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Selenium speciation analysis for the investigation of selenium uptake for the hydroponically cultivated garlic samples

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Selenium is a significant nutrient source for humans and plants. Currently, inorganic selenium, including selenate and selenite, is used to cultivate selenium-rich crops to manage people's selenium deficiency problems. Garlic, being a major accumulative plant in the Allium genus, can absorb selenium concentrations beyond 1000 mg/kg when grown in soils rich in selenium. In this study, garlic samples were germinated in a soilless medium and transfered to hydroponic cultivation medium containing three different levels of sodium selenite (Na_2SeO_3). The total amount of selenium in the roots and leaves of lyophilized 150 µM garlic extracts was 43.8 ± 33.2 and 62.7 ± 16.4 mg/kg (n = 4), while the total amount of selenium in the enzyme-extracted leaves and roots was 10.3 ± 2.0 and 10.6 ± 5.9 mg/kg (n = 4). Furthermore, selenium speciation analysis revealed that MeSeCys and SeMet as the main organoselenium compounds in garlic. Additionally, unknown selenium species were detected, indicating the need for further research to identify them.

Keywords *Allium sativium*, Selenium enrichment, Hydroponic cultivation, Garlic

Garlic is a horticulture crop that has been propagated vegetatively for a very long time[1]. Garlic has been utilized since ancient times, not only for enhancing the taste of food, but also for its therapeutic properties[2]. The primary reason for its health benefits is the existence of allicin molecules[3]. Garlic possesses a diverse range of molecules and minerals including carbohydrates, fiber, protein, magnesium, potassium, etc[4]. Organosulfur molecules contribute to the medicinal, culinary and insecticidal properties of garlic. Garlic's organosulfur components contribute to its pungent and astringent odour[5].

Selenium has a close relationship with sulphur. Selenium (Se) can act as a substitute for sulfur (S) in several metabolic processes[6]. Due to the analogous chemical and physical characteristics of selenium and sulfur, selenium can be used as a substitute for sulfur in the metabolic processes of plants. This results in their rivalry in the absorption, transportation, and incorporation in plants. Selenate (SeO_4^{2-}) is chemically reduced using the same assimilation mechanism as sulfate (SO_4^{2-}), and it becomes part of Se-containing amino acids, such as selenocysteine (SeCys) and selenomethionine (SeMet). This mechanism substitutes the amino acids cysteine and methionine, which contain sulfur SeCys and SeMet are presumed to be capable of being integrated into proteins[7].

Selenium is a vital micronutrient that is necessary for human health in small amounts[8]. This element, as an essential dietary element for humans, promotes the improvement of human antioxidant and immunological activities[9]. Hence, a scarcity of selenium in the human body can lead to various health ailments, including stunted growth, cardiovascular disorders, cancer, and numerous other complications. The World Health Organization (WHO) suggests that individuals consume a daily amount of selenium ranging from 55 to 200 mg[10]. The organism can absorb selenium by dietary intake. Selenium is of great importance due to its potential to have both positive and negative effects on human health, with a very small margin between a deficit and toxicity[11]. The toxicity and bioavailability of selenium can be influenced by its chemical form[12]. Each variant of selenium serves a distinct purpose in the metabolic processes of the human body[13]. Selenium exists in both inorganic and organic forms in nature and has four distinct oxidation states. Inorganic selenium compounds include selenite

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(SeO_3^{2-}), selenate (SeO_4^{2-}), selenide (Se^{2-}), and elemental selenium (SeO)[14]. Moreover, selenomethionine (Se-Met) and selenocysteine (Se-Cys) are involved in various biological activities. The amino acids that include selenium are essential components of protein structures and can be found in various food products[15]. Se-amino acids including methyl-selenocysteine (methyl-SeCys), Se-Met and SeCys have much higher antioxidant activity compared to their sulfur compounds[16]. The analogue's biosynthetic activity, sulfur, joins Se to form Se-amino acids (SeCys and SeMet). Proteins that substitute Se-amino acids for S-amino acids (cysteine and methionine) can produce harmful and abnormal proteins. In order to preserve crops, it is essential to determine the optimum levels of Se for biofortification. This will help to keep the Se content of grains or edible parts within safe limits, avoiding any potential toxicity[17]. Plants use the S-assimilation pathway for Se metabolism, which replaces sulfur in important S-amino acids such as cysteine (Cys) and methionine (Met), as well as their associated proteins[18]. Crops are humans' primary source of Se intake. However, the selenium level of crops generally inadequate to supply the selenium needs of humans. Hence, the approach of increasing the selenium level in the edible section of plants, commonly referred to as Se biofortification, provides an effective way for addressing selenium insufficiency According to reports, the use of exogenous Se can not only increase the amount of Se in crops but also inhibit heavy metal absorption by crops from the soil[19].

In literature, garlic has been analyzed qualitatively and quantitatively using anion exchange chromatography (AEC), size exclusion chromatography (SEC), hydride generation atomic fluorescence spectrometry (HG-AFS)[16], atomic absorption spectrometry (AAS)[11], double-channel atomic fluorescence spectrophotometry (AFS)[19] and gas chromatography mass mass spectrometry (GC–MS)[20]. It was typically chosen for IP-RP-HPLC because the system is known to be effective in determining the speciation of selenium in plant samples[21].

The extent of selenium insufficiency in the population of developing nations, including Türkiye, remains uncertain, and there has been limited researches conducted to assess the selenium levels in the edible portions of food crops. In order to increase the Se content while reducing the accumulation of heavy metals in edible parts of garlic, hydroponic cultivation was used, including the sprouting phase. The study's main goal was to conduct in-depth research on the enrichment of hydroponically grown garlic with different concentrations of selenite. The goal of this study was to find out how garlic takes in selenite and what kinds of selenium are being produced in the garlic body.

Materials and method

Instrumentation

The determination of total selenium in all samples were performed by inductively couple plasma tandem mass spectrometry (ICP-MS/MS) model 8800 ICP-QQQ (Agilent Technologies, Japan) and it was hyphenated with Agilent 1100 series HPLC system equipped with an auto sampler and a binary pump for measurement of selenium species in the samples.

The elimination of spectrum interferences caused by the matrix in totally digested samples was accomplished by implementing a mass shift technique using O_2 for the isotopes ^{76}Se, ^{78}Se, and ^{80}Se and simply collision gas of H_2 was utilized in speciation analysis to reduce molecular interferences resulting due to the plasma. All operating parameters for the speciation and total analyses of Se were applied according to the previously conducted study in our research group[22].

Digestion of garlic samples was carried out by using Mars 5 microwave digestion unit (CEM Corporation, USA) in a temperature- and pressure-regulated program.

The Agilent 1100 series HPLC system was utilized for the separation of analytes. The outlet of the column was directly connected to the ICP-MS/MS nebulizer via PEEK tubing. For speciation analysis, a Phenomenex Synergi Hydro-RP C18 column (250 × 4.60 mm, 4 µ) was employed.

Reagents

All reagents used in the study were analytical grade unless otherwise stated. Elga Veolia's PURELAB Flex system was used to produce ultrapure deionized water for mobile phases, as well as all sample and standard preparations.

In the enzymatic digestion of garlic samples, Protease XIV (from *Streptomyces griseus*) and Proteinase K (from Tritirachium album) were employed and both of them were Sigma-Aldrich, Germany. Tris-hydroxymethane (min. 99%, ITW Reagents) was used as a buffer solution (pH 7.5) and the prepared solution was utilized in the enzymatic digestion procedure.

Reverse phase ion pairing chromatography (RP-IP-HPLC) was used for the speciation analysis of selenium and the mobile phase containing 3.0% (v/v) methanol was prepared using heptafluoorobutyric acid (HFBA) which was purchased form Alfa Aesar with 99% purity. In order to obtain 1000 mg/kg Se for selenate and selenite, and 100 mg/kg Se for the other organo-selenium species, appropriate amounts of sodium selenate (Na_2SeO_4) (anhydrous 99.8 + %, Alfa Aesar), sodium selenite (Na_2SeO_3) (Alfa Aesar, 99% min), seleno-DL-cystine $Se(Cys)_2$ (Sigma, USA), seleno-methylselenocysteine (MeSeCys) (95%, Sigma, USA) and selenomethionine (SeMet) (Sigma, USA) were dissolved in deionized water. For speciation analysis, stock solutions were kept at + 4.0 °C, and working solutions were prepared daily by serial dilution using stock solutions.

In the determination of total selenium, sub-boiled HNO_3 produced by Milestone SubPUR sytem from Emsure grade nitric acid (Merck, 65%) and H_2O_2 (Merck, 35%, w/w) were used in sample digestion and also further sample preparation steps. Certified reference material of NIST coded as SRM 3149 were used for plotting calibration curve in total Se determination.

Cultivation of selenium-enriched garlic

The origin of garlic samples used throughout the study was Kastamonu/Türkiye. Garlic cloves were kept in a refrigerator at + 4.0 °C for two weeks. Selenium enrichment studies were carried out by using bulbs of the garlics. Garlic samples were rinsed with deionized water and completely dried at ambient temperature. Then,

Sample code	# Replicate	Fortified by	Concentration, µM
Control	5	–	–
1	4	Se(IV)	50
2	5	Se(IV)	100
3	5	Se(IV)	150

Table 1. Garlic samples in hydroponic cultivation.

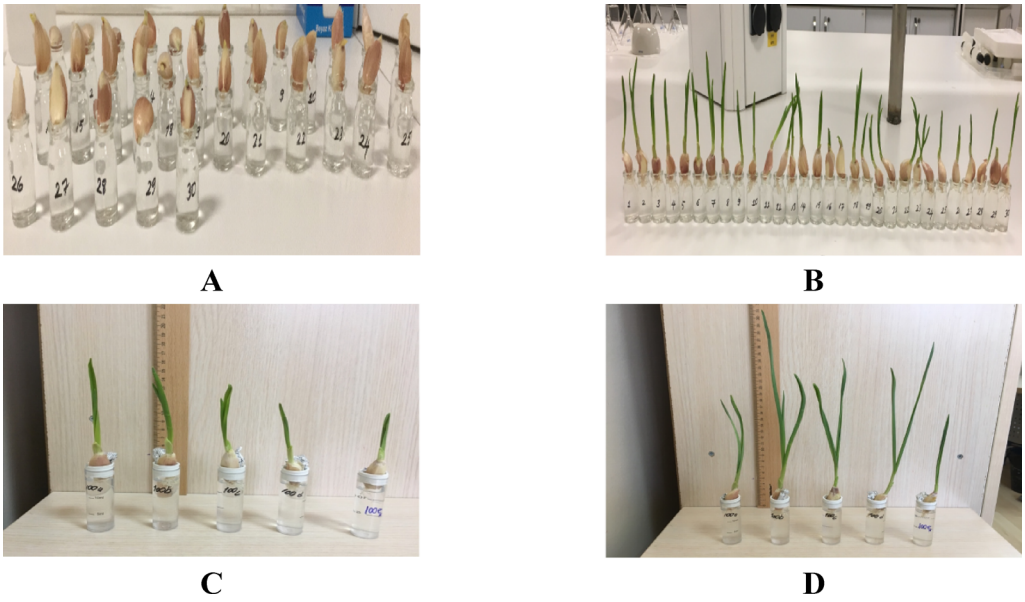


Fig. 1. Sprouting garlic in a hydroponic medium (**A**), Sprouting garlic (4th day) (**B**), Selenium-enrichment process in hydroponic medium (day 0) (**C**), Harvest time for garlic enriched with selenium (9th day) (**D**).

the garlic samples were weighed and sprouted in tap water, which is a soilless medium, at room temperature for 4 days. At the end of the sprouting period, samples were transferred into selenium-enriched nutritional solution which was prepared by adding 0.50 g of plant nutrient into tap water and spiking with an appropriate amount of sodium selenide. The garlic samples were cultivated in three different selenium-enriched media containing 50 µM, 100 µM, and 150 µM sodium selenit (Se(IV)) in 14 g tap water (Table 1). Additionally, control samples were prepared without spiking selenium solution in order to assess the impact of selenium presence for the garlic growth. The samples were kept to be grown in the hydroponic medium for 10 days under regular daylight and room temperature conditions.

The growth of the garlic plants was carefully observed day by day during the cultivation period. Changes in the color, size, or general condition of the plants were visually recorded on a daily basis. The plant's height was also measured each day and recorded. The roots were also examined for signs of yellowing or abnormal development. During the growing period, the nutritional solutions were checked every two days and increased to 14 g to maintain ideal growth conditions for the garlic plants by using tap water. Figure 1 shows a visualization of the experimental setup for the cultivation of garlic. At the end of the 10th day, the hydroponic environment was terminated before yellowing/ripening began. Root and leaves were separated from the harvested plants. The roots and leaves of the garlics were weighed separately; the length of the leaf was measured by using a ruler, and the nutrient medium was diluted from 14 to 50 g with water. The root and leaf of garlic were cut into smaller pieces with a plastic knife to increase the surface area of the samples for more efficient lyophilization. After lyophilization, garlic samples were stored at – 80 °C.

Quantification of total selenium

Total selenium was determined in the root and leaf of lyophilized garlic and in enzymatically digested solutions of them. In addition, nutrient solutions at the end of growth were analyzed for total selenium remaining after cultivation. The digestion procedure which was developed and validated in our previous research study on leek samples was applied for mineralization of the samples described above as the matrixes are quite similar with those previously[22]. The program consists of increase in temperature to 135 °C in 5.0 min, followed by a further increase to 180 °C in 5.0 min, held for 20 min and then cooled to ambient temperature. To digest the samples, approximately 10 mg of lyophilize root and leaf of garlic was weighted and transferred into vessels. Then, 3.0 mL of sub-boiled HNO_3 solution (65% v/v), 1.0 mL of 30% (w/w) H_2O_2, and 1.0 mL of H_2O were added to the vessels. The digested samples were diluted to 10 g with ultrapure deionized water.

The nutritional solutions were also digested to evaluate selenium levels remained. The vessels contained approximately 0.15 mL of nutritional solution samples, all from the control and selenium hydroponic mediums. It was carried out by adding 2.0 mL of sub-boiled HNO_3, 1.0 mL of 30% H_2O_2, and 2.0 mL of H_2O. The digested samples were diluted to 10 g with ultrapure deionized water.

For the analysis of enzymatically extracted root and leaf of garlic solutions, 2.0 mL of solution was weighted into vessels together with 2.0 mL of sub-boiled HNO_3 solution (65% v/v), 1.0 mL of 30% H_2O_2. The digested samples were diluted to 10 g with ultrapure deionized water.

The total selenium amount in all digested samples was determined applying a matrix-matched external calibration method and whole sample and standard preparation steps were performed gravimetrically.

Extraction procedure by the help of enzyme

The enzymatic hydrolysis process was used to release selenoamino acids from proteins. Enzymatic extraction protocol described by Ari et al.[22] was applied to both lyophilized roots and leaves of selenium enriched garlic. 10 mg of garlic samples were mixed with 5.0 mL of an extraction solution made from 5.0 mg of protease XIV and proteinase K prepared in 30 mM Tris–HCl with 1.0 mM $CaCl_2$ (pH: 7.5). Protease was added to hydrolyze the peptide bonds. The solutions were centrifuged and filtered with 0.45 µm filters after shaking at 50 °C for 18 h. The root and leaf of the garlic samples cultivated in 150 µM sodium selenit (Se(IV)) fortified medium were analyzed to demonstrate the representative extraction efficiencies of the proposed method. The evaluation of the extraction yields was carried out by comparing the total selenium content in the enzymatically extracted solutions and in solid samples. The mineralization of these extracted solutions was carried out according to the procedure described in Sect. "Quantification of total selenium". The total selenium in the solution was then quantified using ICP-MS/MS, employing a matrix-matched external calibration technique under optimized tuning parameters.

Selenium speciation analysis

HPLC-ICP-MS/MS was used to perform speciation analysis (inorganic and organic Se) on enzymatically extracted root and leaf of garlic samples. All standards and garlic samples were prepared gravimetrically, and measurements were performed using an external calibration approach.

For speciation analysis, a Phenomenex Synergi Hydro-RP C18 (250 × 4.60 mm, 4µ) column was used. 0.10% (v/v) HFBA, 3.0% (v/v) MeOH, and a pH 6.0 mixture were utilized as a mobile phase with 1.0 mL/min of flow rate, and 20 µL of the injection volume. Optimum instrumental and chromatographic conditions are applied as it was used by Ari et al.[22] and the enzymatically extracted samples were analyzed directly without applying further dilution.

Quantification of $Se(Cys)_2$, SeMet, and MeSeCys in garlic was achieved using the external calibration method. The calibration curves for selenoamino acids were created separately for both the root and leaf of garlic. Calibration plots of both samples were prepared for selenoamino acids in the concentration range of 0.49–100.9 ng/g. For the leave samples, the regression coefficients of the calibration curves were recorded as 0.9997, 0.9998, 0.9979 for $Se(Cys)_2$, MeSeCys, and SeMet, respectively. Similarly, the regression coefficients of the calibration curves for the root samples were calculated as 0.9995, 1.0000, 0.9990 for $Se(Cys)_2$, MeSeCys, and SeMet, respectively (Table 1).

System analytical performances in terms of limit of detection (LOD) and quantification (LOQ) values were tested with 0.50 ng/g standard solution in 3.0% MeOH for sodium selenit (Se(IV)), Se(VI), $Se(Cys)_2$ and SeMet, while it was evaluated using 5.0 ng/g standard solution in 3.0% MeOH for MeSeCys. The following equations were used in calculation of LOD and LOQ.

$$\mathrm{LOQ} = 10\mathrm{sd} + \mathrm{C_{std}}$$
$$\mathrm{LOD} = 3\mathrm{sd} + \mathrm{C_{std}}$$

The calculated LOD and LOQ valued for all selenium species are given in Table 2.

Result and discussion

Hydroponic cultivation of garlic

The average weight of Taşköprü garlic before hydroponics was known; thus, the roots and leaves were evaluated for the effect of selenium fortification on the growth of garlic samples. Average weight for different parts of the garlic samples are given in Table 3. The results showed that inorganic selenide supplementation significantly increased the growth of both the root and leaf of garlic compared to the control plants.

	LOD, ng/g (n = 6)	LOQ, ng/g (n = 6)
Se(IV)	1.04	1.53
Se(VI)	0.76	1.11
$Se_{(MeSeCys)}$	6.6	7.9
$Se_{(SeMet)}$	0.75	0.99
$Se_{(SeCys2)}$	0.74	1.08

Table 2. LOD and LOQ for selenium species in the employed instrumental conditions.

Se (μM) (n = 5)	Before cultivation, (in fresh form)	After cultivation, (in dried form)	
	Root, g	Root, g	Leaf, g
0	2.29 ± 0.67	1.70 ± 0.75	1.52 ± 0.14
*50	1.95 ± 0.43	1.99 ± 0.10	2.08 ± 0.09
100	1.82 ± 0.40	2.03 ± 0.42	2.14 ± 0.16
150	2.02 ± 0.48	1.54 ± 0.07	1.66 ± 0.15

Table 3. The effect of selenium on the growth parameters of garlic. *n = 4.

When only the increase in mass of the roots is evaluated, it is observed that even the dry masses of the plants growing in the medium supplemented with 50 μM and 100 μM sodium selenit (Se(IV)) are higher than their initial wet weights, while the control group remains at a dry mass, which is considered to be equivalent only to water loss[22]. However, roots cultivated in 100 μM selenide fortified medium showed a similar behavior with the control group. Therefore it is concluded that 150 μM sodium selenit (Se(IV)) concentration may be toxic level for garlic plants and lead to reduced growth.

High Se levels can inhibit photosynthesis and nutrient transport. Studies have shown that changes in the mineral balance of plants, specifically the buildup of large amounts of phosphorus, may have caused problems with their growth and a decrease in their biomass when there were high selenium levels in the nutrient solution[23].

Investigation of selenium uptake rate and translocation of selenium in edible parts of garlic samples

One of the advantage of hydroponic cultivation is to be able to calculate the uptake rate of fortified analyte by plants. In this study, theoretically total selenium amount in the nutrient solutions in 50 μM, 100 μM and 150 μM sodium selenit (Se(IV)) were calculated as 3.9 mg/kg, 8.0 mg/kg and 11.9 mg/kg, respectively. The amounts of total selenium in each nutritional solutions after the completion of growing process of the plants were measured by ICP-MS/MS and reported as 0.85 ± 0.26, 2.5 ± 0.8 and 2.6 ± 0.4 mg/kg, respectively. Therefore, the relative average uptake amount were found as 78%, 69% and 78% for each concentration levels, respectively. These data support that garlic samples were efficiently uptaking Se from the solutions during the growth period. As discussed in Sect. "Hydroponic cultivation of garlic", the evaluation of the increase in the masses of samples shows that selenium application alone significantly improved the growth parameters of garlic which is in agreement with previous reports[24]. It is thought that selenium's effect on plant growth stems from its ability to detoxify heavy metals, thereby positively contributing to growth[25].

In order to investigate typical localization of Se, the total selenium amount of the roots and leaves of the garlic samples cultivated in 150 μM selenium enriched growth medium were separately determined by ICP-MS/MS. Total selenium contents of lyophilized root and leaf of garlic samples were found as 43.8 ± 33.2 and 62.7 ± 16.4 mg/kg (n = 4), respectively. According to the data obtained, the difference in Se accumulation ability between roots and leaves of garlic samples seems to be significant considering the standard deviations on the average values. Therefore, it can be concluded that the conversion of Se(IV) into organic forms of selenium takes time in the root and are more likely to be accumulated in leaves rather than the root parts in a given enough time during the cultivation time period. These findings are consistent with those reported in the literature that selenium levels are generally higher in the stems and leaves of most plants than the roots[26].

Investigation of extraction efficiency rate

Total selenium amounts in the extracts were determined as described in Sect. "Quantification of total selenium" by ICP-MS/MS and extraction efficiencies of the applied method in roots and leaves were tested in the highest sodium selenit (Se(IV)) fortified samples which is 150 μM.

The total amount of selenium in the enzyme-extracted leaves and roots was 10.3 ± 2.0 and 10.6 ± 5.9 mg/kg (n = 4), respectively. The average extraction efficiencies measured for root and leaf were calculated as (33 ± 22)% and (17 ± 3)% (n = 4) respectively. These low extraction efficiency values indicate that about 70–80% of total Se in each part of the garlic cannot be extracted. In our previous study[22], the extraction efficiency rates of the proposed extraction protocol for leaves and stems of leek samples (n = 53) were recorded as (70 ± 20)% and (67 ± 19)%, respectively. On the other hand, efficiencies of different enzymatic extractions procedures applied on Allium families were also reported as significantly higher than the observed values in this study[27]. Therefore, the authors are suspecting form that the particle size of the garlic samples is not small enough as in our previous study, hence the extraction efficiency were found to be relatively low compared to previously reported studies.

Selenium speciation analysis

It is reported that, SeMet breaks down into free SeCys via the transsulfuration pathway and enters metabolic reactions[28]. Food sources that are rich in selenium, particularly organic selenium such as selenomethionine (SeMet), can enhance the absorption and use of selenium in the human body[26]. Plants can contain selenium in the form of inorganic or organic molecules, which can become high-molecular-weight compounds such as proteins. Studies have shown that higher plants have a Se pathway. After being absorbed by the roots, Se is metabolized within the plant, resulting in the formation of organoselenium compounds. These compounds can either be stored within the plant or released into the air via volatilization[21]. In Allium plants (including garlic), "Alliins" are the primary source of active compounds and flavors. Many selenides, which are possible breakdown

products of selenium compounds (Se compounds) and are similar to "alliins" (Se-"alliins"), have also been found in selenium-enriched garlic[29].

Although experimental results based on the rate of uptake showed garlic could collect high amounts of selenium, further investigation into the mechanism of selenium translocation in garlic samples was required to determine selenium amount in the edible sections. The nutritional solutions of control garlic samples contained selenium below the detection limits of ICP-MS. Therefore, while the results from the measurements of the selenium-enriched samples were consistently analyzed, speciation analysis was not performed on the control samples whose Se concentrations were expected to be significantly lower than those of the selenium-enriched samples. For the speciation of organoselenium species in the plant, a number of ion-pairing agents are used in a reverse phase high performance liquid chromatgrams (IP-RP-HPLC) coupling with ICP-MS[30]. In this study, the presence of selenium species in different parts of garlic samples were determined by means of IP-RP-HPLC-ICP-MS/MS system using HFBA as ion-pairing agent which has capability of separating more organoselenium species than trifluoroacetic acid (TFA), pentafluoropropanoic acid (PFPA) and triethylammonium acetate (TEAA)[21,31]. Inorganic selenium species are not separated as efficiently as organoselenium species in this separation method as seen Fig. 2. However, it helps to qualitative determination of inorganic selenium species in the extracted samples. As any significant peak belonging to selenite or selenate were detected in the IP-RP-HPLC system, any futher chromatograpic separation such as strong anion exchange HPLC system were not applied in this study.

Through sulfate transporters, selenium is taken up by plant roots and subsequently transported into the xylem of the leaves, where it undergoes sulfur assimilation in chloroplasts to produce SeMet, SeCys, and other organic selenium. Selenite is rapidly transformed into organoselenium compounds in the root, while selenate is carried to the xylem and transferred to the shoot, where it is integrated into organoselenium compounds and transported throughout the plant similarly to S[32]. This chromatographic separation method identified only $Se(Cys)_2$, SeMet, and MeSeCys and unknown species in the roots and leaves of the garlic samples (Fig. 2). All the species detected in garlic samples including the unknown species were consistent with the literature review. Raw garlic was observed to contain different Se species, including MeSeCys, Se(VI), SeMet, and unknown species[16,20]. Garlic (*Allium sativum*), onions (*Allium cepa*), leeks (*Allium ampeloprasum*), and broccoli (*Brassica oleracea*) all have high amount of Se-methylselenocysteine (MeSeCys), which makes up about half of the total Se[33]. Our study also demonstrated that the most dominant species present in garlic are MeSeCys and SeMet. Moreover, it should be clearly stated that the recorded peak areas for unknown peaks in both leaves and root of garlic samples are as detectable as SeMeCys and SeMet in all samples. As summarized in Table 4, the percentage distribution of selenium species (SeMet and MeSeCys) in leaves and roots of garlic samples across different sodium selenite Se(IV) treatments reveals distinct tissue-specific metabolic responses to selenium exposure. In general, the selenium level in the stems and leaves of most plants is greater when compared to the roots[34].

In leaves, SeMet is the dominant form at lower sodium selenite Se(IV) concentrations (50–100 µM), accounting for approximately 60–62% of the total measured selenium species. However, at 150 µM sodium selenite Se(IV), the proportion shifts markedly in favor of MeSeCys (57.3%), suggesting a metabolic shift toward MeSeCys biosynthesis at higher selenium levels. This shift may reflect an adaptive detoxification mechanism, as MeSeCys is known to be a less toxic, non-proteinogenic form of selenium that can be stored or excreted more safely than SeMet.

In roots, the SeMet and MeSeCys proportions remain more balanced across all sodium selenite Se(IV) concentrations, with a nearly 1:1 ratio at 100 and 150 µM. This indicates that roots exhibit a relatively stable

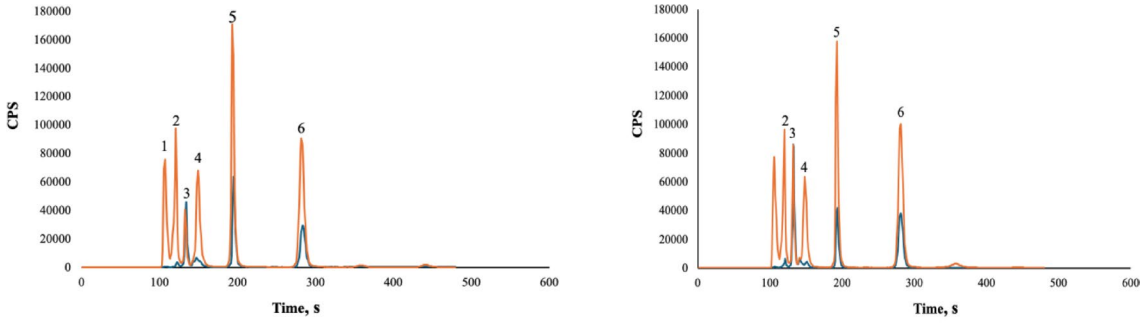


Fig. 2. Chromatograms obtained by IP-RP-HPLC-ICP-MS/MS (**A**)—20 ng/ mL spiked into enzymatically extracted garlic (root) and—enzymatically extracted garlic (root) samples supplemented by 150 µM sodium selenite (Se(IV)); (1) Se (VI), (2) Se(IV), (3) Unknown, (4) $Se(Cys)_2$, (5) MeSeCys, (6) SeMet. (**B**)—20 ng/ mL spiked into enzymatically extracted garlic (leaf) and—enzymatically extracted garlic (leaf) samples supplemented by 150 µM sodium selenite (Se(IV)); (1) Se (VI), (2) Se(IV), (3) Unknown, (4) $Se(Cys)_2$, (5) MeSeCys, (6) SeMet.

	Leaves		Root	
	$Se_{(MeSeCys)}$ mg/kg	$Se_{(SeMet)}$ mg/kg	$Se_{(MeSeCys)}$ mg/kg	$Se_{(SeMet)}$ mg/kg
50 µM Se(IV), (n = 4)	1.8 ± 0.9	2.7 ± 1.1	1.4 ± 0.6	2.1 ± 1.1
100 µM Se(IV), (n = 5)	3.8 ± 4.3	6.2 ± 3.3	4.4 ± 1.9	4.4 ± 3.1
150 µM Se(IV), (n = 5)	8.6 ± 5.9	6.4 ± 1.4	3.7 ± 1.5	3.9 ± 2.5

Table 4. Summary of average concentration values of organoselenium species quantified in leaves and roots.

selenium speciation profile, potentially due to a more passive role in selenium metabolism compared to leaves or due to the early-stage processing of Se before translocation to aerial parts. Overall, the data suggest that selenium speciation is dose- and tissue-dependent. While SeMet dominates at lower concentrations, higher selenium exposure induces a shift toward MeSeCys, especially in leaves, likely as a protective response to mitigate Se toxicity. SeMet is the primary selenocompound found in cereal grains, grassland legumes, and soybeans. On the other hand, MeSeCys is the primary selenocompound found in Se-enriched cruciferae plants, including garlic, onions, sprouts, broccoli, and wild leeks[35]. MeSeCys has been previously reported in the literature as a selenium compound with notable anticancer potential[33] which was also found as one of the most dominant organoselenium species detected in the garlic samples in this study.

In the literature, it is shown that plants accumulate selenite and transform it into organic selenium species via the metabolism pathway[33]. In this study, the pathway was used to investigate selenium accumulation and transformation in the garlic samples. While uptake selenium was transformed into organoselenium species, all the species were not qualitatively and quantitatively determined. Although the existing separation method has been commonly used in the literature, further studies are needed to be conducted to determine the observed unknown species which contributed significantly to the Se signal. In the literature, unknown peaks in garlic were identified by accurate mass determination, further confirming the identities of the structurally characterized Se species[30,36].

Conclusion

Environmental and genetic factors can influence a plant's growth. This study investigated the effect of selenium enrichment, which affects plant metabolism, and selenium supplementation on garlic growth, uptake, transport, extraction efficiency, and differentiation. Hydroponic garlic can effectively absorb selenite, but its growth may be inhibited when the concentration exceeds 100 µM. This study also revealed that garlic plants took the selenium efficiently, with the uptake rates of 78% at 50 µM and 69% at 100 µM sodium selenit (Se(IV)). Selenium is more likely to accumulate in garlic leaves, indicating a time-dependent conversion of inorganic Se into organic forms, aligning with earlier studies. Selenium speciation analysis confirmed that MeSeCys and SeMet are the dominant organoselenium compounds present in garlic, with differing concentrations in roots and leaves. These results suggest that selenium accumulation and transformation into organoselenium compounds are influenced by factors such as the plant's growth stage, the specific organ, and selenium concentration. Moreover, the study detected unknown selenium species, as indicated by notable peaks in chromatographic analysis, suggesting further research is needed to identify and characterize these unidentified compounds. The enzymatic extraction method used in this study is relatively inefficient and may affect the representativeness of morphological analysis. Morphological analysis identified MeSeCys and SeMet as the main organic selenium forms, and there were also significant unknown selenium species, which are worthy of further study. Future studies should employ advance techniques such as ESI–MS/MS or LC-HRMS to elucidate the identity of the unknown chromatographic peaks.

Data availability

Data will be made available with reasonable request from corresponding author.

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