filtration (0.45 μm), the mixture was frozen until analysis Intestinal digestion: unfiltered gastric extract, adjusted to pH 7.0 (50% w/v NaOH), plus intestinal juice was shaken for 4 h. Finally, bioaccessible fraction was separated by filtration	Total As bioaccessible was 36 ± 3% and 44 ± 7% (for gastric and gastric + intestinal digestion, respectively), and 68 ± 27 and 78 ± 15% (for gastric and gastric + intestinal digestion, respectively) for clams and seaweed, respectively Inorganic As bioaccessible in clams was 98 ± 10% and 86 ± 42% (for gastric and gastric + intestinal digestion, respectively) and 54 ± 12 and 45 ± 7% (for gastric and gastric + intestinal digestion, respectively) for seaweed MA + DMA and arsenosugars account for ~1.2–1.9% and 43–46%, respectively, of the bioaccessible As in seaweed	[33]
MA, DMA, AB, TMAO, TETRA	Sole, Greenland halibut and DORM-2	*Idem* to [26], except for sample mass (5.0 g)	Total arsenic bioaccessibility from raw samples was 97.7%, 98.3% and 79.2% (for DORM-2, sole and Greenland halibut, respectively) Cooking process did not affect the bioaccessibility in sole (102.1%), but As bioaccessibility was increased to 100% in Greenland halibut AB bioaccessibility was very high for samples of raw (68%, 107% and 100% for DORM-2, sole and Greenland halibut, respectively) and cooked (115% and 124% for sole and Greenland halibut, respectively) DMA (32%) and TMAO (80%) bioaccessibilities were only obtained from DORM-2 and Greenland halibut, respectively, for which concentrations were over quantification limits For AB in DORM-2, the arsenic-uptake percentages obtained by Caco-2 cells were 12% for As transport	[34]

As(III), As(V), MA, DMA, AB, AC, TMAO	TORT-2, DOLT-3, CRM 6327 and DORM-3	Saliva: 6.8 g of KH_2PO_4 and 77 mL of 0.2 M NaOH to 1 L with DIW, pH 6.5 (0.2 M NaOH) Gastric juice: 2.0 g of NaCl, 3.2 g of pepsin and 7.0 mL of conc. HCl to 1 L with DIW (pH 1.2) Intestinal juice: 6.8 g of KH_2PO_4, 10 g of pancreatin and 77 mL of 0.2 M NaOH to 1 L with DDW, pH to 6.8 (0.2 M NaOH) Continuous leaching procedure: Sample (200 mg) wrapped in an Isopore membrane filter (0.4 mm, 47 mm diameter) is placed in PTFE tubing (8 cm long, 3/16 in o.d., 1/8 in i.d.) between two quartz-wool plugs. The mini-column was connected to the nebulizer of ICP-MS by PTFE tubing (35 cm long, 0.8 mm i.d.). Simulated saliva, gastric juice and intestinal juice were pumped into the mini-column (heated at 37°C). After 5 min for saliva and 1.0 h for gastric and intestinal juices, released As was analyzed in real time Batch method: Sample (200 mg) was placed into test tubes with a certain volume of reagent (6 mL for saliva or 24 mL for gastric and intestinal juices). The tubes were shaken for the same time as for continuous leaching at 37°C and then centrifuged	Total As bioaccessible quantities released (saliva + gastric + intestinal juices) was up 42–59% when using an on-line continuous leaching method For the batch method, As was mainly released by saliva, but small fractions were obtained after treatments with gastric juice. The lower extracted fraction did not exceed 10% for all cases. Moreover, the sum of released total As was 52–69% when using the batch method	[35]
As(III), As(V)	Freshwater crayfish	Gastric juice: 1.25 g/L pepsin, 0.5 g/L sodium citrate, 0.5 g/L malic acid, 1 mL/L glacial acetic acid and 0.15 M NaCl at pH 1.8 (conc. HCl) Intestinal juice: 0.05 g pancreatin and 0.175 g bile extract Gastric digestion: 0.1–0.15 g of yabby abdominal muscle sample, plus 10 mL of gastric juice and pH 1.8 (conc. HCl). The mixture was incubated in a shaker (275 rpm at 37°C, 1 h) Intestinal digestion: gastric digest at pH 7.0 (saturated Na_2CO_3), plus intestinal juice, and pH at 7.0. After incubation (37°C, 4 h), mixtures were centrifuged (3800 rpm, 30 min), and solutions were filtered (0.45 μm)	Total arsenic bioaccessibility was up to 69%, and As(III) and As(V) were the most bioavailable species	[36]

Table 1 *(continued)*

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	Sample matrix	*In-vivo*/*In-vitro* assay	Results	Ref.
Se species				
$SeCys_2$, SeMetCys, SeMet and Se(IV)	Radish	Gastric juice: 6.0% w/v pepsin, 0.15 M NaCl and pH of 1.8 (conc. HCl) Intestinal juice: 1.5% w/v pancreatin, 0.5% w/v α-amylase in 0.15 M NaCl Gastric digestion: 2.5 g of sample plus 7.5 mL of gastric juice. The mixture was incubated in a thermostatic bath (at 37°C, 4 h) Intestinal digestion: gastric digest adjusted to pH 6.8 (saturated Na_2CO_3) plus 5 mL of intestinal juice was incubated (37°C, 4 h, shaking periodically) before energetically shaken (1.0 min). After centrifugation (7500 × g, 30 min), solutions were filtered (0.22 μm)	Results suggest that almost all selenium content in radish is potentially bioaccessible after GI digestion	[37]
SeMet Se-MeSeCys, γ-glu-Se-MeSeCys	Garlic	Gastric juice: 2 g of NaCl and 3.2 g of pepsin in 7 mL of 12 M HCl. This mixture was diluted to 1 L with MilliQ water; the final pH was 1.2 Intestinal juice: 77 mL of 0.2 M NaOH solution and 500 mL of DIW to 6.8 g KH_2PO_4 in 250 mL DIW. Finally, 10 g of pancreatin was added and the mixture was made up to 1 L; the pH of this solution was 6.8 200 mg aliquots of solid sample were placed into test tubes with a 2 mL of reagent (fresh human saliva, gastric and intestinal juices). The tubes were shaken in a hot water bath (37°C, 140 rpm) for 15 min for saliva and 24 h for gastric and intestinal fluid. After centrifugation, extracts were frozen (−20°C) until further analysis	No given data	[38]

SeMet, $SeCys_2$	Brazil nuts	Gastric juice: 2 g of NaCl and 3.2 g of pepsin in 7 mL of 12 M HCl. This mixture was diluted to 1 L with DIW; the final pH was 1.2 Intestinal juice: 77 mL of 0.2 M NaOH solution and 500 mL of DIW to 6.8 g KH2PO4 in 250 mL DIW. Finally, 10 g of pancreatin was added and the mixture was made up to 1 L; the pH of this solution was 6.8 Gastric digestion: 400 mg of sample was added to 3.0 mL of gastric juice The mixture was incubated in a thermostatic bath under mechanical shaking in dark (at 37°C, 24 h, 150 rpm) Intestinal digestion: gastric digest plus 3.0 mL of intestinal juice was added and incubated under mechanical shaking in dark (37°C, 24 h, 150 rpm) After centrifugation the extract were frozen (−20°C) until further analysis	No given data	[40]
Se(IV), Se(VI), SeCys, SeMet	Cod	Gastric juice: 1.0% w/v pepsin, 0.15 M NaCl in DIW, pH of 2.0 (conc. HCl) Intestinal juice: 1.5% w/v pancreatin, 0.5% w/v amylase and 0.15% w/v bile salts, in 0.15 M NaCl Gastric digestion: 5 g of cooked cod was added to 10 mL of gastric juice. The mixture was incubated in an oven fitted with a shaking tray (at 37°C, 4 h, shaking periodically) Intestinal digestion: gastric digest at pH 4.8 (saturated $NaHCO_3$) plus 10 mL of intestinal juice, pH adjustment to 6.9, and incubation (37°C, 4 h, shaking periodically). After centrifugation (2500 rpm, 120 min at 10°C), the solutions were stored (4°C) until further analysis	No given data	[41]
SeMet	Swordfish, sardine and tuna	Gastric juice: 6.0% w/v pepsin, 0.15 M NaCl in DIW, pH of 1.8 (conc. HCl) Intestinal juice: 1.5% w/v pancreatin, 0.5% w/v amylase and 0.15% w/v bile salts, in 0.15 M NaCl Gastric digestion: 25 g of fish sample was added to 75 mL of gastric juice and shaken (1.0 min). The mixture was incubated in a thermostatic bath (at 37°C, 4 h, shaking periodically) Intestinal digestion: gastric digest at pH 6.8 (saturated Na_2CO_3) plus 50 mL of intestinal juice. Incubation (37°C, 4 h, shaking periodically) before energetically shaking (1.0 min). After centrifugation (1575 × g, 60 min), the solutions were filtered (0.45 μm) and frozen until further analysis	Bioaccessible percentages for total Se and SeMet were 50–83% and 14–19%, respectively	[42,43]

Table 1 *(continued)*

	Sample matrix	*In-vivo*/*In-vitro* assay	Results	Ref.
SeMet	Baker's yeast and yeast tablets	Gastric juice: 2 g of NaCl and 3.2 g of pepsin in 7 mL of 12 M HCl. This mixture was diluted to 1 L with DIW; the final pH was 1.2 Intestinal juice: 77 mL of 0.2 M NaOH solution and 500 mL of DIW to 6.8 g KH_2PO_4 in 250 mL DIW. Finally, 10 g of pancreatin was added and the mixture was made up to 1 L; the pH of this solution was 6.8 Gastric digestion: 6–10 mg of sample was added to 2.0 mL of gastric juice. The mixture was incubated in a thermostatic bath under mechanical shaking (at 37°C, 48 h, 200 rpm) Intestinal digestion: gastric digest plus 2.0 mL of intestinal juice was added and incubated under mechanical shaking (37°C, 48 h, 200 rpm). After centrifugation, the extract were filtered (0.22 μm) and frozen (−20°C) until further analysis	Bioaccessible percentages for total Se were 55–80%. SeMet was the major species but no data were given on SeMet bioaccessibility percentage	[44]
SeMet	Selenized yeast	Gastric juice: 1.0% w/v pepsin, 0.15 M NaCl in DIW, pH of 2.0 (conc. HCl) Intestinal juice: 3.0% w/v pancreatin, 1.5% w/v α-amylase, 1.0% w/v bile salts in 0.15 M NaCl at pH 6.8 Gastric digestion: 10–300 mg of sample was added to 0.1–3.0 mL of gastric juice. The mixture was incubated in a thermostatic bath (at 37°C, 4 h) Intestinal digestion: gastric digest at pH 6.8–7.0 (2.0 M Na_2CO_3) plus 0.1–3 mL of intestinal juice and incubation (37°C, 4 h, shaking periodically). After centrifugation (6000 × g, 40 min at 4°C), the extract was concentrated by lyophilization and stored under argon atmosphere at −20°C until further analysis	Bioaccessible percentages for total Se and SeMet were 89 ± 3% and 41 ± 2%, respectively, for yeast. Low SeMet recovery (26–37%) was found for yeast-based nutritional supplements	[45,46]
SeMet	Wheat	Human gastric and duodenal juices: these solutions were obtained from a pooled batch of six individual healthy persons Gastric digestion: 1.0 g of sample was added to 10 mL of 0.9% NaCl at pH 5.5 and 100 μL of human gastric juice. The mixture was incubated in a thermostatic bath (at 37°C, 2 h, pH 2.0) Duodenal digestion: gastric digest at pH 7.5 (1.0 M NaOH) plus 400 μL of human duodenal juice and incubation (37°C, 3 h). After centrifugation (10000 × g, 10 min), the extract was frozen and stored until further analysis	Bioaccessible percentages for total Se were ∼70%	[47]

Hg species				
MeHg	Swordfish, sardine and tuna	Gastric juice: 6.0% w/v pepsin, 0.15 M NaCl, pH of 1.8 (conc. HCl) Intestinal juice: 1.5% w/v pancreatin, 0.5% w/v amylase and 0.15% w/v bile salts, in 0.15 M NaCl Gastric digestion: 25 g of fish sample was added to 75 mL of gastric juice and shaken (1.0 min). The mixture was incubated in a thermostatic bath (at 37°C, 4 h, shaking periodically) Intestinal digestion: gastric digest at pH 6.8 (saturated Na_2CO_3) plus 50 mL of intestinal juice and incubation (37°C, 4 h, shaking periodically) before energetically shaking (1.0 min). After centrifugation (1575 × g, 60 min), the solutions were filtered (0.45 μm) and frozen until further analysis	MeHg bioaccessible percentage was 17%, 13% and 9% for swordfish, sardine and tuna, respectively	[42,43]
MeHg	Caribou, bearded seal, ringed seal, walrus, narwhal, beluga, Arctic char	Carbohydrate nutrition solution: 5.01 g $KHCO_3$, 2.92 g NaCl, 0.5 g arabinogalactan, 1.0 g pectin, 0.5 g xylan, 2.1 g potato starch, 0.2 g glucose, 1.5 g yeast extract, 0.5 g peptone, 2.0 g mucin, 0.25 g L-cysteine/L of DIW acidified to pH 2 via the addition 12 M HCl Small intestinal solution: 12.5 g/L $NaHCO_3$, 6.0 g/L Oxgall, 0.9 g/L pancreatin) Colon SHIME solution: 10^8 total aerobes CFU/mL and 10^8 total anaerobes CFU/mL, included fecal coliforms, clostridia, enterococci, staphylococci, bifidobacteria, and fungi Dynamic SHIME model: 100 mL of carbohydrate nutrition solution was added to the stomach every 8 h. After 2 h residence time, nutrition solution was pumped into the small intestine and mixed with 50 mL of small intestinal solution. After 2 h, the nutrition/small intestine mixture was pumped into the colon stage. The SHIME was maintained at 37°C under nitrogen atmosphere Static SHIME model: – Stomach stage: 0.2 g of sample, 8 mL of diluted HCl (pH 1.5) and 6.8 μL of concentrated pepsin were incubated under horizontally shaking (37°C, 2 h, 70 rpm) – Intestinal stage: 4 mL of small intestinal solution was added to stomach mixture (pH 6.5 ± 0.1) and the headspace was replaced with a nitrogen atmosphere using an anaerobic glove box. The mixture was incubated under horizontally shaking (37°C, 2 h, 70 rpm) – Colon stage: 8 mL of colon SHIME solution from the dynamic SHIME colon was added to the intestinal digests. The pH was corrected to 6.3 ± 0.1 and the headspace was replaced with a nitrogen atmosphere. The colon digests were incubated under horizontally shaking for an additional 18 h After SHIME fluid syringe filtered (0.45 μm) and ultra-filtered (10 kDa membrane), the extract preserved in 5% HCl, and frozen prior to analysis.	MeHg bioaccessible percentages were between 1.0% (caribou and ringed seal) and 93% (Arctic char)	[49]

MA, Monomethylarsonic acid; DMA, Dimethylarsinic acid; DIW, De-ionized water; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; PBS, Phosphate-buffered solution; MEM, Minimum essential medium; AB, Arsenobetaine; TMAO, Trimethylarsine oxide; TETRA, Tetramethylarsonium ion; AC, Arsenocholine; PTFE, Polytetrafluoroethylene; $SeCys_2$, Selenocystine; SeMet, Selenomethionine; SeMetSeCys, Seleno-methylselenocysteine; GI, Gastrointestinal; γ-glutamyl-Se-Me-SeCys, γ-glutamyl-selenomethylselenocysteine; SeOMet, Selenomethionine-

Table 2. Measurement conditions and quality control for developed methods

Analytical Technique	Separation conditions	Extraction procedure for total	Extraction procedure for speciation previous *in-vivo/in-vitro* methods	Quality control	Ref.
As species					
HPLC-ICP-MS	PRP-X-100 (250 × 4.1 mm i.d., 10 μm) anion-exchange column Injection volume: 50 μL Column temperature: 40°C Mobile phases flow rate: 1.5 mL/min Mobile phase composition: 20 mM $NH_4H_2PO_4$ (pH 5.6) Elution program: isocratic	Rice (0.5 g) plus HNO_3 (10 mL) (overnight at RT) and then heated from 75°C to 140°C for up to 10 h (volume of 1 mL). Cooling, dilution (20 mL) and filtration (0.45 μm)	Rice (0.25 g) plus 2.0 M TFA (2.0 mL) was heated on a heating block (100°C for 6 h). After evaporation to dryness, residue was dissolved in DIW, filtered (0.22 μm) and made up to 20 mL with DIW The extracts were stored at −20°C	Bush branches and leaves DC73349 (As certified value of 26.18 ± 3.14 mg/kg) and analytical recovery studies	[18,28]
HPLC-HG-AFS	PRP-X-100 (250 × 4.1 mm i.d., 10 μm) anion-exchange column Injection volume: 100 μL Column temperature: 25°C Mobile phases flow rate: 1.0 mL/min Mobile phase composition: Mobile phase A: 5 mM $NH_4H_2PO_4$ (pH 5.75) Mobile phase B: 100 mM $NH_4H_2PO_4$ (pH 5.75) Gradient program: 100% A, 4.0 min 50% A, 6.0 min 100% A, 5.0 min	Raw and lyophilized cooked rice (0.5 g), rice bioaccessible fraction (2.0 g), cell monolayers, raw seaweed (0.25 g) or lyophilized bioaccessible fraction (0.2–3.0 g), 20% w/v $MgNO_3$ + 2% w/v MgO (2.5 mL) plus 50% v/v HNO_3 (5.0 mL) was evaporated to dryness and mineralized (450°C). After white ash dissolution (6.0 M HCl), mixture was reduced with 5% w/v KI and 5% w/v ascorbic acid (5.0 mL), filtered and diluted to 25 mL with 6.0 M HCl	Raw and lyophilized cooked rice (1.0 g), raw seaweed (0.5 g) or lyophilized bioaccessible fraction (0.2 g) with DIW (4.1 mL) and HCl conc. (18.4 mL) was left overnight. 1.5% (w/v) hydrazine sulfate (1.0 mL) and HBr (2.0 mL) were then added. Inorganic As was extracted into $CHCl_3$ (3 × 10 mL, shaking 3.0 min) and centrifuging (2000 rpm, 5.0 min)). Inorganic As was back-extracted (1.0 M HCl (10 mL) and shaking (10 min)). After centrifugation (2000 rpm), 20% w/v $MgNO_3$ + 2% w/v MgO (2.5 mL) plus HNO3 conc. (10 mL) were added and then dry ashed Clean up: soluble fraction (10 g) at pH < 2 (4.0 M HCl) was passed through an AG 50 W-X8 H^+ (100–200 mesh) strong cation-exchange resin, which was then washed with 0.01 M HCl (20 mL) and DIW (20 mL). After lyophilization, the dry residue was redissolved with DIW (5 mL) and filtered (0.45 μm)	– Rice flour SRM1568a (As certified value of 0.29 ± 0.03 μg/g), – Fucus sp. IAEA-140/TM (As certified value of 42.2–46.4 μg/g), – Lagarosiphon major BCR 60 aquatic plant (As indicative value of 8.0 μg/g) and – Ulva lactuca BCR-279 sea lettuce (As certified value of 2.89–3.29 μg/g)	[27,30,31]
HPLC-HG-AFS	PRP-X-100 (250 × 4.1 mm i.d., 10 μm) anion-exchange column Injection volume: 100 μL Mobile phases flow rate: 1.0 mL/min Mobile-phase composition: Mobile phase A: 20 mM NH_4CO_3 (pH 9.0) Mobile phase B: DIW Mobile phase C: 20 mM NH_4CO_3 (pH 10.3) Gradient program: 5% A and 95% B, 7.0 min 100% A, 7.0 min 100% C, 6.0 min 5% A and 95% B, 5.0 min	*Idem* [27,30,31], except that raw seaweed and lyophilized bioaccessible fraction mass were 0.25 g and 0.2 g, respectively	Raw or cooked seaweed (1.0 g) with 1:1 v/v MeOH/DIW (20 mL) was mechanical shaken for 15 min, and then centrifuged (2000 rpm, 10 min). The extraction process was repeated twice more. After evaporation to dryness (T<50°C), dry residue was re-dissolved in 3.0 mL of DIW, centrifuged (12000 rpm, 10 min) and filtered (0.45 μm)	– Fucus sp. IAEA-140/TM (As certified value of 42.2–46.4 μg/g) and – Ulva lactuca BCR-279 sea lettuce (As certified value of 2.89–3.29 μg/g)	[32]

HPLC-ICP-MS, HPLC-HG-AAS	HPLC conditions for As(III), As(V), MA, DMA separation: PRP-X-100 (150 × 4.6 mm i.d., 10 µm) anion-exchange column Mobile phases flow rate: 0.8 mL/min Mobile phase composition: 20 mM ammonium bicarbonate (pH 7.0) Elution program: isocratic HPLC conditions for arsenosugars and AB separation: Chrompack Ionosphere C (100 × 3.0 mm i.d., 5.0 µm) cation-exchange column Mobile phases flow rate: 1.0 mL/min Mobile phase composition: 20 mM pyridinium formate (pH 2.7) Elution program: isocratic HPLC conditions for As(III), As(V), MA, DMA separation: PRP-X-100 (150 × 4.6 mm i.d., 10 µm) anion-exchange column Mobile phases flow rate: 1.0 mL/min Mobile phase composition: 20 mM $NH_4H_2PO_4$ (pH 7.0) Elution program: isocratic	–	–	– Dog fish muscle tissue DORM-2 (As certified value of 18.0 ± 1.1 µg/g) and – Hizikia fusiforme seaweed (As bioaccessible certified value of 89 ± 11 µg/g)	[33]
HPLC-HG-AFS	HPLC conditions for MA, DMA, AB and As(V): PRP-X-100 (250 × 4.1 mm i.d., 10 µm) anion-exchange column Injection volume: 100 µL Mobile phases flow rate: 1.0 mL/min Mobile-phase composition: Mobile phase A: 1 mM $NH_4H_2PO_4$ (pH 9.3) Mobile phase B: 20 mM $NH_4H_2PO_4$ (pH 9.3) Gradient program: 100% A, 5.0 min 100% B, 6.0 min 100% A, 4.0 min HPLC conditions for TMAO, TETRA and AC: PRP-X-200 (250 × 4.1 mm i.d., 10 µm) cation-exchange column Injection volume: 100 µL Mobile phases flow rate: 1.0 mL/min Mobile phase A: 100 mM $NH_4H_2PO_4$ (pH 4.5) Mobile phase B: DIW Gradient program: 10% A, 4.0 min 40% A, 7.0 min 10% A, 4.0 min	*Idem* to [30]	Lyophilized raw and cooked samples (1.0 g) were extracted three times with 1:1 v/v MeOH/DIW (20 mL). After evaporation to dryness, extracts were dissolved in DIW (2.0 mL), centrifuged, and filtered (0.45 µm). Soluble fractions (15 g) or basal media (2 mL) were lyophilized, redissolved in DIW (2.0 mL or 1.0 mL, respectively), and filtered (0.45 µm)	Dog fish muscle tissue DORM-2 (As certified value of 18.0 ± 1.1 µg/g)	[34]
HPLC-ICP-MS	IonPac (150 × 4.6 mm i.d., 10 µm) anion-exchange column Mobile phases flow rate: 1.35 mL/min Mobile phase composition: Mobile phase A: 0.5 mM HNO_3, 1.0% MeOH Mobile phase B: 50 mM HNO_3, 1.0% MeOH Gradient program: 100% A, 2.5 min 10% A, 3.0 min 80% A, 5.0 min	–	–	– Lobster hepatopancreas TORT-2 (As certified value of 21.6 ± 1.8 µg/g), – Dogfish liver DOLT-3 (As certified value of 10.2 ± 0.5 µg/g), – Tuna fish CRM 627 (As certified value of 4.8 ± 0.3 µg/g), and – Dog fish muscle tissue DORM-3 (As certified value of 6.88 ± 0.3 µg/g)	[35]

Table 2. (*continued*)

Analytical Technique	Separation conditions	Extraction procedure for total	Extraction procedure for speciation previous *in-vivo*/*in-vitro* methods	Quality control	Ref.
HPLC-ICP-MS	HPLC conditions for MA, DMA, As(III) and As(V): PRP-X-100 (150 × 4.6 mm i.d., 10 μm) anion-exchange column Mobile phases flow rate: 1.0 mL/min Mobile phase composition: 20 mM $NH_4H_2PO_4$ (pH 6.0) Elution program: isocratic HPLC conditions for TMAO, TETRA, AB and AC: Whatman SCX (150 × 4.5 mm i.d., 10 μm) cation-exchange column Mobile phases flow rate: 1.0 mL/min Mobile phase composition: 20 mM pyridine formate (pH 2.7) Elution program: isocratic	–	Lyophilized samples (0.2–0.3 g) were sonicated (30 min) with 1:1 v/v MeOH/DIW (15 mL) and then centrifuged (4000 rpm for 15 min). After evaporation to 1.0 mL (at 40°C), extracts were diluted (3.0 mL), and filtered (0.45 μm)	– Dog fish muscle tissue DORM-2 (As certified value of 17.1 ± 1.6 μg/g) and – Hizikia fusiforme seaweed (73 μg/g and 111 μg/g for gastric and gastric + intestinal digestion, respectively)	[36]
Se species					
HPLC-ICP-MS	PRP-X-100 (150 × 4.6 mm i.d., 10 μm) anion-exchange column Injection volume: 100 μL Mobile phases flow rate: 1.0 mL/min Mobile phase composition: 10 mM ammonium citrate (pH 5.0) Elution program: isocratic	Sample (25 mg) was digested (2.5 mL of HNO_3 and 1.0 mL of H_2O_2, microwave oven). The solutions were then diluted to 25 mL with DIW	Fresh sample (250 mg) or dried sample (25 mg) plus 10 mg of Protease XIV and 3.0 mL of DIW were sonicated (2.0 min). After centrifugation (7500 × g, 30 min), the supernatant were filtered (10 kDa cut-off filter)	–	[37]
LC-ESI-MS^2	Xterra MS C_{18} (250 × 2.1 mm i.d., 5 μm) Injection volume: 10 μL Mobile-phase flow rate: 1.0 mL/min Mobile-phase composition: 0.01% TEACl, 2.0% MeOH at pH 4.5 Elution program: isocratic	Sample (0.1–0.5 g) was digested (5.0 mL of HNO_3 and 1.0 mL of H_2O_2, microwave oven)	Freeze-dried sample (2.5 g) and DIW (20 mL) were placed in a hot-water bath (50°C, 8 h, shaking at 140 rpm). After centrifugation, the extract were filtered and frozen (−20°C) until further analysis	–	[38]
HPLC-ICP-MS	Chrompack IonoSpher 5C (150 × 4.6 mm i.d., 5 μm) Injection volume: 100 μL Mobile phases flow rate: 0.8 mL/min Mobile phase composition: 2 mM pyridinium formate, pH 2.8 Elution program: isocratic	Sample (0.1 g) was digested with 2.0 mL of HNO_3 and 2.0 mL of H_2O_2 for 12 h. Then, total digestion was carried out at 110°C for 20 min in a laboratory pressure cooker. Extracts were made up to 10.0 mL	Sample (50 mg) plus of Protease E (5 mg) and DIW (5.0 mL) were shaken at RT (24 h). After solution centrifugation (5000 rpm, 10 min), the supernatant was filtered (0.45 μm)	White clover CRM-402 (Se certified value of 6.7 ± 0.27 μg/g)	[39]

	Hamilton PRP X-100 (250 × 4.1 mm i.d., 10 μm) Injection volume: 100 μL Mobile phases flow rate: 1.5 mL/min Mobile phase composition: Mobile phase A: 10 mM $NH_4H_2PO_4$, 1% v/v methanol, pH 5.0 Mobile phase B: 50 mM $NH_4H_2PO_4$, 1% v/v methanol, pH 5.0 Gradient program: 100% A, 7.0 min 50% A, 7.5 min 0% A, 20.5 min 100% A, 26 min				
HPLC-ICP-MS, HPLC-ES-MS-MS	Xterra MS C_{18} (250 × 2.1 mm i.d., 5 μm) Injection volume: 10 μL Mobile phases flow rate: 0.16 mL/min Mobile phase composition: 0.01% TEACI, 2.0% MeOH at pH 4.5 Elution program: isocratic	Sample (0.4 g) was digested (5.0 mL of HNO_3 and 1.0 mL of H_2O_2, microwave oven)	Defatted sample (400 mg), using MeOH/$CHCl_3$, was incubated in a shaking bath (37°C for 24 h in dark at 150 rpm) with protease XIV (40 mg) and DIW (3 ml). After centrifugation, the extract were frozen (−20°C) until further analysis	–	[40]
HPLC-ICP-ES-MS	Polysphere IC AN-2 (120 × 4.6 mm i.d.) cation-exchange column Injection volume: 50 μL Mobile phase flow rate: 0.75 mL/min Mobile phase composition: 5.0 mM salicylate (pH 8.5) Elution program: isocratic	Sample (0.2 g) was digested (2.0 mL of HNO_3 and 0.5 mL of H_2O_2, microwave oven)	–	Wheat gluten NIST 8418 (total Se content of 2.58 ± 0.19 μg/g)	[41]
HPLC-ICP-MS	PRP-X-200 (550 × 4.1 mm i.d., 10 μm) cation-exchange column Injection volume: 100 μL Mobile phase flow rate: 1.0 mL/min Mobile phase composition: 4.0 mM pyridine formiate, H_2O:MeOH (93:3) (pH 2.8–4.7) Elution program: isocratic	Sample (0.5 g of wet fish or 0.2 g of dry fish) were digested (2 mL of HNO_3 and 0.5 mL of H_2O_2, at 43% power output). The solutions were then diluted to 25 mL with DIW	Sample (0.1–0.2 g) plus protease E (0.02 mg) and Tris-HCl buffer (3.0 mL, pH 7.5) were sonicated (120 s, 20 W). After centrifugation (4000 rpm, 20 min), extract were ultrafiltered (10-kDa cut-off filter) and diluted to 10 mL	Antarctic krill Murst-ISS A2 (Se certified value of 7.37 ± 0.91 μg/g)	[42,43]
HPLC-ICP-MS, HPLC-ES-MS^2	Xterra MS C_{18} (250 × 2.1 mm i.d., 5 μm) Injection volume: 10 μL Mobile-phase flow rate:: 0.16 mL/min Mobile-phase composition: 0.01% TEACI, 2.0% MeOH at pH 4.5 Elution program: isocratic	–	–	–	[44]
SEC-ICP-MS, HPLC-ICP-MS, ESI-Q-TOF-MS, MALDI-TOF-MS	Fractionation: Superdex 200 HR 10/30 SEC (300 × 10 mm i.d., 13 μm) with a exclusion limit of 1300 kDa for HMW (10 –600 kDa) Injection volume: 6 × 200 μL Mobile-phase flow rate: 0.6 mL/min Mobile-phase composition: 10 mM ammonium acetate (pH 8.5) Elution program: isocratic	Sample (0.1 g) was digested (3.0 mL of HNO_3 and 0.5 mL of H_2O_2, microwave oven). Extracts were made up to 20 mL	–	Yeast SEAS 6 (Se indicative value of 1374 ± 101 μg/g)	[45]

Table 2. (*continued*)

Analytical Technique	Separation conditions	Extraction procedure for total	Extraction procedure for speciation previous *in-vivo*/*in-vitro* methods	Quality control	Ref.
	Separation: Shidex GS-220 HQ (300 × 7.6 mm i.d., 6 μm) with exclusion limit of 3 kDa LMW Injection volume: 6 × 200 μL Mobile-phase flow rate 0.5 mL/min Mobile-phase composition: 20 mM or 50 mM ammonium acetate (pH 7.0) Elution program: isocratic Characterization: Discovery BIO Wide Pore C_8 microbore (150 × 2.1 mm i.d., 3 μm) Mobile-phase flow rate: 175 μL /min Mobile-phase composition: Mobile phase A: 0.1% HFBA in 5.0% MeOH Mobile phase B: 0.1% HFBA in 95% MeOH Gradient program: 5% methanol, 10 min 5–75% methanol, 45 min 75–90% methanol, 50 min 90% methanol, 60 min				
HPLC-ICP-MS	Hamilton PRP X-100 (250 × 4.1 mm i.d., 10 μm) Injection volume: 100 μL Mobile-phase flow rate: 0.9 mL/min Mobile-phase composition: Mobile phase A: 10 mM $NH_4H_2PO_4$, 2% v/v methanol, pH 7.0 Mobile phase B: 100 mM $NH_4H_2PO_4$, 2% v/v methanol, pH 7.0 Gradient program: 100% A, 3.0 min 0% A, 12 min 0% A, 15 min	*Idem* [45]	Procedure 1: sample (10 mg) plus Protease XIV (1.0 mg) in 100 mM Tris-HCl buffer (pH 7) was incubated in a shaking water bath (at 37°C, 20 h). After centrifugation (4000 × g, 40 min at 4°C), the extracts were filtered (0.45 μm) and frozen (−20°C) until further analysis Procedure 2: sample (10 mg) was incubated in a shaking bath a 37°C with 4% (w/v) driselase (150 μL) in 30 mM Tris-HCl buffer in the presence of 1.0 mM PMSF for 4 h. Then, protease XIV (1.0 mg) was added. The samples were incubated for 20 h at 37°C. After centrifugation (4000 × g, 40 min at 4°C), the extracts were filtered (0.45 μm) and frozen (−20°C) until further analysis	Yeast SEAS 6 (Se indicative value of 1374 ± 101 μg/g)	[46]
HPLC-ICP-MS	Superdex 75 (300 × 10 mm i.d., 10 μm) Injection volume: 100 μL Mobile phases flow rate:: 0.7 mL/min Mobile phase composition: 100 mM ammonium acetate, pH = 7.5 Elution program: isocratic	–	Sample (0.2 g) plus 0.9% NaCl (pH 5.5) were sonicated (3.0 min, 30 Hz). After centrifugation (10000 × g, 10 min), the procedure was repeated twice and supernatants pooled	SRM 1567A Wheat flour (Se certified value of 1.1 ± 0.2 μg/g)	[47]

HPLC-ICP-MS	Superdex 75 (300 × 10 mm i.d., 10 μm) Injection volume: 100 μL Mobile phases flow rate: 0.7 mL/min Mobile phase composition: 100 mM ammonium acetate, pH = 7.5 Elution program: isocratic	Sample (0.1 g) was digested (2.5 mL of HNO_3 and 1.5 mL of H_2O_2, by heating at 65°C for 4.5 h)	0.2 g of defatted white egg (using cyclohexane) plus 30 mM Tris-HCl buffer (5.0 mL, pH 7.5) were incubated in a water bath (37°C, 1.0 h). After centrifugation (14500 rpm, 15 min), extracts were analyzed 0.2 g of defatted yolk egg (using cyclohexane) plus 7.0 M urea (0.5 mL) plus 30 mM Tris-HCl buffer (5.0 mL, pH 7.5) were sonicated (ultrasonic probe, 2.0 min). After centrifugation (3000 rpm, 5 min), extracts were analyzed	NIST 8415 Whole Egg Powder (Se certified value of 1.39 ± 0.17 μg/g)	[48]
Hg species					
GC-AFS	Capillary fused silica column SGL-1 (15 m × 0.53 mm i.d.) coated with 1.5 μm dimethylpolysiloxane Helium flow rate: 10 mL/min Injector temperature: 250°C Temperature program: Initial temperature: 40°C Final temperature: 200°C Ramp temperature: 15°C/min	Sample (0.5 g of wet fish or 0.2 g of dry fish) was digested (2 mL of HNO_3 and 0.5 mL of H_2O_2, at 43% power output). The solutions were then diluted to 25 mL	Sample (0.3 g) plus 5 M HCl (5.0 mL) were sonicated (5.0 min). Extracted Hg species were converted into bromide derivates (RHgBr) and extracted into methylene chloride (5.0 mL, 5 h shaking). After centrifugation (10 min), organic solvent (4.0 mL) was mixed (20 min) with sodium thiosulfate (1.0 mL 0.01 M) and centrifuged (1.575 × g). Aqueous layer (800 μL) plus KBr/$CuSO_4$ (300 μL) and CH_2Cl_2 (300 μL) were shaken (1.0 min) and centrifuged	Tuna fish CRM-463 (MeHg certified value of 2.85 ± 0.16 μg/g)	[42, 43]
LC-CV-AFS	–	Sample was microwave acid digested	–	– Lobster hepatopancreas TORT-2 (As certified value of 21.6 ± 1.8 μg/g), – Dogfish liver DOLT-2 (Hg certified value of 1990 ± 100 ng/g) and – Dogfish muscle tissue DORM-2 (Hg certified value of 4.64 ± 0.26 μg/g)	[49]

HPLC-ICP-MS, High-performance liquid chromatography-inductively coupled plasma mass spectrometry; RT, Room temperature; DIW, De-ionized water; EPA, Environmental Protection Agency; TFA, Trifluoroacetic acid; HPLC-HG-AFS, High-performance liquid chromatography-hydride generation atomic fluorescence spectrometry; HPLC-HG-AAS, High-performance liquid chromatography-hydride generation atomic absorption spectrometry; MA, Monomethylarsonic acid; DMA, Dimethylarsinic acid; AB, Arsenobetaine; TMAO, Trimethylarsine oxide; TETRA, Tetramethylarsonium ion; AC, Arsenocholine; LC-ESI-MS^2, Liquid chromatography electrospray ionization tandem mass spectrometry; TEACl, Tetraethylammonium chloride; HPLC-ES-MS^2, High-performance liquid chromatography-electrospray tandem mass spectrometry; HPLC-ICP-ES-MS, High-performance liquid chromatography-inductively coupled plasma mass spectrometry-electrospray mass spectrometry; SEC-ICP-MS, Size-exclusion chromatography- inductively coupled plasma mass spectrometry; ESI-Q-TOF-MS, Electrospray ionization-time of flight-mass spectrometry; HMW, High-molecular-weight compounds; LMW, Low-molecular-weight compounds; MALDI-TOF-MS, Matrix-assisted laser desorption/ionization-time-of-flight-mass spectrometry; HFBA, Heptafluorobutyric acid; PMSF, Phenylmethylsulfonyl fluoride; HG-AAS, Hydride generation atomic absorption spectrometry; GC-AFS, Gas chromatography-

inorganic As reached 63–99%; and As(V) was the main species found. In Caco-2 cells, As retention, transport, and total uptake (retention + transport) varied (i.e. 0.6–6.4%, 3.3–11.4% and 3.9–17.8%, respectively). The lack of statistical correlation between the soluble total As added to the Caco-2 cells and the total uptake observed indicates that, although As may be bioaccessible after GI digestion, other soluble components from rice may cause differences in the extent of absorption.

Finally, He et al. [29] demonstrated the feasibility of evaluating the *in-vivo* bioaccessibility and metabolism of As in rice through a simple mass-balance approach during a short period of controlled diet. Although only two participants were involved in the study, that was sufficient for method-development purposes because many samples were collected over time. The amounts of drinking water, food and urine, together with total-As and As-species concentrations in these samples were monitored to construct a mass balance of As intake and excretion. The experiment was designed to determine whether urinary As excretion responded to dietary changes with food containing different, but still normal, concentrations of As. In the first five days, the two volunteers on a wheat diet had an average As daily intake of 15.4 ± 2.6 μg and 9.6 ± 0.7 μg, respectively. In the next five days, these volunteers switched to a rice diet, increasing the average As daily intake to 36.4 ± 2.8 μg and 34.1 ± 7.7 μg, respectively. Daily excretion of urinary As, mostly as DMA, doubled from 9.8 ± 0.3 μg to 21.0 ± 3.0 μg, and from 6.5 ± 0.8 μg to 11.6 ± 4.5 μg, respectively. The percentage of ingested As excreted in urine remained constant at around 58% for one volunteer before and after the rice diet, and was around 69% for the other.

Concerning edible seaweeds, although the high As content in this foodstuff is mainly organic As (arsenosugars), there are bioavailability studies dealing with both organic and inorganic species, so there were also studies of the bioaccessibility of inorganic As contents from edible seaweeds [*Hizikia fusiforme* (brown seaweed), *Porphyra sp.* (red seaweed) and *Enteromorpha sp.* (green seaweed)] by using an *in-vitro* GI digestion [30,31]. The bioaccessibility of total As in red seaweed (67.2%) was slightly higher than that observed in brown seaweed (62.3%), and these values were double that obtained from green seaweed (32%). This might be due to a different capability of the enzymes in the *in-vitro* method for releasing As present in each sample, or differences in the composition of the seaweeds, which might affect the solubility of As forms. The authors did not observe the same tendency for inorganic As [As(III) + As(V)] in raw seaweed, and the bioaccessibility was higher in brown seaweed (74.7%) and green seaweed (77.2%) than it was in red seaweed (48.6%).

The effect of cooking edible seaweed on As bioaccessibility was also considered. Results showed that total and inorganic As bioaccessibility increased significantly after cooking, attaining 79.9% and 72.6%, respectively, in *Porphyra sp.*, and 65.7% and 87.9%, respectively, in *Hizikia fusiforme*. Boiling may decrease fiber content and denature proteins, permitting greater accessibility of enzymes during proteolysis. The effect of cooking on proteins, in which inorganic As is bound through sulfhydryl groups, might affect the efficiency of the digestive enzymes used on releasing As from each sample. Because As(III) is bound to protein groups with sulfur residues and cysteine, the boiling might facilitate dissolution of As(III) in cooked seaweed, and consequently the dissolution of inorganic As.

Speciation studies for inorganic As in the bioaccessible fraction have revealed different As(III)/As(V) ratios in foodstuffs when raw or cooked. For raw seaweed, the major species was As(III) after both gastric and GI stages, whereas the major As species found after cooking depended on the batch analyzed.

The bioaccessibility of arsenosugars {e.g., glycerol ribose (3-[5A-deoxy-5A-(dimethylarsinoyl)-b-ribofuranosyloxy]-2-hydroxypropylene glycol, As(328); phosphate ribose (3-[5A-deoxy-5A-(dimethylarsinoyl)-b-ribofuranosyloxy]-2-hydroxypropyl-2,3-ydroxypropyl phosphate, As(482); sulfonate ribose (3-[5A-deoxy-5A-(dimethylarsinoyl)-b-ribofuranosyloxy]-2-hydroxypropanesulfonic acid, As(392); and, sulfate ribose (3-[5A-deoxy-5A-(dimethylarsinoyl)-b-ribofuranosyloxy]-2-hydroxypropyl hydrogen sulfate, As(408)} from edible seaweeds (*Hizikia fusiforme*, *Undaria pinnatifida* and *Porphyra sp.*) was also studied by Almela et al. [32] using *in-vitro* GI digestion. These authors tested the effect of various types of cooking (boiling, soaking, soaking and boiling, baking, toasting over a flame) on arsenosugars and other As species present in raw seaweed. Results showed that the cooking procedures do not alter the As species in *Undaria pinnatifida* and *Porphyra sp.* However, they produced a substantial increase (from 2–5-fold higher) in the content of As(V) from *Hizikia fusiforme*. In all of the seaweeds analyzed, the bioaccessibility of arsenosugars was high (>80%), and it was not affected by the cooking procedures.

The bioaccessibility of arsenosugars (glycerol ribose, phosphate ribose, sulfonate ribose, and sulfate ribose), As(III), As(V), MA, and DMA from seaweed (*Fucus sp.*) and from softshell clams (*Mya arenaria*) harvested in an As-contaminated marine area was also evaluated by Koch et al. [33]. MA plus DMA and arsenosugars account for ~1.2–1.9% and 43–46%, respectively of the bioaccessible As in seaweed.

Laparra et al. [34] also discussed the bioaccessibility of As species from raw and cooked seafood (sole, *Solea solea*; Greenland halibut, *Reinhardtius hippoglossoides*; and DORM-2 fish protein) by *in-vitro* GI digestion with pepsin, pancreatin and bile extracts. After centrifugation, the bio-accessible fractions were analyzed by

high-performance liquid chromatography (HPLC) with thermooxidation-hydride generation (HG)-atomic fluorescence spectroscopy (AFS) to estimate the bioaccessibility of AB, MA, DMA, TMAO and TETRA (tetramethylarsonium ion). Results showed bioaccessibility values of 67.5–100% for AB, 30% for DMA, 45% for TETRA, and >50% for TMAO. In addition, the bioaccessible contents of total As and organic As were not modified after cooking, and high values, ~100%, were found for cooked samples.

Transport by Caco-2 cells from organoarsenical standards and DORM-2 was also evaluated in this study. Results for DORM-2 showed that transport was only significant for AB (12%), with far higher efficiency than in the case of the standard solution.

The bioaccessibility of As(III), As(V), MA, DMA, AB, AC, and TMAO from several seafood reference materials {i.e. TORT-2 (lobster hepatopancreas), CRM 627 (tuna fish), DOLT-3 (dogfish liver) and DORM-3 (fish muscle)} was also assessed by Dufailly et al. [35]. The method involved use of KH_2PO_4 for saliva digestion, and pepsin and pancreatin for gastric and intestinal digestion, respectively. After centrifugation, the bioaccessible fraction was analyzed by HPLC with inductively coupled plasma mass spectrometry (ICP-MS). To assess the maximum bioaccessibility, these authors also developed an *in-vitro* dynamic model in which artificial saliva, and gastric and intestinal juice were successively pumped through the sample contained in a mini-column (about 200-mg aliquots of solid sample wrapped in a membrane filter placed in a PTFE tubing 8 cm long, 3/16 in o.d., and 1/8 in i.d.). The mini-column was directly connected to the nebulizer of an ICP-MS instrument. In contrast to the conventional batch method, this approach allowed continuous monitoring of the progressive release of As after passing the three simulated digestive juices. Results showed that saliva alone was sufficient to release, in less than 5 min, the total bio-accessible As that had been previously mobilized by saliva and gastric juices in the batch mode. In addition, this approach avoided potential contamination because sample preparation was minimized and conducted in a closed system.

Finally, the bioaccessibility of As(III), As(V), MA, DMA, AB and TETRA from the abdominal muscle of the yabbies (freshwater crayfish) was assessed by Williams et al. [36]. A highly bioaccessible As content from the abdominal muscle of these animals, up to 69%, was found. Results, after speciation analysis, showed that the inorganic As species, As(III) and As(V), predominate. However, As(V) was found to be extracted from the tissue only during intestinal digestion. In addition, the bioaccessible As percentage was higher (41–78%) from tissues containing concentrations of As higher than those containing low As concentrations (As bioaccessible percentage within the 12–38% range).

2.2. Se species in food samples

Because Se has largely been recognized as an essential nutritional element for human life, and Se deficiency is a significant problem in several countries, numerous studies have been conducted to establish the bioaccessibility and the bioavailability of Se from several fortified foodstuffs (e.g., radish, green onion, chives, yeast and yeast-based nutritional supplements, wheat, chicken eggs and chicken meat). Fortification of these materials was performed with several Se species, {e.g., Se(IV), Se(VI) and SeMet}. Foodstuffs containing high Se concentrations (e.g., Brazil nuts, yeast and fish) were also studied. These studies focused on assessing bioaccessible and bioavailable Se species from: raw and cooked vegetables [37–39] (i.e. radish, onion, chives and garlic) and nuts [40]; raw and cooked fish [41–43]; yeast and yeast-based nutritional supplements [44–46]; wheat [47]; and, chicken eggs [48].

Pedrero et al. [37] assessed the potential bioavailability of Se and Se species from radish (*Raphanus sativus*), belonging to the *Brassicaceae* family. The concentrations of selenocysteine ($SeCys_2$), SeMet and Se-methylselenocysteine (SeMetSeCys) in fresh samples remained almost unaltered after a simulated GI digestion, and the analysis of the GI digests showed that almost 100% of Se in the fresh plants is found in the bioaccessible fraction.

Dumond et al. [38] treated garlic (*Allium sativum*) samples with saliva and simulated gastric and intestinal fluids, and observed the same Se species, SeMet, SeMetSeCys and γ-glutamyl-selenomethylselenocysteine (γ-glutamyl-Se-Me-SeCys), and additional unknown Se-compounds in saliva and in both simulated fluids. Data about bioaccessibility percentages for each species were not given.

Kápolna et al. [39] used *in-vitro* GI digestion to assess the bioavailability of Se from selenized green onion and chive samples. As a consequence of Se fortification with Se(VI), 80–90% of the total Se content (mainly as selenate) became bioaccessible in the simulated GI digests. Unknown Se compounds were detected, and they contributed approximately 2% to the total bioaccessible Se content. Because the toxicity of Se(VI), Se supplementation with this Se species is inappropriate. Otherwise, a lower percentage of bioaccessible Se was obtained from Se(IV)-fortified green onion (30%) and chives (12%), and from SeMet-fortified green onion (20%) and chives (22%). Se(VI), Se(IV) and SeMet species were quantified in the bioaccessible fraction from Se(IV)-fortified green onion and chives. Certain unknown Se compounds (3.0%) were also detected. Concerning SeMet-fortified green onion and chives, Se(IV), SeMet, MeSeCys, and selenomethionine-Se-oxide (SeOMet) species were quantified in the GI digests. Unknown Se compounds were also observed, and they represented ~2–10%. When assessing bioaccessible Se from Brazil nuts [40],

Bertholletia excelsa, (one of the products exhibiting the highest Se content), $SeCys_2$ and SeMet were the main compounds found after simulated gastric and intestinal digestion. Data on bioaccessibility percentages for each species were not given in this study.

Crews et al. [41] investigated Se(IV) and SeMet contents in *in-vitro* GI digests from cooked cod. They found that bioaccessible total Se was around 61.3%. Data about bioaccessibility percentages for Se(IV) and SeMet species were not given.

Cabañero et al. [42,43] assessed Se bioaccessibility from fish [swordfish (*Aphanopus carbo*); sardine (*Sardina pilchardus*); and, tuna (*Thunnus spp.*)] using an *in-vitro* approach. Se bioaccessibility was different depending on the type of fish. Higher Se-solubility percentages were observed in the GI supernatants from swordfish and sardine (76% and 83%, respectively) compared with those obtained for tuna (50%). Simulated human gastric and intestinal digestion led to the identification of SeMet in the GI digests from the three fishes, and similar bioaccessible percentages were observed for SeMet (16%, 14% and 19%, for swordfish, sardine and tuna, respectively).

Results from yeast (*Saccharomyces cerevisiae*) and yeast tablets (Selenium-ACE, Bioplex, SelenoPrecise) [44], showed that total Se bioaccessible was 55–80%. The main compound extracted by both gastric and intestinal fluids was SeMet. Two other minor Se compounds were identified as $SeCys_2$ and SeOMet (a degradation product of Se-methionine). Otherwise, data about bioaccessibility percentages of these species were not given.

Hinojosa et al. [45,46] investigated potentially bioavailable Se-containing compounds in selenized yeast (SEAS 6) and yeast-based nutritional supplements (Arkovital, Verdalia and Selenium-ACE). Results obtained for yeast showed that SeMet was the major compound identified in the GI extracts, while SeOMet was its main degradation product after medium-term and long-term sample storage. Results showed that 89% of total Se was extracted after the GI approach, but, surprisingly, only 41% was quantified as free SeMet, so, although this free selenoamino acid appears to be the most abundant Se species in the GI digests, half the total SeMet in the extracts still seems to be associated with Se-containing peptides after *in-vitro* GI digestion. The remaining extracted Se species (20%) were found to be in the form of low-molecular-weight compounds. Concerning yeast-based nutritional supplements, low SeMet percentages (26–37%) were observed, the remaining dissolved Se being other unknown Se compounds (probably Se-containing peptides).

Govasmark et al. [47] and Lipiec at al. [48] assessed the bioaccessibility of Se species in Se-fortified wheat and chicken eggs, respectively. The Se yield rose to 70% in wheat when using simulated gastric digestion and the bioaccessible fraction did not increase when using simulated duodenal digestion. For chicken eggs, data on the bioaccessibility percentages for each Se species were not given in the study.

2.3. Hg species in food samples

Despite the high toxicity of Hg, as established after the Minamata Bay poisoning by consumption of contaminated fish and shellfish and the high MeHg concentrations reported in fish according to the Rapid Alert System for Food and Feed (RASFF), bioaccessibility and bioavailability studies on Hg species in foods are rare, compared with As and Se *in-vivo* and *in-vitro* GI methods.

Fish is the most studied sample [42,43,49] and, due to MeHg being the major Hg species in fish, studies are mainly focused on MeHg bioaccessibility. Studies were performed for swordfish (*Aphanopus carbo*), sardine (*Sardina pilchardus*) and tuna (*Thunnus spp.*) using an *in-vitro* approach [42,43]; and, caribou (*Rangifer tarandus L.*), bearded seal (*Erignathus barbartus E.*), ringed seal (*Phoca hispida S.*), walrus (*Odobenus rosmarus L.*), narwhal (*Monodon monoceros L.*), beluga (*Delphinapterus leucas P.*), and arctic char (*Salvelinus alpinus L.*) using an *in-vitro* SHINE model [49].

Similarly to SeMet, the bio-accessibility of Hg depends on the type of fish analyzed (swordfish, sardine or tuna) [42,43], but, in general, low MeHg bioaccessibility (9–17%) was found for all tested fish [42,43].

Laird et al. [49] used the SHIME model to assess Hg bioaccessibility in fish samples. GI microbes may affect Hg and MeHg bioaccessibility from foodstuffs, either increasing or decreasing bioaccessibility depending upon the type of food. Results indicated that Hg and MeHg bioaccessibility is inversely related to Hg concentration in the material. In addition, GI microorganisms have been found to affect the speciation of the ingested Hg via methylation or demethylation of Hg species in the GI lumen.

3. As, Se and Hg degradation and inter-conversion

Despite the relatively mild operating conditions used by *in-vitro* GI methods (Table 1), it is possible that degradation and transformation of chemical forms of an element could occur when studying bioaccessibility and bioavailability from the viewpoint of speciation.

In-vitro digestion procedures reported do not modify the chemical forms of organoarsenic species standards (AB, DMA, MA, TMAO, TETRA, and AC) [34], and recoveries of 88% (TETRA) to 114% (DMA) were reported for the bioaccessible fractions. In addition, unknown species were not detected, indicating that degradation of these organoarsenic species standards did not occur under the simulated GI conditions [34].

Studies were also performed for DORM-2 (AB certified value of 16.3 ± 0.5 μg/g), which was digested after

being spiked with an AB content similar to the certified AB value. Results indicated that, after spiking with AB, no increase in the dissolved MA content was observed, and AB recovery was 96.4 ± 7.0% [34]. In addition, these authors also showed the absence of species interconversion by using the Caco-2 cell line during evaluation of transport in both the apical and basal media.

Similarly, Almela et al. [32] did not observe arsenosugar (glycerol ribose, phosphate ribose, sulfonate ribose and sulfate ribose) degradation from seaweeds as a result of *in-vitro* digestion tests (pH 1.0, 37°C for 2 h).

However, Gamble et al. [50] and Van Hulle et al. [51] showed that these arsenosugars and dimethylarsinoylethanol (DMAE) were found to degrade within a short time into a compound with a mass of 254 Da under simulated gastric juice extraction (pH 1.1, 38°C for 48 h and 60°C for 7.5 h) [50] or (pH 1.2, 37°C, 4 h) [51]. An acid hydrolysis mechanism was proposed for the formation of the degradation product from each native arsenosugars by hydrolysis at the C-1 carbon on the ribose ring.

For Se, although dipeptide γ-glu-Se-Me-SeCys is the major species in garlic, SeMetSeCys is the main compound found in the GI digests, so Dumont et al. [38] suggested that γ-glu-Se-MeSeCys is converted to Se-MeSeCys during human digestion.

Similarly, Káplona et al. [39] assumed that the degradation of organic Se species (MeSeCys, $SeCys_2$ and SeMet) present in green onion and chives took place during the digestion process because the concentrations found for inorganic Se compounds were significantly higher than those obtained after a conventional extraction approach. Furthermore, the main initial Se forms could not even be detected in the GI extract. Because of the significant pH change between the gastric and the intestinal tracts, two oxidation processes take place – selenite is oxidized to selenate, and SeMet is oxidized to SeOMet. However, Se-species transformations were not observed for SeMet and $SeCys_2$ in Brazil nuts [40] or SeMet in fish [42,43] during GI-simulated conditions.

Dumont et al. [44] found low SeCys (80%) and SeMeSeCys (70%) recoveries in yeast samples after simulated digestion, while high SeMet and $Se(Cys)_2$ recoveries (90–95% as total Se) were found after GI digestion. This might be due to volatile Se compounds formed during digestion. These authors also found that a small amount of SeMet was transformed to SeOMet during digestion.

Govasmark et al. [47] and Lipiec at al. [48] showed that simulated gastric and duodenal digestions of chicken eggs and wheat lead to degradation of high-molecular-weight compounds containing SeMet and SeCys to low-molecular-weight peptides and amino acids. The process is ~50% effective with egg whites and ~ 80% effective with egg yolks; the average molecular weight of the digest of the egg whites is lower than that of egg yolks and overlaps with the amino-acid fraction.

GI digestion allows the conversion of as much as 90% of the initially present Se-containing proteins in the amino acid and the small peptide fraction, which is supposed to be bioaccessible.

Finally, MeHg transformation was reported during simulated digestion processes [42,43].

4. Measurement conditions and quality control

Table 2 summarizes relevant analytical data for As, Se and Hg speciation, separation and detection conditions, and QC data for *in-vivo* and *in-vitro* methods. It also includes extraction methods to assess total As, Se and Hg (acid extraction assisted by conventional heating, mechanical shaking or microwave energy, mainly), and conventional extraction methods for isolating As, Se and Hg species (acid or solvent extraction assisted by mechanical shaking and ultrasound energy, and enzymatic hydrolysis using Protease XIV and Protease E) from food samples.

Analytical methods are based on the coupling of LC (ion-exchange, size-exclusion, and ion-pair reversed-phase HPLC) and gas chromatography (GC) to specific detection systems for As, Se and Hg, usually ICP-MS, AFS and atomic absorption spectrometry (AAS). Electrospray ionization (ESI)-MS and matrix-induced laser desorption/ionization (MALDI)-MS have also been used for confirmation and identification of some detected Se compounds.

Quantitative validation of the *in-vivo* and *in-vitro* assays for quality assurance (QA) and QC is needed prior to application of the approach to specific samples or general studies. At present, validation of these approaches is incomplete due to the lack of enough *in-vivo* data, and because of the lack of suitable certified reference materials (CRMs) for human-bioaccessibility studies. As can be seen in Table 2, although several CRMs are used in bioaccessibility studies, these CRMs are not certified for As, Se and Hg content in the bioaccessible and bioavailable fraction. However, some validated data are available for total As in Hijiki (*Hizikia fusiforme*), a Japanese edible seaweed, for which bioaccessibility control limits were established [33]. As can be expected, there are no CRMs with bioaccessible and bioavailable As, Se and Hg species content.

5. Conclusions and future development

Several *in-vivo* and *in-vitro* GI methods have been developed and applied to assess bioaccessibility and bioavailability of As, Se and Hg species from raw and cooked foodstuffs. The approaches developed mainly

comprised *in-vitro* procedures based on solubility (gastric plus intestinal digestion, including the SHIME model) and on the use of Caco-2 cells (approaches that also simulate retention, transport, and total uptake of the target organometallic species). *In-vivo* methods based on the use of swine have also been described. The bioaccessibility and the bioavailability of As, Se and Hg species are affected by sample-matrix composition, the cooking procedure for food, and the experimental conditions (gastric and intestinal pH, incubation temperature and residence time). Regarding species degradation and transformation during *in-vitro* procedures, good stability was observed for most As species, except for certain arsenosugars that have been reported to degrade under certain specific GI digestion conditions. However, important transformations during the *in-vitro* process were reported for Se species, [e.g., conversion of γ-glu-Se-MeSeCys to Se-MeSeCys, and organic Se species (MeSeCys, $SeCys_2$ and SeMet) degradation to inorganic Se].

As a consequence of the different GI models (e.g., SHIME or intestinal epithelial) used to assess bioaccessible or dialyzable fractions, future development in this area will focus on assessing an international standardized protocol for assessment of *in-vivo* and *in-vitro* approaches. The development of CRMs (together with laboratory guidelines for their use) could provide appropriate materials for QA and QC procedures in all laboratories. Because bioaccessibility and bioavailability studies for Hg species are scarcer than for other organometallic species (e.g., As and Se), and due to the high toxicity of Hg and its main organometallic species (MeHg), further developments to assess bioaccessibility and bioavailability of Hg species in marine foodstuffs are necessary. Similarly, bioaccessibility and bioavailability studies must also be developed for organometallic species of other trace elements (e.g., tin, germanium or lead).

Finally, because of the increasing importance of functional foods, mainly foodstuffs fortified with essential trace elements, speciation studies are essential for their bioaccessible and bioavailable fractions.

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