Selenium enrichment in broccoli sprouts as an initial step in the preparation of Certified Reference Material

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Abstract
Selenium is considered one of the essential elements in animals and humans. The main source of selenium intake for humans is the diet. Selenium deficiency has one of the most concerning issues worldwide. Broccoli sprouts are five-to-six-day old broccoli plants, and they may exhibit stronger chemoprotective effects. Thus, the enrichment of selenium in broccoli sprouts can enhance anticancer properties. As a result of conducted study, four batches of selenium-enriched sprouts have been produced in order to verify the efficiency of selenium uptake by broccoli sprouts under commercial conditions. Sodium selenite solution (10 mg L^{-1}) was used to prepare selenium-enriched broccoli sprouts. Sodium selenite is available for biological uptake by plants. The results might be different while the addition of sodium selenite in each step of the germination process could enhance the concentration of selenium in broccoli sprouts. Therefore, it could be potentially selected as a candidate for certified reference materials.

1. Introduction

The healthy development and growth of life functional organisms depend on acquired nutrients that one of which is called micronutrients. A semi-metal, selenium is among one of the essential micronutrients. Selenium exists with distinct chemical forms in soils such as metal selenides (Se^{2-}), elemental selenium (Se^0) microelement forms (SeO_4^{2-}, SeO_3^{2-}), and organic selenium compounds [1]. The major source of selenium uptake is via diet for humans which includes seafood, vegetables, and meats [2]. The uptake of selenium should be limited in human as it is poisonous when exceeds a minimum requirement of intake [3]. The selenium content of food may have varied based on its geological conditions, such as soil conditions, climate conditions, protein content, and food processing [4]. As implications of selenium deficiency on human health have become understood, interest in the dietary amount of this mineral has grown [5]. Thus, agronomic

selenium biofortification is an interesting research topic, mainly applied by the use of fertilizers enriched with selenium to soils and crops [6–8]. The presented study handles the hydroponic system applied to broccoli sprouts with the addition of sodium selenite mixture.

The study is implemented to evaluate the changes in selenium content in four differently prepared spouts sets which are: i) control sprouts (natural content of selenium); ii) soaking seeds in sodium selenite solution; iii) Se-enriched sprouts (everyday watering with sodium selenite solution); iv) Se-enriched sprouts (everyday watering with sodium selenite solution and soaking seeds in sodium selenite solution). It is also important to be notified that the nutritional bioavailability of selenium from plants is mostly determined by selenium compounds. These palatable sprouts are usually served in a variety of diets, which is beneficial for human health, including anti-cancer protection [9]. Therefore efforts were made to examine the potential certified reference material (CRM) with the use of Se-enriched sprouts for the aim of a study of total selenium content in four different conditioned batches. This investigation was conducted utilizing selenium enhanced sprouts to produce reference materials for the complete analysis of selenium. This is a beginning attempt and its good outcome will allow the development of analytical methods for selenium determination as well as extend the information about the organic form of selenium's beneficial influences on living creatures.

2. Experimental

2.1 Reagents and chemicals

Chemicals and reagents used were in the analytical grade. Deionize water from the Milli-Q water purification system unit (Merck Millipore, Germany) was used in preparations of solutions in case of total Se determination. Germination solutions were developed using sodium selenite dissolution in tap water (Sigma Aldrich, Germany). Sprout seeds were purchased from Hurtownia PRIMA (Piła, Poland). The samples were digested with HNO_3 (Suprapur, 65%, Merck, Darmstadt, Germany). Samples were diluted in deionized water from a Milli-Q for total Se measurement.

2.2 Processing of sprouts

Seed germination was done using tap water and with a solution containing 10 mg L^{-1} of Na_2SeO_3 (as a controlled growing media). The seeds were steeped in the appropriate solutions (250 mL) for 6 hours before germination. The seed had been put in commercial germination bowls for sprouting. Germination was performed at room temperature (20–24 °C) and during the day and night, under the prevalent light and dark conditions during the time of day and night. The entire germination time was around 178 hours, which includes also the time for

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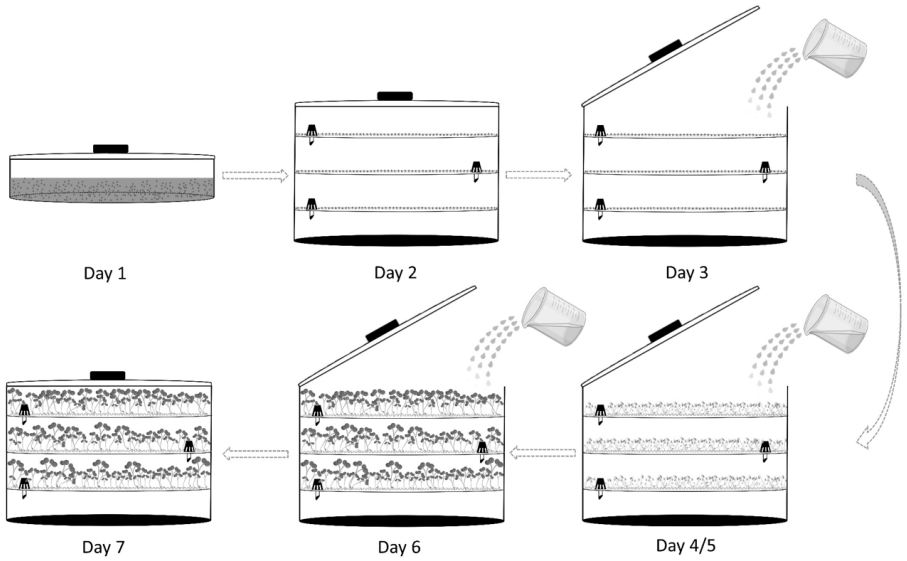

Fig. 1 Preparation steps of broccoli sprouts.

Table 1
The preparation of broccoli sprouts with applicable conditions is discussed.

Time period	Conditions
Day 1	10 g of seeds soaked in solution[a]; stored in dark place
Day 2	Watering twice a day[a]; stored in dark place
Day 3	Watering twice a day[a]; stored in dark place
Day 4/5	Watering twice a day[a]; exposed to indirect sunlight
Day 6	Watering twice a day[a]; exposed to indirect sunlight
Day 7	Cultivation of sprouts

[a] 250ml of corresponding solution.

soaking the seeds in water. The sprouts had been washed twice a day with 250 mL of the appropriate solutions throughout the germination process. All sprouts were rinsed after harvesting with deionized water (2×500 mL) to prevent contamination by the culture solution of the sprout surface. The sprouts were then placed in PE screenable bags and kept in a freezer at –20 °C. All samples were freeze-dried. The dry samples were then ground into a blender purchased from First Austria, transferred into screenable bags, and stored in a freezer at –20 °C. Around 5 g of sample was obtained for broccoli sprouts. The germination process is graphically described in Table 1 and Fig. 1.

Four batches have been generated of Se-enriched sprouts that includes: i) control sprouts; ii) sprouts seeds with the solution addition of 10 mg L^{-1} Na_2SeO_3 into (while soaking seeds) iii) watering the sprouts (with 10 mg L^{-1} Na_2SeO_3); iv) combination of point ii) and iii).

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2.3 Instrumentation

For germination commercial germination bowls (E-TRADE, Pruszków, Poland), equipped with a drain and a plastic lattice at the bottom of the bowl to keep the seeds at constant moisture without overwatering them, were used. The blank was utilized as the sample matrix with target element level beneath the detection limit by microwave plasma atomic emission spectroscopy (MP-AES) analysis. Lyophilization was performed in a Labconoco FreeZone 6 system (USA). For total selenium analysis, samples were digested in a microwave digestion system Muliwave GO (Anton Paar, Austria). The mineralization process was performed in 8 mL of suprapur grade 65% HNO_3 solution (purchased from Merck). The masses of the mineralized samples were around 1 g. Each mineralization process was performed in two cycles to prevent rapid pressure increase. Total selenium determination was performed under MP-AES supplied by Agilent.

Calibration solutions used were prepared with 20% concentrated nitric acid of ICP grade. The wavelength used was 196.026 nm. The standard addition method was performed in means to find out the matrix influence of the material.

3. Results and discussion

All sprout samples were evaluated by the measurement of total selenium content (Table 2). The substantial rise in the total selenium content in samples has been achieved via watering the sprouts with a 10 mg L^{-1} sodium selenite solution. It is worth noting that irrigation with the 10 mg L^{-1} sodium selenite solution should not have negative consequences during sprouts development, such as rotting, molding, and growth suppression.

A preliminary study suggests that matrix highly influences the results of the measurement of selenium in sprouts. Therefore, the standard addition method was used to evaluate the effect of the matrix on the results. The method of standard addition is widely used when the aim is to obtain accurate quantitative results. As an initiative step, calibration solutions were prepared with 20% HNO_3 and deionized water to meet this requirement. The comparison of calibration curves (with the used of 20% HNO_3 and with use of deionized water) is depicted in Fig. 2. Two measurements (sample sprouts, sample sprouts and addition of known standard with concentration of 4 ppm of selenium) were taken of four replicates for each of the batches. The selenium standard was added prior to the mineralization process.

Table 2 shows the selenium concentration in the samples with obtained solution concentration and intensity obtained under MP-AES. It was found that batches iii) and iv) are overaged the concentration as observed in obtained calibration curve in Fig. 2B. It would be notified that CRM (ERM e BC210a, wheat flour, certified total selenium content of 17.23± 0.91 mg kg^{-1} dry weight) was also analyzed for total selenium, and that was digested in the same way as samples. It

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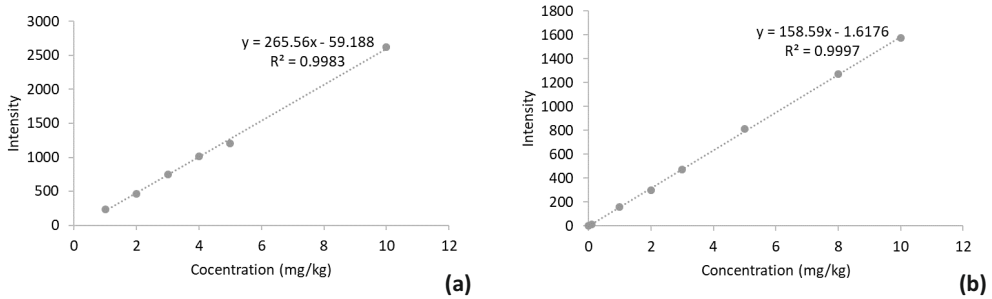

Fig. 2 (a) Calibration standards made from of deionized water. (b) Calibration standards made from addition of 20 % HNO_3 and deionized water.

Table 2
Obtained results under MP-AES with used of standard-addition method.

Batch	Sample	*c*(Se) / ppm	Intensity	Linear regression
i)	Normal sprouts	1.26	199.17	$Y = 133.79\,X + 199.17$
	Normal sprouts + added 4 ppm concentrated Se standard	4.62	734.32	
ii)	Soaked seeds (with 10 mg L^{-1} Na_2SeO_3 before germination)	0.74	116.15	$Y = 175.40\,X + 116.15$
	Soaked seeds (with 10 mg L^{-1} Na_2SeO_3 before germination) + added 4 ppm concentrated Se standard	5.15	817.75	
iii)	Watering the sprouts (with 10 mg L^{-1} Na_2SeO_3)	11.61	1846.86	$Y = 233.62\,X + 1846.9$
	Watering the sprouts (with 10 mg L^{-1} Na_2SeO_3) + added 4 ppm concentrated Se standard	17.49	2781.35	
iv)	Combination of batch ii) and iii)	12.94	2058.47	$Y = 126.28\,X + 2058.5$
	Combination of batch ii) and iii) + added 4 ppm concentrated Se standard	16.12	2563.57	
CRM	ERM-BC210a wheat flour (selenium)	2.76	438.14	$Y = 325.43\,X + 438.14$
	ERM-BC210a wheat flour (selenium) + added 4 ppm concentrated Se standard	10.94	1739.85	

could be notified in Table 2 that this CRM has a huge difference in obtained solution concentration. The availability of CRMs for it is total selenium content is very less in the market.

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4. Conclusions

A preliminary effort was made to evaluate CRMs for the total selenium analysis by MP-AES. The needs are required of CRMs certified for their total selenium content in the matrix form of plant materials. The used standard addition method could be helpful to evaluate the particular type of matrix effect. The matrix effect has a major impact on the obtained results of the samples. The standard addition method allows detecting the mistake in a quality controlled analytical run. An instrumental sensitivity would possibly influence the value of the elemental concentration. To minimize or eliminate the matrix effect, the sample preparation step would be optimized so that samples can be removed from interfering compounds, changing the MP-AES parameters. It would be helpful to solve the problem to obtain reliable results for total selenium content in the samples in the future. It is important to notify that once a kind of CRM will be produced, which would play important role in Quality Assurance and quality control (QA/QC) elements.

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