Chapter 13
Two Sides of Selenium: Occurrence and Determination of Selenium Forms in Food and Environmental Samples Using Analytical Methods

Joanna Zembrzuska, Bożena Karbowska, and Iwona Gołębiewska

Abstract Historically, selenium has been considered a toxic element, suggesting its elimination from the diet. However, it has been recently discovered that it is a crucial trace element for human physiology, playing an important role in the metabolism, production of hormones, and functionality of the immune system. Among others, selenium has been found to exhibit protective effects in the etiology of cancer that are related to its activity against oxidative stress on cell membranes as well as the stabilizing effect on DNA and enhancing the cellular immune responses. Selenoproteins are involved in human metabolism, and so their deficiency causes degeneration of tissues and organs, resulting in an increased risk of various degenerative diseases. Bioavailability of selenium differs depending on the form it is supplied in, organic species being the ones that are absorbed to the highest extent in the human intestine. Therefore, a preferred supplementation method is through dietary routes, including the selenium-rich foods obtained through soil fertilization, livestock fodder fortification, or utilizing functional foods. This warrants an efficient analytical methodology for determining the selenium content and its chemical forms in the food products. The total, often trace amount of selenium, is usually determined by radiometric, electroanalytical, and spectroscopic methods. However, determining the speciations of selenium is an equally important task. Most analytical methods developed for selenium speciation analysis in food products focus on the determination of selenates(IV), selenates(VI), and selenoproteins. Among all the techniques used for selenium speciation analysis, liquid chromatography com-

J. Zembrzuska (✉) · B. Karbowska
Poznan University of Technology, Institute of Chemistry and Technical Electrochemistry, Poznań, Poland
e-mail: joanna.zembrzuska@put.poznan.pl; bozena.karbowska@put.poznan.pl

I. Gołębiewska
Université Catholique de Louvain, Faculty of Bioscience, Engineering & Earth and Life Institute, Applied Microbiology/Bioengineering, Louvain-la-Neuve, Belgium
e-mail: iwona.cybulska@uclouvain.be

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bined with mass spectrometry and excitation in inductively coupled plasma is undoubtedly characterized by the greatest analytical capabilities and the widest application.

Introduction

Selenium (Se) has been the object of scientific interest for many years. Although it was considered a toxic element by scientists for a long time, clinical studies conducted with this element have elucidated important information regarding its positive impact on human health. As a result, selenium was included as a trace element necessary for the proper functioning of the human body. Undoubtedly, it deserves to be called one of the most interesting elements necessary for maintaining health. Among other micronutrients, it is distinguished by a small difference between the toxic and therapeutic dose. Both an excess and a deficiency of selenium in the diet can be a cause of diseases. Ongoing studies are conducted in many research groups around the world, to obtain scientific confirmation regarding the effectiveness of chemical forms of selenium in preventing the formation and development of malignant tumors. Despite many scientific reports on this topic, the exact scope of this element's activity is still unknown.

Selenium: Characteristic of the Element

Selenium (Se) with an atomic mass of 78.96 u belongs to the group of chalcogens (the 16th group of the periodic table) and therefore has chemical properties similar to sulfur. Selenium speciation is a complex issue. It occurs in nature at oxidation levels ranging from −II, −I, 0, and + IV to +VI, in both inorganic and organic forms. It exists in solid, liquid, and gas phases, in the form of six stable isotopes, among which the isotope with a mass equal to 80 U is the most common (49.61%). Selenium is found in six allotropic forms, three amorphous forms, and three annular crystalline forms. Elemental selenium is characterized by a melting point of 494 K and a boiling point of 958 K (Tinggi 2003). The basic forms of selenium occurring in the environment and the human body are presented in Table 13.1.

Water-soluble selenite and selenate can be reduced to insoluble elemental selenium by nonspecific reduction using sulfates (Lenz et al. 2008; Tucker et al. 1998) or nitrates (Sabaty et al. 2001). Selenium oxyanions can be reduced to elemental selenium aerobically or microaerobically by various bacterial strains (Hunter 2007; Hunter and Kuykendall 2006). Formation of elemental selenium is desirable since the resulting forms are less soluble and thus less bioavailable (Combs et al. 1996; Fernández-Martínez and Charlet 2009). Insoluble elemental selenium can be converted into a mobile form by reoxidation to soluble oxyanions (mainly selenite)

Table 13.1 Common selenium compounds (Sentkowska 2019)

Name	Chemical formula
Selenium(IV) oxide	SeO_2
Selenate(IV) acid	H_2SeO_3
Selenate(VI) acid	H_2SeO_4
Sodium selenite(IV)	Na_2SeO_3
Sodium selenite(VI)	Na_2SeO_4
Hydrogen selenide	H_2Se
Diethyl selenide	CH_3-CH_2-Se-CH_2-CH_3
Dimethyl selenide	CH_3-Se-CH_3
Dimethyl diselenide	CH_3-Se-Se-CH_3
Dimethyl selenium sulfide	CH_3-Se-S-CH_3
Trimethyl selenium ion	$(CH_3)_3Se^+$
Dimethyl selenoxide	$(CH_3)_2SeO$
Selenocysteine	H-Se-CH_2-CH(NH_2)-COOH
Selenomethionine	CH_3-Se-$(CH_2)_2$-CH(NH_2)-COOH
Selenocystine	HOOC-(NH_2)CH-CH_2-Se-Se-CH_2-CH(NH_2)-COOH
Selenocystamine	NH_2-CH_2-CH_2-Se-Se-CH_2-CH_2-NH_2
Se-methylselenocysteine	CH_3-Se-CH_2-CH(NH_2)-COOH
Selenohomocysteine	H-Se-CH_2-CH_2-CH(NH_2)-COOH
Selenocystathionine	HOOC-CH(NH_2)-CH_2-Se-$(CH_2)_2$-CH(NH_2)-COOH

under aerobic conditions (Dowdle and Oremland 1998; Losi and Frankenberger Jr 1998; Sarathchandra and Watkinson 1981). Solubilization of elemental selenium can alternate based on a reduction to dissolved selenide (Herbel et al. 2003), which easily reacts with metal cations and forms strong metal selenium deposits (Seby et al. 2001).

Selenium is an essential element in organisms and is involved in intracellular redox homeostasis and thyroid hormone metabolism (USHHS 2003), similar to selenoproteins. Historically, glutathione peroxidase was the first identified enzyme containing selenium which protects the cell from oxidative damage (Flohe et al. 1973; Rotruck et al. 1973). Later, selenium was identified in different varieties of the selenium proteins, including at least 25 selenoproteins in humans (Papp 2007), but the function of many compounds belonging to this group remains unknown.

The Discovery of Selenium (the Origin of Selenium)

The medieval physician Paracelsus, considered the father of toxicology, once said, "All things are poison and nothing is without poison, only the dose permits something not to be poisonous" (Krieger 2001). Selenium is a particularly good example of this saying, as most living organisms require small amounts of selenium in food to stay healthy, while higher intake can cause illness and even death. Selenium was

discovered in 1817 by the Swedish chemist Jöns Jacob Berzelius and its name comes from the Greek goddess of the moon "Selene." Since the discovery of selenium, for the next hundred years there have been literature publications dealing mainly with inorganic selenium compounds found in nature in the form of minerals. In 1957, there was a breakthrough in terms of investigating the properties of the element, in which the relationship between selenium and the health of animal organisms was demonstrated (Hefnawy and Tortora-Perez 2010; Lopez et al. 2010; Rayman 2000; Tinggi 2003; Vinceti et al. 2020).

Selenium in Soils

Selenium is present in the soil at all basic oxidation levels: −II, 0, IV, and VI, which depends on humidity, free oxygen concentration, and pH, as well as the redox potential. Apart from the geochemical properties, the behavior of selenium compounds in the soil is also determined by the multivalence of the element. The transformation of inorganic selenium into organic forms is a very important process that occurs in soil. The dominant reaction in this environment is the biomethylation carried out in plants, fungi, bacteria, and other microorganisms. The volatile alkyl derivatives resulting from this process – dimethyl selenide $(CH_3)_2Se$ and dimethyl diselenide $(CH_3)_2Se_2$ – significantly impact the geochemical circulation of this element in nature. Selenates(VI) in the form of SeO_4^{2-}, which occur in an alkaline environment, constitute a thermodynamically stable group of compounds. They are well soluble in water, which results in easier leaching from the soil, transport to groundwater, and uptake by plants. Selenates(IV) occur under poorly oxidative conditions and can be reduced to the Se^0 elemental form. Selenides (Se^{2-}) present in the acidic environment are poorly mobile and difficult to access for plants. The organic matter associated with mud and clay fractions significantly affects selenium content, further influencing its mobility and bioavailability in the soil (Pezzarossa and Petruzzelli 2001; Tam et al. 1995; Wang and Gao 2001).

It is estimated that selenium is present in the earth's crust in the amount of 0.05–0.5 mg Se·kg^{-1} (Amoatey and Baawain 2019; Lemly 2004), with total selenium concentrations in rocks (mainly in sandstone, quartzite, and limestone) constituting 40% of the earth's crust (Wang and Gao 2001). It is reported that the global content of selenium in most soils (classified as poor in selenium) is equal to 0.4 mg Se·kg^{-1}, while in seleniferous soils (rich in selenium) this content may reach up to 1200 mg Se·kg^{-1}. Such selenium-rich soils occur in the USA, Canada, Columbia, Great Britain, China, and Russia (Fordyce 2005).

The average selenium content in low selenium soil in the Keshan area in China is equal to 0.121 mg·kg^{-1}, with a range of 0.059–0.19 mg·kg^{-1} (Sun et al. 1985; Guilherme et al. 2017). Other low values have also been reported globally: 0.198 mg·kg^{-1} and 0.23 mg·g^{-1} in former Yugoslavia (Jović 1998; Maksimovic et al. 1992) and less than 0.1 mg·kg^{-1} in New Zealand, Hungary, and Finland (Westermarck et al. 1977). The low selenium content in the soil in the Al-Kharj region (Saudi

Arabia) can be attributed to the nature of the soil in the study area, which is well-drained, with a predominance of limestone and clays with a sandy top layer (Al-Sahel Mphil et al. 2009).

Selenium is bound to natural sulfides, such as pyrite, chalcopyrite, and sphalerite (Wiberg et al. 2001). However, selenium ore deposits have no economic significance (Butterman and Brown 2004). Selenium is also present in high sulfur carbons. Although the world average is equal to only 1.6 and 1.0 mg Se kg^{-1} (hard and brown coals, respectively) (Yudovich and Ketris 2006), in the USA, Russia, and China there are regions of increased selenium content in carbons up to 43 mg Se kg^{-1}. Furthermore, black slate and volcanic tuff may contain high concentrations of 22 and 32 mg kg^{-1} of selenium in the Daba region, China (Kunli et al. 2004).

Selenium in Surface Waters

Selenium occurs in natural waters in the following forms: H_2SeO_3, $HSeO_3^-$, SeO_3^{2-}, $HSeO_4^-$, or SeO_4^{2-}. Its content ranges from 0.1 to 60 ng·L^{-1} for ocean waters and from 0.1 to 400 µg·L^{-1} for surface and groundwaters. According to the World Health Organization (WHO), the acceptable content of Se in drinking water is 10 µg·L^{-1} (Barceloux 1999). In natural water, this element is found in trace amounts as a result of weathering of minerals, soil erosion, and volcanic activity. Its levels vary from region to region but are usually below 10 ng·mL^{-1}. Surface waters can absorb selenium from the atmosphere by dry and wet deposition, from adjacent waters that may contain selenium, from surface runoff, and from subsurface drainage. In the study regarding direct discharges from an oil refinery in the San Francisco Bay, the average concentration of selenium in wastewater was equal to 0.067 mg·L^{-1} with a range of 0.0066–0.156 mg·L^{-1} (Barceloux 1999; Cutter 1989). Approximately 50–76% of total selenium in the wastewater occurred in the form of selenate(IV) (Cutter, 1989).

The most oxidized forms of selenate(VI) and selenate(IV) found in surface waters exhibit high bioavailability and a tendency for bioaccumulation (Dungan and Frankenberger Jr. 1999).

Selenium in Plants

Although selenium is not considered as an essential plant nutrient, more selenium species have been identified in plants than in animals, e.g., inorganic compounds such as selenate Se(VI) and selenite Se(IV), as well as organic forms such as selenomethionine (SeMet), selenocysteine (SeCys), Se-methyl-selenocysteine (Se-methyl-SeCys), γ-glutamyl-Se-methyl-selenocysteine (γ-glutamyl-Se-methyl-SeCys), and selenoproteins. Some plants also volatilize selenium as dimethyl selenide or dimethyl diselenide (Aureli et al. 2012; Ogra et al. 2007; Torres et al. 2016).

The concentration of selenium in plants depends on the region and soils used to cultivate the plants. Plants can also absorb volatile selenium from the atmosphere. In species sensitive to selenium, the concentration threshold of this metalloid in shoot tissues ranges from 2 mg kg^{-1} dry mass (d.m.) in rice to 330 $mg \cdot kg^{-1}$ d.m. in white clover. Selenium hyperaccumulators can tolerate concentrations exceeding even 4000 $mg \cdot kg^{-1}$ d.m. without any signs of adverse effects on their growth. Such hyperaccumulators include, among others, plants belonging to the *Compositae*, *Leguminosae*, *Cruiferae*, and *Allium* families (Wierzbicka et al. 2007; Ellis et al. 2003; Terry et al. 2000).

The majority of crops and grasses usually contains less than 25 $mg \cdot kg^{-1}$ d.m. of selenium and does not tend to accumulate this element in tissues in concentrations exceeding 100 $mg \cdot kg^{-1}$ d.m. even during growth on soils with a high content of this element.

The content of selenium in cereals and cereal products is usually in the range of 4–267 $\mu g \cdot kg^{-1}$ d.m. Potatoes contain approximately 0.43 $mg \cdot kg^{-1}$ d.m., tomatoes and onions 0.03 $mg \cdot kg^{-1}$ d.m, carrots approximately 0.40 $mg \cdot kg^{-1}$ d.m., broccoli approximately 1.0 $mg \cdot kg^{-1}$ d.m., cauliflower 0.44 $mg \cdot kg^{-1}$ d.m., cabbage 0.72 $mg \cdot kg^{-1}$ d.m., and lettuce 0.36 $mg \cdot kg^{-1}$ d.m. In case of legumes, the content ranges as follows: soybean 435 $\mu g \cdot kg^{-1}$ d.m, peas 1345 $\mu g \cdot kg^{-1}$ d.m, and beans 938 $\mu g \cdot kg^{-1}$ d.m. Fruits (e.g., pears, apples, and plums) contain up to several $\mu g \cdot kg^{-1}$ d.m.

Various types of nuts were also analyzed for selenium content; lower levels were found in cashews (*Anacardium occidentale* – 0.27 $mg \cdot kg^{-1}$ d.m.), walnuts (*Juglans regia* – 0.03 $mg \cdot kg^{-1}$ d.m.), hazelnuts (*Corylus avellana* – 0.02 $mg \cdot kg^{-1}$ d.m.), peanuts (*Arachis hypogea* – 0.04 $mg \cdot kg^{-1}$ d.m.), pecans (*Carya pecan* – $mg \cdot kg^{-1}$ d.m.), and macadamia nuts (*Macadamia whelanii* – 0.07 $mg \cdot kg^{-1}$ d.m) (Barclay et al. 1995; Ihnat 1989; Pennington et al. 1995).

It is worth emphasizing that the differences in selenium content are not only species-based but also population-based. They depend on the area where a given plant species grows.

Selenium in Fish and Bird Organisms

In the aquatic environment, selenium is a particular threat to wildlife. Bioaccumulation (increased concentration in the body compared to the surrounding environment) and biomagnification (increasing concentration due to chain transfer) increase the risk of toxic selenium forms and are a threat to the wildlife. As a result, selenium concentration in the tissues of lower invertebrates or fish can reach levels up to 2000 times higher than the selenium concentration in water (Wu 2004). It has been shown that adverse effects on fish can occur at a selenium concentration in water of 5 $\mu g \cdot L^{-1}$, (Frankenberger et al. 2004; Hamilton 2004). The most thoroughly studied case of selenium contamination in wildlife took place at the National Wildlife Refuge Kesterson Reservoir, California, USA (Hamilton 2004; Presser and Luoma

2007), where selenium-rich subsurface drainage water entered the ponds in the wildlife reserve. In some acid leachates, concentrations were as high as 4200 μg $Se \cdot L^{-1}$ (Kharaka et al. 1996; Stefaniak et al. 2018). Contamination with selenium caused the death of many fish and waterbird populations. Also, developmental abnormalities were found in nesting birds in 20% of nests, whereas more than 40% contained one or more dead embryos (Ohlendorf 2002). Selenium caused deformation and reduced the survival of various fish species, e.g., bluefin larvae (*Lepomis macrochiros*).

Impact of Selenium on Human Health

Selenium in the Human Diet

Selenium has been reported to play an important role in human and animal nutrition as one of the antioxidant microelements. However, awareness regarding the importance of this element is scarce. The sources of selenium in food are often characterized with high protein content and include Brazilian nuts (up to 6.86 $\mu g \cdot g^{-1}$), milk products (up to 0.55 $\mu g \cdot g^{-1}$), or beef (up to 0.47 $\mu g \cdot g^{-1}$) (Fairweather-Tait et al. 2010; Ip et al. 2000; Smrkolj et al. 2005). Garlic and onions contain up to 0.50 $\mu g \cdot g^{-1}$ of selenium, making them a good source of this element without the high protein content. Selenium is present in these vegetables in the form of γ-glutamyl-Se-methylselenocysteine or Se-methylselenocysteine (Finley 2005).

Selenium plays an important role in human physiology. It participates in the metabolism, production of hormones, and functionality of the immune system. It is involved in cell growth and in modulating the action of transcription factors and cell signaling systems. It has been reported that appropriate selenium intake prevents diabetes, infertility, cancer, and cardiovascular diseases (Hendrickx et al. 2013). The functioning of the endocrine system and healthy inflammatory responses depend on the correct supply of this element (Ruseva et al. 2013). Furthermore, selenium exhibits protective activity against the toxic effects of metals such as lead, cadmium, arsenic, mercury, and some organic compounds (Rosen and Liu 2009).

Selenium is crucial for the correct functioning of the thyroid gland (along with iodine), as it is involved in the deiodination of thyroxine (T4) to triiodothyronine (T3) through the selenoprotein enzyme – iodothyronine deiodinase. Thus, selenium deficiency results in impaired iodine removal and, in turn, dysfunctions of the thyroid gland (Rosen and Liu 2009). Also, this element is also one of the neurotransmitters crucial for the correct functioning of the central nervous system (Lipinski 2015; Rayman 2012).

Selenoprotein P (SEPP1) is involved in the protection of the organism against free radicals and the damage they cause. Furthermore, SEPP1 is a heavy metal chelator, which forms nontoxic selenium–metal complexes (Pappa et al. 2006; Rayman 2012). Other proteins have also been identified to take part in important biological

processes: selenoprotein W is involved in muscle metabolism (Holben and Smith 1999), selenoprotein S in control of redox balance in cells, and selenoprotein R in probable antioxidant function (Brozmanová et al. 2010; Dokoupilová et al. 2007; Papp et al. 2007; Rosen and Liu 2009).

Selenium is also crucial for immune system regulation (Ruseva et al. 2013). It stimulates the immune system to increase the production of antibodies (e.g., IgG and IgM) and increases the activity of T cells and macrophages (Drutel et al. 2013). It acts in synergy with vitamin E, contributing to the limitation of the aging process and aiding cell regeneration. This microelement has been linked to inhibition of the progression of HIV infection into AIDS (Kamwesiga et al. 2011). Selenium also exhibits antibacterial and antiviral properties and alleviates the course of disease in patients infected with hepatitis, including hepatitis A (HAV) and hepatitis E (HEV) (Szucik et al. 2014), in addition to its protective properties against hepatitis B and C (Rayman 2012).

When supplied in the diet, approximately 85–95% of selenium quantity in food is absorbed in the intestine. However, the bioavailability of selenium differs depending on the form it is supplied in. Organic selenium compounds are absorbed with the extent of 90–95%, while inorganic compounds are only accessible at 10%. Immediately after entering into the bloodstream, selenium is bound by red blood cells, albumins, and globulins of the blood serum and is further transported to the tissues. It can also penetrate the placenta. The highest amounts of this element are found in the skeletal muscles, liver, renal cortex, pancreas, thyroid gland, pituitary gland, and testis, but selenium also accumulates in hair and nails (Kieliszek and Błażejak 2016).

As selenium absorption depends on the form in which it is being consumed, the best way of supplying the correct amount is through a proper diet. Enrichment of food with compounds containing selenium may be conducted through fertilization of soils with selenium compounds in order to obtain plants enriched with this element or through the enrichment of fodder with selenium compounds to obtain, e.g., selenium-rich eggs. Enrichment of soil or fodder represents an indirect method of selenium supplementation. Soil fertilization with selenium compounds is referred to as “biofortification” and presents one of the most efficient methods of resolving the societal issue of selenium deficiency. In contrast, a direct method of dietary supplementation with selenium is based on the intake of dietary supplements which constitute a source of this element (Kieliszek and Błażejak 2013; Mehdi et al. 2013; Ogawa-Wong et al. 2016; Ramos et al. 2010).

As the significance of the concept of functional foods is currently increasing, the use of microorganisms for selenium accumulation has been explored (Kieliszek et al. 2016). Through this method, it is possible to introduce increased amounts of selenium into grain products such as baker’s goods, produced using sourdough with the addition of bacteria and yeasts enriched with selenium (Stabnikova et al. 2008). Selenium yeasts are effective and safe sources of selenium in its most bioavailable form (selenomethionine), and its absorption is enhanced by vitamins present in the yeast biomass (mainly vitamins B and E) (McSheehy et al. 2006). The accumulation and retention of selenium originating from selenium yeast by the human organism

are estimated at between 75% and 90% (Dumont et al. 2006b; Gaikwad and Rajurkar 2016).

Currently, WHO recommends selenium intake of 70 μg day^{-1}, whereas in most European countries the dietary intake ranges between 30 and 50 μg day^{-1} (Kieliszek and Błażejak 2013). Selenium toxicity is estimated to start at an intake level of 400 μg day^{-1}. Consuming high doses of selenium can result in adverse health effects such as hair loss, diarrhea, and emesis (Fordyce 2007).

Protective Properties of Selenium Against Cancer Development

The anticancer properties of selenium are mainly linked to its antioxidant activity. In addition to the free radical scavenging function of selenium, the significant impact of this element on the cytotoxic activity of natural killer (NK) cells can be highlighted in the activity against tumor development (Rayman 2012). Clinical studies have shown that selenium may also protect against the occurrence of prostate, lung, and colorectal cancers (Brozmanová et al. 2010). In turn, decreased amounts of selenium in blood plasma can result in becoming more prone to cell damage and thus to the occurrence of certain cancer diseases. When comparing the individuals suffering from lung, prostate, liver, and stomach cancers to healthy individuals, it has been found that the level of selenium content in plasma of the cancer patients was decreased in comparison to healthy individuals (Wasowicz et al. 2003). Furthermore, a correlation between geographical differences of selenium content in the soil, the element consumption in the diet, and mortality related to cancer of various organs has been reported (Jönsson-Videsäter et al. 2004).

The presence of selenium as a selenocysteine residual in the four active centers of the GSH-Px (glutathione peroxidase enzyme), as one of the principal antioxidant systems in the organism, was identified, which suggests that a deficiency of this element would result in an impairment of the GSH-Px activity. The main action of this enzyme is to catalyze the reduction of the organic and inorganic hydroperoxides produced during the oxidative stress of phospholipids in the membrane and metabolic oxidation of the xenobiotics (Tato Rocha et al. 1994). Efficient removal of the free radicals maintains the integrity of membranes, therefore reducing the risk of cancer and slowing the aging process (Chan et al. 1998; Juhasze-Toth and Csapo 2018). Tissues with high metabolic activity, such as liver, heart, diaphragm, and striated muscle, are highly vulnerable to oxidative stress. This fact explains the selenium deficiency in cases of hemolysis, hepatic necrosis, or impairment of the immune and inflammatory function (Tato Rocha et al. 1994). Therefore, this element has high importance for the prevention of the cellular injury associated with these diseases, because an impairment in the GSH-Px activity cannot be compensated with other non-Se-dependent antioxidant systems.

Based on current research, selenium compounds such as methylselenocysteine (MeSeCys) and γ-glutamyl derivatives were identified as agents exhibiting the highest anticancer activity (Szucik et al. 2014). Se-methylselenocysteine is an active

compound detected in selenium yeast cells. However, it is known that the anticancer effect of methylselenocysteine depends on the expression of β-lyase. It has been observed that MeSeCys supplementation can significantly reduce the incidence of metastasis and tumors in the lungs can result in the reduction of tumor size in mice. Supplementation with a mixture of soy proteins containing high selenium amounts has demonstrated similar results (Chen et al. 2013).

As already mentioned, the protective effects of selenium in the etiology of cancer diseases are related to its activity against oxidative stress on cell membranes as well as the stabilizing effect on DNA and enhancing the cellular immune responses (Kieliszek and Błażejak 2013; Stabnikova et al. 2008). It has also been found that selenium inhibits tumor cell proliferation via the effect exerted on the expression of p53 tumor suppressor gene and Bcl-2 apoptosis suppressor gene (Zablocka and Biernat 2010). However, the anticarcinogenic effect of selenium depends on the chemical form of the element administered, its dosage, and type of agent which induces the development of cancer (Gromadzińska et al. 2008; Venza et al. 2015; Zachara 2015). Although administered selenium compounds exhibit different protective effects, they are metabolized to a final product (methylselenol) which exerts the most potent anticarcinogenic effect (Lavu et al. 2016; Zeng et al. 2009).

According to the Nutritional Prevention of Cancer indications, a dose of 200 μg Se·day^{-1} in the form of selenium yeasts decreases the risk of stomach, colon, rectal, prostate, and lung cancers (Hoffmann and Berry 2008; Lavu et al. 2016). Some reports have been carried out regarding the selenium-enriched broccoli, which was effective in inhibiting the formation of colon tumors (Gong et al. 2012).

Kenfield et al. (2015) have reported that supplementing selenium at doses higher than 140 μg·day^{-1} may increase the risk of death in metastatic prostate cancer patients, which warrants caution in administering the course of treatment for this group (Kenfield et al. 2015). In contrast, the studies presented by Heras et al. (2011) have shown that selenium supplementation at 200 μg·g^{-1} prevents the occurrence of high-grade tumors. The 15 kDa selenoprotein (Sep15) and TrxR1 (thioredoxin reductase 1) proteins are of particular importance (Heras et al. 2011).

Selenium Deficiency

Selenium deficiency is a socially important dietary aspect as it has been linked to some of civilization disease prevention, and a diet lacking in trace amounts of this microelement can cause a long list of health issues. This is especially true for individuals with dietary difficulties, such as phenylketonuria (Alves et al. 2012). As selenoproteins are involved in human metabolism, their deficiency causes degeneration of tissues and organs (Pedrero and Madrid 2009). Most health issues are related to joint and muscle tissues (including cardiovascular and reproductive degeneration), but can also affect the nervous system (Kryczyk and Zagrodzki 2013). Examples of selenium deficiency-related conditions include the Keshan disease (dilated cardiomyopathy) or Kashin–Beck disease (endemic osteoarthropathy)

(Pedrero and Madrid 2009). The Kashin–Beck disease can cause rheumatoid arthritis and growth disorders as well as bone and cartilage damage, leading to necrosis. Other conditions caused by selenium deficiency can include increased risk of asthma (related to impaired activity of glutathione peroxidase) or inducing AIDS progression. Selenium deficiency has also been positively correlated with sudden infant death syndrome (SIDS) (Navarro-Alarcon and López-Martınez 2000; Patelski and Dziekonska 2012; Vinceti et al. 2018).

Toxicity of Selenium

Adverse effects of consuming excessive amounts of selenium have been noticed in the first half of the twentieth century (Khanal and Knight 2010). It has been reported that inorganic forms of selenium have higher toxicity compared to the organic forms (Thiry et al. 2012).

Acute excessive consumption of selenium compounds can lead to various symptoms from the digestive system (including diarrhea and vomiting) and also neurological disorders (Fordyce 2007; Navarro-Alarcon and López-Martınez 2000; Vinceti et al. 2018). Selenosis, chronic selenium overdosing, manifests as hair loss, infertility, digestive and nervous system issues, and thyroid and liver dysfunctions. Certain hematological abnormalities have also been associated with selenium toxicity. Severe selenium toxicity level has been established at 2 μg per g of serum (Khanal and Knight 2010; Li et al. 2012). The toxicity mechanism has been attributed to the DNA damage by the free radicals generated when selenium is overdosed, which corresponds to impaired functions of produced proteins (Letavayová et al. 2008). Symptoms of selenosis have been observed in individuals consuming over 850 ug·day^{-1} (Mistry et al. 2012), and in patients supplementing this microelement at a dose of 600 μg·day^{-1} to aid rheumatoid arthritis, improvements in their treatment were observed with no signs of selenosis (Rayman 2004).

Methods for Determining Selenium Forms in Biological Materials

Speciation analytics is currently one of the most rapidly growing branches of analytical chemistry. It is of high importance because the proper functioning of living organisms is affected by the form in which the element occurs and not its total content. Therefore, it plays an important role in clinical analysis, toxicity determination, quality control of pharmaceuticals and food products, health risk assessment, and study of biochemical cycles of chemical compounds.

Interest in selenium speciation is constantly increasing due to the properties of selenium compounds that can be both toxic and necessary for the proper function-

ing of the human body (Cuderman and Stibilj 2008). Understanding the chemical forms of selenium in plants seems particularly important because of the possibility of using them, after prior enrichment, as a source of dietary supplementation with this element. Selenium-enriched yeast, garlic, onion as well as nuts and mushrooms are used to study selenium speciation.

In the case of speciation analysis, it is important to know the total content of an element and its chemical forms. This approach forces the use of separate procedures that provide an answer regarding the total content of Se and its chemical forms. These procedures differ in terms of sample preparation as well as in the selection of the analytical technique for the determination of the analyte. If the determination of the total selenium content is the goal, it is important to prepare the sample in a form in which the determination is possible. For solid samples, mineralization of the samples is necessary, which leads to its complete dissolution. If the determination of the chemical forms during sample preparation is intended, the employed methods should not affect the chemical forms of the tested element. The extraction procedures are most commonly used for this purpose. The procedure for preparing the material for the Se speciation is shown in Fig. 13.1.

Preparation of a Sample of Plant Material for Speciation Analysis

Mineralization – Determination of the Total Se Content

Determination of the total content of an element in the tested material requires its full mineralization. Food samples are wet mineralized in a closed system, most often in a mixture of HNO_3 and H_2O_2 (Denovics et al. 2002; Hsieh and Jiang 2013; Krawczyk-Coda 2019).

Extraction – Determination of Selenium Chemical Forms

Three extraction procedures are most often used for the preparation of samples in which the chemical forms of selenium are determined: extraction with water (or other solvents), enzymatic hydrolysis, and sequential extraction (Zembrzuska et al. 2014).

Water extraction is a frequently used technique for separating selenium compounds from food, usually with ultrasound-assisted hot water (Gosetti et al. 2007). This technique can be used to separate inorganic forms of selenium and water-soluble organic forms of this element (mainly selenoproteins). Water extraction is not a very efficient technique as only 10% of selenium can be isolated (Wróbel et al. 2004).

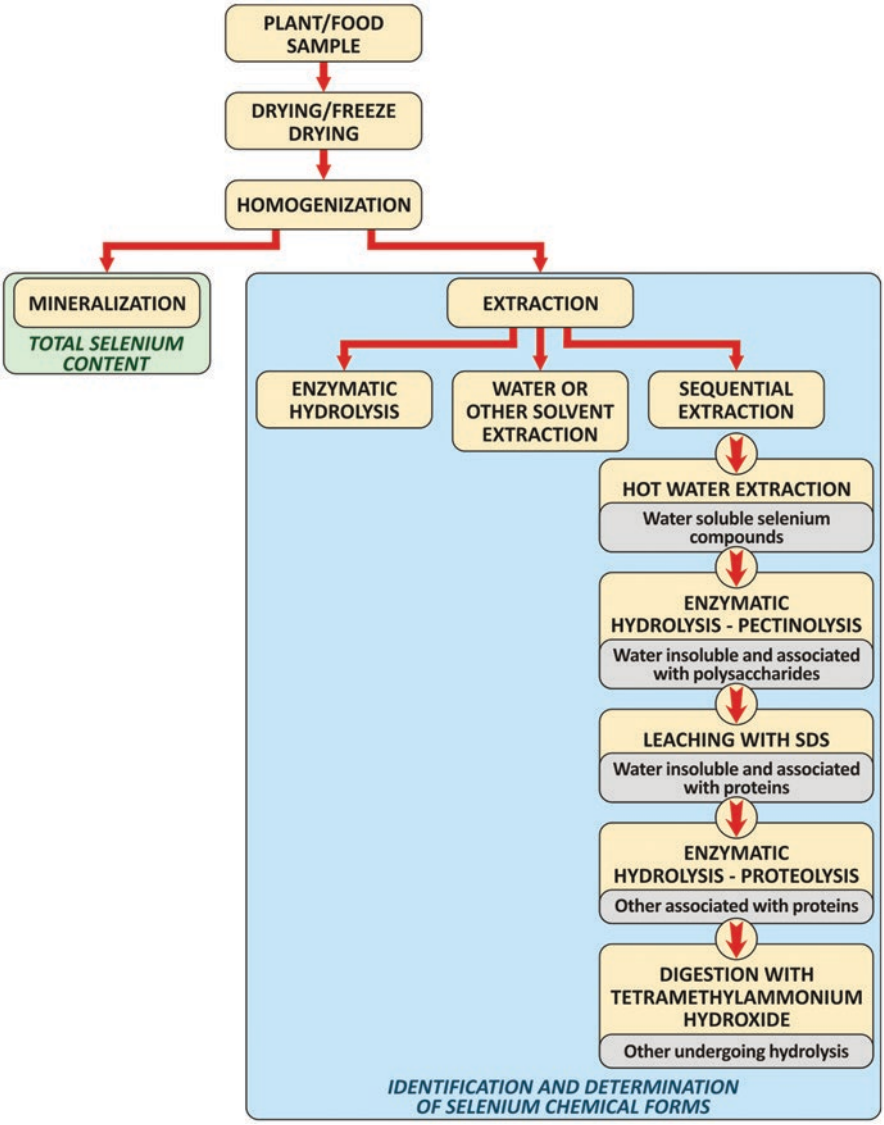

Fig. 13.1 The procedure for preparing the material for the Se speciation

Very often, the extraction of selenium compounds from biological materials is carried out with the use of a surfactant – sodium dodecyl sulfate (SDS), which denatures proteins and is used to elute selenium proteins from animal tissues (Wang et al. 2009; Wróbel et al. 2004).

Aside from water, many solutions can be used in the classic extraction of selenium compounds, such as hydrochloric acid (Cuderman et al. 2010; Montes-Bayon

et al. 2002), tetramethylammonium hydroxide, and methanesulfonic acid (Wang et al. 2009).

In case when selenium is incorporated into protein structures, it is necessary to use enzymatic extraction to isolate selenium compounds, which is based on enzymatic hydrolysis using proteolytic enzymes (Encinar et al. 2003; Vale et al. 2010). Proper selection of enzymes, pH of the hydrolyzed solution, and temperature is important for successful enzymatic extraction (Wang et al. 2009). The specificity of the enzyme plays an important role. Several specific and nonspecific enzymes can be used. Protein-cleaving protease is one of the most commonly used nonspecific enzymes (Casiot et al. 1999; Zou et al. 2018). Goenaga-Infante et al. (2008) used proteases and lipases to determine selenomethionine in pharmaceutical yeast tablets. The authors employed the catalysis for the hydrolysis of ester bonds in water-insoluble lipid substrates. Protease XIV and proteinase K are the enzymes most commonly used to extract selenium from plants, which were used to extract selenium compounds from edible fungi (Stefánka et al. 2001, Denovics et al. 2002). Proteinase K, aminopeptidase, and carboxypeptidase Y have been successfully used to extract selenium compounds from yeast (Wang et al. 2009).

The complementary separation of five basic fractions of selenium compounds from biological materials is possible through the use of sequential extraction. This method, which included five stages, was proposed by Encinar et al. (2003) to isolate the chemical forms of selenium present in yeast. The fractionation resulted in the isolation of selenium compounds: (1) water-soluble (hot water extraction), (2) water-insoluble and associated with polysaccharides (enzymatic hydrolysis – pectinolysis), (3) water-insoluble and associated with proteins (leaching with SDS), (4) other, associated with proteins (enzymatic hydrolysis – proteolysis), and (5) other, undergoing hydrolysis (digestion with tetramethylammonium hydroxide).

Total Selenium Determination

The total, often trace amount of selenium is usually determined by radiometric, electroanalytical, and spectroscopic methods. The most commonly used radiometric method is neutron activation analysis (NAA). This is one of the most sensitive methods of elemental analysis. In this technique, the sample is irradiated in the first stage in a reactor, followed by gamma radiation measurement. The advantages of this technique include no mineralization stage of the sample and no need for sample sacrifice. NAA has been used to determine the total selenium content of dietary supplements (Zembrzuska et al. 2014). Voltammetry is the most commonly used electroanalytical method. There are reports regarding the use of electrochemical methods to determine trace amounts of selenium, e.g., in mushrooms (Piech et al. 2014); however, these methods are very susceptible to matrix effects. Hence, their use in the study of complex environmental samples is very limited. The last group of methods used to determine the total amount of selenium includes spectroscopic methods, which rely on molecular spectrometry and atomic spectrometry. Molecular

spectrometry is based on the relationship between absorbance and the concentration of a colored substance in a solution. A suitable reagent is added to the sample to form colored complexes with the test element. The formation of colored complexes, e.g., with 2,3-diaminonaphthalene, is often used for the determination of selenium. Selenium in feed and cereal mixtures was determined using this method (Ramachandran and Kumar 1996). Due to the selectivity of molecular spectrometry, spectrophotometric methods can be used to selectively determine the content of Se(IV) in the presence of Se(VI). Molecular spectrometry methods are commonly used for the determination of selenium, although their sensitivity is often too low to carry out trace testing.

The following atomic spectrometry methods are widely used for trace amounts of selenium: flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS) (Méndez et al. 2002), high-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GFAAS) (Krawczyk-Coda 2019), atomic fluorescence spectrometry (AFS) (Stefánka et al. 2001), optical emission spectrometry (OES), and mass spectrometry (MS). In spectroscopic techniques, the atomization process occurs under the influence of high flame temperature, electrothermal atomizer, or plasma, most often inductively coupled plasma (ICP). In most cases, the sample is introduced into the atomizer as a solution. Selenium can also be initially separated from the solution by a hydride generation (HG) and introduced into the atomizer in this form with a carrier gas (Tuzen et al. 2007).

The most common and also the most sensitive analytical technique is inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS allows for the determination of very low elemental contents, but in the case of selenium, the possibility of interference should also be taken into account (Gawor et al. 2020; Wysocka et al. 2003).

Determination of Chemical Forms of Selenium

Most analytical methods developed for selenium speciation in food products focus on the determination of selenates(IV), selenates(VI), and selenoproteins (Połeć-Pawlak et al. 2005). Selenium compounds are mainly separated by chromatographic methods (Yu et al. 2019). The research uses various variants of gas and liquid chromatography as well as electrophoretic methods. The selection of the separation technique primarily depends on the chemical and physical properties of the substances present in the sample.

Gas chromatography (GC) is mainly used for the separation of volatile alkyl selenides (Uden et al. 1998) and selenoproteins after the derivatization process (Pelaez et al. 2000). Gas chromatography–mass spectrometry (GC-MS) was used to identify selenium forms in yeast (Iscioglu and Henden 2004). Electron capture detector (ECD) and flame ionization detector (FID) are most often used for the detection of GC-separated forms of selenium.

Nevertheless, selenium compounds are separated by liquid chromatography rather than gas chromatography. The quantitative and qualitative determination of selenium forms described in the literature is mainly based on combined techniques. Among them, high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC-ICP-MS) is the most popular (Auger et al. 2004; Encinar et al. 2003; Gao et al. 2018; Gawor et al. 2020; Lipiec et al. 2010; Tsopelas et al. 2005; Wróbel et al. 2004). Due to the different forms of selenium present in the tested samples, chromatography with different separation mechanisms is used: size-exclusion (Acosta et al. 2018; Moreno et al. 2004; Pyrzyńska and Sentkowska 2019), anion or cation exchange (Cai et al. 1995; Chassaigne et al. 2002), reversed-phase (Do et al. 2001; Gao et al. 2018; Hsieh and Jiang 2013; Tsopelas et al. 2005; Zembrzuska et al. 2014), and hydrophilic interaction (Sentkowska and Pyrzynska 2018).

In addition to HPLC-ICP-MS technique, high-performance liquid chromatography–electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) is increasingly used to identify and quantify selenium compounds (Dumont et al. 2005; Dumont et al. 2006a; Gawor et al. 2020; Gosetti et al. 2007; Vu et al. 2018; Zembrzuska et al. 2014). The application of this technique allows to identify masses of apparent molecular ions of selenium compounds by searching in the mass spectrum of signal groups corresponding to the isotopic composition of selenium, confirm the structure of the determined compounds based on pseudomolecular ion fragmentation, and quantify them using the MRM mode (multiple reaction monitoring). HPLC-ESI-MS/MS was used to determine the chemical forms of selenium in dietary supplements (Dumont et al. 2005; Gosetti et al. 2007; Infante et al. 2005; Zembrzuska et al. 2014) as well as in onions (Sentkowska and Pyrzyńska 2018), yeast (Bierła et al. 2018), and brazil nuts (Dumont et al. 2006a).

The chemical forms of selenium separated on a column are sometimes subjected to atomic absorption spectrometry detection. In this case, the column fractions are first collected, and then the element content is tested. ETAAS (Do et al. 2001) or Hydride Generation Atomic Absorption Spectroscopy (HGAAS) (Marchante-Gayón et al. 1996) are the most frequently used detectors in such cases, due to the low detection limit of selenium and the low volume of solution needed for the determination.

In addition to chromatographic techniques, selenium compounds are also separated by electrophoretic techniques, primarily capillary electrophoresis (CE). The main advantages of this technique include low sample volume, low reagent consumption, short analysis time, and high separation efficiency. The combination of this technique with ICP-MS results in its increasing use for the study of selenium speciation (Kannamkumarath et al. 2002). Moinicou et al. (2002) used this technique to determine chemical forms in yeast. In addition to CE, isotachophoresis (ITP) is another electromigration technique used to determine the selenium (Grass et al. 2002). It was used to determine SeMet and selenocysteine in beer (Zembrzuska and Matusiewicz 2010).

Among all the techniques used for selenium speciation analysis, liquid chromatography combined with mass spectrometry and excitation in inductively coupled

plasma is undoubtedly characterized by the greatest analytical capabilities and the widest application. This technique is characterized by very high sensitivity and allows simultaneous multi-element determinations at the $ng \cdot L^{-1}$. The used analytical procedures provide detailed information regarding the chemical forms of selenium in the samples. Unfortunately, there are no procedures for testing selenium speciation which can be used routinely and widely. Speciation testing requires special treatment for each sample.

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