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Trace element speciation in food: State of the art of analytical techniques and methods*

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Abstract: Some elements in food are notoriously toxic, whereas others are considered essential for human health. Information on the exact chemical form in which an element is present in food is of paramount importance to determine the safety and nutritional quality of food. This critical review discusses the state of the art of analytical approaches to speciation of trace elements in food products. The topics addressed include (i) responding to regulations concerning some toxic elements (As, Hg, Sn); (ii) quality control of food and feed supplements; and (iii) characterization, in terms of element speciation, of nutritional plants (natural and genetically modified) and food supplements produced by biotechnology. The maturity of analytical techniques allowing the determination of individual well-defined metal species is highlighted. On the other hand, the recent developments of multidimensional hyphenated techniques and the democratization of electrospray high-resolution mass spectrometry (Orbitrap) start permitting fine characterization of element speciation in natural products.

Keywords: analytical chemistry; chemical speciation; food; food chemistry; food supplements; mass spectrometry; metals; speciation; trace elements.

INTRODUCTION

Surveillance of the concentrations of trace elements, toxic and considered as essential nutrients, in food is carried out by governmental agencies in many countries on a routine basis. However, it has become evident in the last two decades that food safety and nutritional quality are dependent on the chemical form in which an element occurs in food. Indeed, it is the concentration of particular element species that determines the toxicity, essentiality, and bioavailability of a trace element. Consequently, information on the speciation of elements in food is of paramount importance for studies of human nutrition and health [1].

Speciation measurements are increasingly important for regulatory agencies to ensure that a product fulfils the criteria of food safety in terms of the absence of the toxic element species [2]. On the other hand, information on speciation permits the food industry to gain a competitive advantage and to

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improve product quality, especially for food supplementation. Last but not least, information on speciation is fundamental to improving biotechnological production processes to ensure the presence of introduced elements in the desired form (e.g., organic Se yeast) and to develop new biotechnologies aiming at increasing the bioavailability of essential minerals (e.g., Zn and Fe).

The choice of analytical method to determine element speciation is critically dependent on the objective. Relatively simple and well-established methods are available for the determination of the most notorious toxic target species including inorganic As, methylmercury, or tributyltin (still of concern in shellfish consumed in southeast Asia) [3]. The same applies to selenomethionine (SeMet) of which the concentration has become the most common parameter of the evaluation of the quality of Se-rich yeast [4]. Quality control and assurance in all these cases is fairly straightforward as calibration standards exist and certified reference materials (CRMs) can be used.

A different situation occurs for speciation analysis of natural products, such as, e.g., plants that are the primary source of essential elements in food. The knowledge of the speciation of many metals is scarce, and speciation studies are often exploratory aiming at the detection and identification of previously unreported metal species. The increasing use of biotechnologies to produce food supplements rich in organically bound trace elements claims for analytical methods capable of fine characterization of these products, of the reproducibility assessment of the production process, and of the verification of the provenance of the product available on the market. Relevant analytical methods are at an early stage of development recently boosted by the advances in multidimensional chromatographic separation techniques and multistage high-resolution mass spectrometry [5].

The aim of this critical review is to summarize the state of the art of analytical methods for trace element speciation in food and food supplements, to present an opinion on the role of element speciation in regulations, and to highlight the need for new analytical methods for the characterization of food ingredients, either natural or issued from novel biotechnologies.

STATE OF THE ART AND ADVANCES IN ANALYTICAL APPROACHES

Determination of individual well-defined element species

A number of methods targeting individual organometallic contaminants in food such as methylmercury, organolead, and organotin compounds were developed in the 1990s [1]. Calibration standards were either available or could be readily synthesized, which allowed the optimization of efficient extraction and derivatization methods. The presence of a metal–carbon covalent bond assured a reasonable stability of the target species integrity during sample preparation. The volatility of the species allowed the use of gas chromatography (GC). Its inherent advantages, such as the high separation efficiency and the absence of the condensed mobile phase, enabled a sensitive (down to the sub-pg level), element-specific detection by atomic, fluorescence, or emission spectrometry [3]. The increasing market share of inductively coupled plasma-mass spectrometry (ICP-MS) and the availability of commercial GC-ICP-MS interfaces have spurred the growing number of reports on ICP-MS [6]. Some features of ICP-MS such as femtogram detection limits or multi-isotopic capacity are impressive but do not represent a real advantage over the older techniques, which are fully sufficient to answer the question whether a food sample contains toxic species above the legal threshold or not.

ICP-MS offers, however, an unmatched performance as high-performance liquid chromatography (HPLC) detector, which allowed the development of a numbers of methods for organometallic species that could not be determined by GC. The HPLC-ICP-MS coupling has become a routine technique for the determination of SeMet, cobalamine, and organoarsenic species [3].

An emerging trend in speciation analysis is the use of LC-MS-MS [7]. Recent improvements of electrospray source design and sensitivity of triple quadrupole analyzers allow much lower detection limits to be obtained in comparison with ICP-MS. The ultrasensitive MS-MS systems are still much more expensive than ICP-MS but they are versatile and not acquired by analytical laboratories for spe-

ciation analyses only. The wider availability of LC-MS-MS may soon lead to the situation that speciation of organometallic molecules will lose its uniqueness and the determination of heteroatom-containing species will be as trivial as that of any organic molecule.

Exploratory studies for trace element species in natural materials and products issued from biotechnologies

In contrast to anthropogenic contaminants, the element speciation in natural products is usually unknown and target analytes have often not been identified. HPLC-ICP-MS coupling is for the moment irreplaceable as a method for screening for unknown metal species because it responds quantitatively to any molecule containing a given hetero-element, regardless of its coordination environment. The HPLC separation mechanism chosen should assure the complete analyte recovery and the baseline separation of the analyte compounds. Size-exclusion chromatography (SEC) is the mostly used technique despite its poor resolution. Veritable maps of heteroatom-tagged molecules can be obtained by multidimensional chromatography, but the control of the recovery and interactions of metal species (especially of coordination complexes) with the stationary phase remains a challenge [8,9].

The identification of the detected species had remained extremely difficult until recently as electrospray quadrupole MS did not offer sufficient sensitivity in the full scan mode and electrospray time-of-flight mass spectrometry (TOF-MS) turned out to be extremely vulnerable to co-eluting impurities because of the limited intrascan dynamic range [10]. The situation has changed since the advent of electrospray Orbitrap systems. Its intrascan dynamic range makes it much more tolerant to co-eluting impurities, and the molecular mass accuracy of 1–3 ppm is maintained at the different stages of fragmentation down to MS^4 [11]. Element isotopic profiles can be readily recognized in any of the spectra.

In vitro bioavailability assessment

A good measure of bioaccessibility of an element can be obtained from tests simulating digestion processes in the stomach and small intestine [12]. Enzymolysis procedures rely on the measuring of the soluble amount of elements obtained after sequential in vitro treatments of food by hydrolases and proteases (pepsin and pancreatin) applied in conditions similar to those existing in vivo. These protocols need to be validated and standardized but an agreement of the results between the in vitro studies and the corresponding in vivo experiments was reported [12]. Note that in vitro methods are not speciation methods in the IUPAC sense of the term [13], as no species are actually either identified or quantified. Several procedures based on this principle developed and applied to a variety of foods were recently reviewed [12].

SPECIATION OF TOXIC ELEMENTS (FOOD SAFETY)

Arsenic

As is a notorious toxic element which often occurs at very high concentrations in seafood (>50 mg kg^{-1} wet weight); the latter being the major contributor of As to the European diet [14]. The element features a very complex chemistry with over 50 compounds reported to be present in seafood [15]. The most abundant species in animal organisms is arsenobetaine, whereas arsenoribosides (arsenosugars) prevail in algae. Other classes of organoarsenic species, such as thioarsenicals and arsenolipids, should also be mentioned. However, the toxicity is mostly associated with As(III). Therefore, for the purpose of food safety, speciation of As is limited to the measurement of As(III) or, more precisely, because As(III) interchanges during sample storage and preparation with the less toxic As(V), of the "total inorganic As". It is usually determined by hydride generation atomic absorption spectrometry (AAS) using commercial instrumentation and routine analytical procedures.

Facing the lack of toxicological data for most of As species and indications of a possibly similar behavior of arsenosugars and inorganic As in terms of bioaccessibility, a recommendation was recently made to report partly speciated As concentrations in food commodities in three fractions: (i) toxic inorganic As as arsenate (after oxidation); (ii) arsenobetaine as established nontoxic As; and (iii) potentially toxic As, which includes arsenosugars and other organoarsenic compounds [16].

The analytical methodology for As speciation is based on the coupling of HPLC-ICP-MS, which also allows quantification [17–19]. An often overlooked aspect of fundamental importance is the yield of extraction of As species from the sample, which should always be optimized and given in scientific reports. A single HPLC separation mechanism is seldom sufficient to attain baseline resolution of organoarsenic species when ICP-MS detection is used [20,21]. Therefore, HPLC-electrospray ionization-MS-MS (HPLC-ESI-MS-MS) has the vocation to become a privileged technique for quantification when standards are available [22]. There is still a potential for the detection of unknown As metabolites, which is expected to be explored in the near future using HPLC-Orbitrap MS^n.

As concentrations in terrestrial foods are much lower (<0.05 mg kg^{-1}) but the element is present mostly as inorganic As. A special interest has been attracted by As speciation in rice and rice-based products owing to alarmingly high concentrations of inorganic As and of potential exposure of specific groups of the population. The analytical methods available seem to be reliable [23] but a CRM would be of benefit.

Roxarsone (4-hydroxy-3-nitrophenylarsonic acid) has been widely used agriculturally as a chicken-feed additive but is likely to be banned as it was found responsible for high levels of As in a potentially toxic form. The methods for the determination of roxarsone and its metabolites are based on HPLC-ICP-MS and HPLC-ESI-MS-MS [24,25].

Mercury

Methylmercury is a lipid-soluble species capable of bioaccumulation in the aquatic food chain, and thus present in relatively high quantities in large predatory fish (swordfish, tuna). The U.S. Food and Drug Administration (FDA) sets a limit of max. 1 μg/g to be present in edible portions of fish and shellfish, whereas the U.S. Environmental Protection Agency (EPA) guideline recommends a limit on Hg consumption related to body weight 0.1 mg/kg body weight per day [2].

The interest in Hg speciation results in an impressive number of works published on the development of new methods of seafood analysis [26], but the usefulness of the vast majority of these reports is rather limited. Indeed, the analytical chemistry of Hg speciation is still based on the methods developed by Bloom [27] in the late 1980s that have undergone only cosmetic changes since then. The introduction of stable isotopes to speciation analysis of Hg by Hintelmann et al. [28] improved quality control and allowed the detection of a number of sources of errors, such as, e.g., the spurious formation of methylmercury during some extraction procedures.

Methylmercury is released from the sample in strongly acidic conditions, derivatized by ethylation using $NaBEt_4$ and cryotrapped and chromatographed by packed column or capillary GC. Hg produced by pyrolysis is detected by atomic fluorescence spectroscopy (AFS) or AAS. The typical chromatogram contains three peaks corresponding to Hg(0), MeEtHg, and $HgEt_2$. Commercial analyzers based on this principle are available. There are a number of CRMs available for fish and shellfish which should be used for quality assurance. A recent comparison study demonstrated the validity of classical methods by GC-MS and GC-ICP-MS, the better sensitivity of the latter was not found necessary in view of the concentrations of methylmercury present [29].

There is a natural interest in the identification and description of the Hg binding centers in the methylmercury-binding biomolecules. This interest is rather academic as the MeHg-Cys bonds seem to be unstable in gastrointestinal conditions and the toxic species to be accounted for is methylmercury [2].

Tin

Organotins (mainly tributyl- and triphenytin) are used as biocides in wood preservatives and antifouling paints, pesticides, and PCV stabilizers. The biggest concerns relevant to food safety are contamination of shellfish and possible leaching from some plastic packing materials.

The analytical procedures are based on the extraction of organotin compounds with acetic acid, derivatization by sodium tetraethylborate, and GC with plasma source detection [30]. Extraction of organotin compounds can be accelerated by microwave field [31]. The principal analytical technique is GC-ICP-MS, which replaced gas chromatography-microwave induced plasma-atomic emission detection (GC-MIP-AED) with which the original methods were developed [6].

Food contamination by organotin compounds is not a health risk to the European consumer according to the opinion of the European Food Safety Agency [32] and a recent extensive survey [33]. However, the monitoring of seafood from coastal environments in the emerging economies is still an important issue.

Cadmium and lead

Speciation of other notoriously toxic elements, such as Cd and Pb, has been given much less attention. The bioaccessibility of these metals was studied in cocoa because of the alleged anthropogenic contamination resulting in Cd concentrations in marketed chocolate products above the permissible levels [34]. Most of Cd and Pb were found to be present in cocoa in insoluble and not bioaccessible forms [35]. Elevated concentrations of Cd were also reported in fish and shellfish where it occurs complexed by metallothionein, a cystein-rich class of proteins expressed in the presence of Cd and other contaminants [36]. Cd-metallothioneins are unstable below pH 2 (gastrointestinal conditions) dissociating to produce the Cd^{2+} ion so that speciation information does not seem to affect the Cd toxicity.

The risk of contamination of some foods by organic forms of lead is not off the agenda since the ban of the leaded gasoline. Pb was found to be present in fruits, vegetables, and related products in the form of a complex with a polysaccharide di-Ramnogalacturonan II (diRG II) [37] of which the behavior in gastrointenstinal conditions was not studied. The Pb-diRG II complex can be readily determined by the coupling of SEC-ICP-MS [38]. Note that Cd and Pb are readily complexed randomly by a number of proteins that can be detected by SEC-ICP-MS [39]. These data seem to be meaningless from the point of view of the toxicity assessment of these elements and thus of relevant legislation.

SPECIATION AND SUPPLEMENTATION OF ESSENTIAL ELEMENTS: SELENIUM

Se is an essential trace element known for its antioxidant properties. The physiological role of Se is principally awarded to its incorporation into selenoproteins as selenocysteine (SeCys), referred to as the 21^{st} amino acid [40].

Natural selenium-rich plants

Speciation of Se in edible plants has been a hot research topic for the last decade [41]. Particular attention was paid to garlic, mushrooms, and different types of nuts, known for high Se content and beneficial health effects.

SeMet has been the principal species identified mostly because the extraction procedure was designed to release it from the proteins by means of a proteolytic digestion. Specific reported metabolites included: γ-glutamyl-Se-methylselenocysteine and γ-glutamyl-Se-methylselenomethionine in garlic [42], Se-methylselenocysteine in garlic and onion [43,44], selenohomolanthionine in Se-enriched Japanese pungent radish [45], and seleno-cystathionine and its γ-glutamyl derivatives in nuts [8]. These examples of successful identification give the impression to have been achieved by chance rather than

by a systematic approach. Mass balance of the Se species was seldom reported, and several peaks in the chromatograms remained unidentified. Therefore, it is expected that a larger integration of high-resolution Fourier transform-MS (FT-MS) in Se speciation analytical protocols will result in a rapid progress of knowledge about the Se speciation in plants.

Selenium-rich yeast

The most popular form of supplemented Se is yeast grown in the presence of selenite (selenate), which is able to accumulate up to 3000 mg Se kg^{-1} [46,47]. It is an attractive source of Se owing to its relatively low cost and high content of SeMet acting as a precursor for selenoprotein synthesis. The typical analytical demand from the industry concerns the verification of the minimum content of SeMet (usually >60 %) in Se-rich yeast and the demonstration of the absence (<2 %) of selenite and selenate. The SeMet concentration is also a necessary parameter to be measured in the Se-fortified premixes and feeds to prove their organic, i.e., derived from Se-rich yeast, origin.

Se-rich yeast can be characterized by the Se metabolic profile (selenometabolome), which is characteristic of yeast strain and fermentation parameters and can be a precious fingerprint of the origin of the preparations available on the market and of the reproducibility of the production process [48]. The water-soluble fraction of Se metabolites accounts for 15–30 % of total Se and contains over 50 detectable compounds. The Se metabolome may also contain a particular species showing either a specific therapeutic activity or an unaccounted for toxicity.

Determination of selenomethionine, selenocysteine, and inorganic selenium

SeMet is released from the proteins by enzymatic or acid digestion. The total recovery requires relative harsh conditions: 16 h with methanesulfonic acid under reflux [4] or triple digestion with a protease [49]. SeMet is typically determined by HPLC (anion-exchange or reversed phase) with ICP-MS detection. The results from both methods are coherent and were the basis of the establishment of the certified value for SeMet in the SELM-1 CRM (NRCC, Ottawa, Canada) [4]. The availability of this reference material was a tremendous step to improve the quality assurance of the analyses of commercial products.

The determination of SeCys is still a difficult task, and the poorly validated methods dominate in the literature. SeCys is still frequently (mis)identified by matching its retention time with that of a $SeCys_2$ standard, which often elutes very close to the void of the column [50]. The SeCys in the metabolite fraction can be quantified following the identification and quantification of the individual SeCys-containing metabolites present. The question about the presence of SeCys in the protein fraction remains open as there has been no successful report of the derivatization of SeCys in the presence of the overwhelming concentration of SeMet in yeast.

The determination of inorganic Se is not a trivial task as Se(IV) is known to be readily complexed by proteins. Therefore, the complete degradation of sample, e.g., by proteolysis, is necessary prior to analysis for Se(IV) and prior to any statements about its absence.

An emerging question is that concerning the presence of Se(0) nanoparticles in Se-rich yeast. This species has escaped to date the chemical analysis and was identified by microscopy and synchrotron radiation imaging [51]. The methods of quantitative determination of Se(0) remain to be developed.

Fingerprinting and identification of selenium metabolites

The Se speciation in the water-soluble fraction of Se-rich yeast by ESI-MS has been attracting a lot of attention but the success had been limited until 2006 (cf. ref. [41]). The TOF mass analyzers used suffered from a poor intrascan dynamic range; the presence of an intense signal from an easily ionizable matrix molecule co-eluting with the analyte Se compounds suppressed the signal from the latter. A breakthrough was achieved by the development of multidimensional (SEC-AE-RP) separation protocols which allowed the ionization of a number of metabolites and their structural characterization by MS-MS [9]. The increasing availability of FT orbital ion trap or ion cyclotron resonance instruments

offering a larger intrascan dynamic range, and the possibility of accurate mass determination and of multistage fragmentation, has largely facilitated Se metabolomics [10,11]. The previously identified metabolites can be rapidly screened for in the simple MS chromatograms on the basis of their accurate mass on one hand [48], and, on the other hand, the characterization of a number of unknown metabolites by multistage fragmentation mass spectrometry is possible. About 50 metabolites have recently been identified using this method in our laboratory.

Quantitative analysis for selenium-containing proteins

Se is believed to replace methionine and to build into proteins randomly but the degree of expression of particular proteins is dependent on the yeast strain and the fermentation process. Consequently, the quantitative distribution of protein-bound Se can be a valuable parameter of yeast origin and of the reproducibility of the fermentation process.

Because of the non-quantitative character of shotgun proteomics and difficulties with the separation of intact protein by chromatographic techniques, 2D electrophoresis is the separation method of choice. The most abundant Se-containing proteins were detected by laser ablation ICP-MS [52,53]. The identification of the proteins was achieved by in-gel tryptic digestion and electrospray (ES)-MS-MS [53]. Seventeen Se-containing proteins (12 are reported for the first time) were identified. The principal challenge to such protocols is the improvement of the recovery of the protein precipitation, yield of the tryptic digestion and of the peptides extraction from the gel, and of the degree of sequence coverage [53].

Selenium-enriched functional foods

The relatively easy incorporation of Se from the supplemented diet into farmed animals (beef, pork, poultry) results in the availability of several Se-rich foods on the market (meat, milk, eggs). Se from these products seems to be highly bioavailable for selenoprotein synthesis.

The speciation analysis can discriminate between SeCys being a constituent of the true selenoproteins and SeMet replacing the methionine sulfur randomly. A number of methods for the simultaneous determination of SeMet and SeCys in meat [54], milk [55], and eggs [56]. The relatively complex methods were based on the derivatization of the residues of the two selenoaminoacids prior to the enzymatic digestion of the sample, the purification of the amino acid fraction by SEC and the separation and determination of SeMet, SeCys, and inorganic Se by reversed-phase HPLC-ICP-MS [54–56].

The current challenge is study of the expression of selenoproteins. To date, only GSHPx activity was used as a molecular marker of active Se.

SPECIATION AND SUPPLEMENTATION OF ESSENTIAL ELEMENTS: TRACE METALS

The bioavailability of trace metals (e.g., Zn, Cu, Fe, Mn) is known to depend on their chemical form. During the last decade, interest in using metal chelates with amino acids, peptides, or proteins increased considerably owing to their higher bioaccessibility in comparison with inorganic sources [57]. For some elements, e.g., Cr, the use of Cr-yeast contrasts favorably with the use of synthetic organic compounds containing this element of which potential genotoxic effects were recently reported [58].

The principal categories of metal-enriched food/feed products include: chelates or complexes with glycine and some other amino acids [59], yeast grown in the presence of essential metal ions [60], and soybean protein digestate [61] chelating metal ions. The characterization of these biotechnology products in terms of metal speciation and the determination of the active species in premixes and feeds are the current analytical challenges. Verification of batch-to-batch reproducibility and the differentiation of supplements in terms of manufacturer are important parameters for product quality control and fraud prevention.

Synthetic supplements (glycinates)

Glycinates are synthetic products and their structures can be readily established by X-ray crystallography. ES-MS-MS allows the unambiguous characterization of glycinate complexes in aqueous solutions providing metal-specific isotopic patterns in the MS mode and characteristic fragments in the MS+MS mode [62]. The original compounds were reported to be prone to rearrangements and losses of coordination water but the metal-glycine moiety was preserved [62]. Several chromatographic mechanisms were not suitable for the separation of metal-glycinates because of their dissociation on the column. Good results were obtained by the capillary electrophoresis-ICP-MS coupling [62]. The latter was mandatory to achieve limits of detection sufficiently low to enable the analysis of premix samples [62].

Yeast enriched in metals (Zn, Fe, Mn, Cr)

The success of the Se-fortified yeast for supplementation spurs interest of growing yeast in the presence of essential trace elements [60]. Yeast, in addition to other unicellular organisms such as *Lactobacilli* and *Spirulina*, has been a favorite cultivation medium, allowing the incorporation of trace elements into biomass [60]. The two main problems include (i) the need of much higher concentrations of metals than Se because of higher supplementation requirements, and (ii) the fact that metals, in contrast to Se, are bound by coordination and are not incorporated into proteins as Se is.

The percentage of organically bound metal is typically determined by measuring the water-insoluble fraction (considered organic), which is usually between 50–90 % depending on the metal and the product origin. The characterization of the water-soluble fraction by size-exclusion LC-ICP-MS shows indeed that the metal is retained on the column or co-elutes with the inorganic standard, which would suggest that the water-soluble part is inorganic [63,64]. However, recent extended X-ray absorption fine structure (EXAFS) analyses of water extract of Zn yeast indicate that the form may be organic but so unstable that it cannot pass intact through a chromatographic column [65].

The characterization of water-insoluble supplements was carried out by in vitro gastrointestinal digestion showing the solubility of half of the chromium present [63]. It was demonstrated to be in the form of Cr-peptide complexes, which were sufficiently stable to be eluted from the column and characterized by ES-MS [63].

Metal-proteinates

An alternative vehicle for trace elements can be soybean constituents, which have attracted widespread research attention for their purported health benefits and are considered by many animal producers as an alternative source of supplemental proteins. A fairly complex analytical set-up based on size-exclusion fractionation followed by nanoHPLC with the parallel ICP-MS and ES-MS-MS detection allowed the identification of a variety of Cu complexes with peptides in the Cu-rich feed supplements [61].

Increasing essential elements bioavailability in edible plants

More than half of the world's population suffers from Zn and Fe deficiency. The bioavailability of Zn and Fe from the cereal grains is of utmost importance for the wellbeing of the population where these products are the main diet. Complexation of Zn and Fe with phytic acid in rice, wheat, and barley is responsible for their poor bioavailability [66].

One of the approaches to increase the amount of bioavailable Fe and Zn in cereal grains is increasing the expression of the gene-controlling nicotianamine synthesis; the enzyme is responsible for the synthesis of nicotianamine, a low molecular metal chelator facilitating the Fe and Zn transport and increasing their bioavailability [67].

The advances in methodology, especially the coupling of hydrophilic interaction liquid chromatography (HILIC) in parallel with ICP-MS and MS-MS offered a reliable method for the quantification of the nicotianamine complexes and the description of the metal transport in plants [68].

CONCLUSIONS

The bioavailability, beneficial activity, and toxicity of mineral elements in food are a function of the metal concentration, its oxidation state, and the chemical form in which it is present in the sample. The analytical methods for the most common food contaminants: methylmercury, inorganic forms of As, and organotin compounds are well established, and no breakthrough is expected despite the high level of activity resulting in new methods still being published. CRMs should be regularly used to assure the good level of quality assurance. The advent and rapid proliferation of ES high-resolution mass spectrometers allowing multistage fragmentation (MS^n) (Orbitrap) opens new perspectives in the identification of metal-species in edible plants and animals and characterization of natural food supplements. The higher rate of reliable data acquisition owing to this new methodology may boost the development of new biotechnologies aiming at a better incorporation of essential element into natural products or increasing concentration of ligands responsible for the bioavailabity of essential element such as Zn and Fe.

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