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The Influence of Thiol Addition on Selenium Stability and Antioxidant Activity of Beetroot Juice

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Abstract: Determination of selenium species in food samples causes problems due to their possible oxidation and degradation. In this study, the stability of selenium compounds present in beetroot juices after addition of recommended thiols, such as ditiotreitol (DDT) or β-mercaptoethanol (βME), was evaluated. More total selenium was found in homemade freshly squeezed beetroot juice than in that sold as an organic juice. Furthermore, Se(IV) and selenomethionine oxide (SeMetO) were the main Se species found in both juices. During storage at different temperatures, the concentrations of selenomethionine (SeMet) and Se-methylselenocysteine (MetSeCys) decreased, while Se(IV) and SeMetO contents increased. The addition of DDT or βME, regardless their concentration, did not increase the concentration of SeMet at the expense of SeMetO decrease, as it was expected based on the literature data. Moreover, the used thiols affected the quantification of other selenium species, particularly Se(IV). The purchased organic beetroot juice showed higher ability to scavenge free radicals when it was stored at 4 °C.

Keywords: selenium species; stability; antioxidant activity; beetroot juice

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1. Introduction

The interest in selenium compounds grew as we learned more about its role in the human body. Today, it is widely recognized that this element is an essential part of antioxidant enzymes, and has important role in scavenging free radicals, regulating their content during biochemical reactions and protecting cells from oxidative stress [1–3]. Long-term oxidative stress can cause the development of many chronic diseases, such as cancer, diabetes, cardiovascular disease, and immune dysfunction. Moreover, the reduction of reactive oxygen and nitrogen species is important for the prevention of aging-related diseases. From the other side, prolonged intake of selenium in high doses can lead to health problems, such as hair loss, muscle tremors, stomach upset, kidney failure, and enhanced risk of type 2 diabetes [4].

The recommended dietary allowance of selenium is in the range of 50–70 μg per day for adults, and it is based on the concentration of selenium in plasma or serum as well as the activity of glutathione peroxidases (selenium-dependent antioxidant enzymes) in plasma [5,6]. The soils in Europe are relatively poor in Se, thus, selenium consumed with the diet does not always provide sufficient intakes of this element. For this reason, Se-enriched foods and different supplements have been proposed to solve these problems [7,8]. One of the selenium sources in the diet may be the products of plant origin. Some plants could accumulate inorganic selenium species from soil and transform them into active selenoamino acids, which are more bioavailable and less toxic than Se inorganic forms [9,10]. Selenomethionine (SeMet) and Se-methylselenocysteine (MeSeCys) are the main selenoamino acids naturally occurring in dietary sources. It was reported that these compounds display strong antioxidant activities and cytotoxicity against varied cancer cells [11].

As various selenium chemical forms have different biological utilizations, toxicities, and nutrition values, detailed knowledge of the contents of individual selenium compounds is important to evaluate their impact on human health. Moreover, some regulations require specification of the active ingredients of foodstuffs or supplements, thus, speciation analysis is necessary [12–15]. Liquid chromatography and electrophoretic separation, both coupled to inductively coupled plasma mass spectrometry detection, are the most frequently used techniques for selenium speciation analysis [14,15]. However, analysis of selenium species in food samples and supplements creates difficulties due to the possible instability of Se compounds in extraction media and during storage [16–22]. The biggest problems presented in the literature data are connected with the oxidation of selenomethionine during the preparation of various kinds of samples [17,18,21–23]. Amako et al. reported that selenomethionine oxide (SeMetO) had started to appear as soon as selenised yeast, a very popular Se supplement, was dried and tableted [24]. The presence of SeMetO was found even in soil samples [25]. Probably a sample matrix has a great impact on the stability of selenium species. It was found that acidification of a sample significantly increases the stability of inorganic selenium compounds, but such treatment caused a drastic degradation of SeMet in the extract of dietary supplements based on Se-enriched yeast [18]. One of the methods applied for preventing SeMet oxidation is the addition of thiols, e.g., ditiotreitol (DDT) or β-mercaptoethanol (βME) [21,25]. However, as we reported earlier, single addition of these thiols was not enough to prevent the Se oxidation process in tea extracts, but it was proved that the state of equilibrium is established between the oxidized and reduced form of SeMet [22].

In this work, we present expanded studies on the stability of selenium compounds as a function of DTT and βME concentration on the example of beetroot juice samples. In recent years, this vegetable has become more popular due to its possible health benefits for humans. Beetroot plants have the ability to accumulate nitrates, which lower blood pressure to improve athletic performance [26]. Additionally, they reduce inflammation and protect cells from damage. Beetroot also contains a high concentration of various phytochemicals, such as betalains, carotenoids, and polyphenols, as well as vitamins (especially folate) and essential minerals [26,27]. Their chemical composition depends on the variety of beetroot, used processing methods, and storage conditions [28]. However, there is a lack of information about the content of selenium in beetroots and beet products, except for our previous work [29] and the report by Giri et al. [30]. This work includes the study of the stability of selenium compounds present in beetroot juices as a function of thiol addition and storage conditions. The antioxidant activity of juice samples stored at different temperatures using DPPH radical assay was also established. From the consumer's point of view, understanding the proper storage conditions can help fresh beetroot juices to maintain their health-promoting properties.

2. Materials and Methods

2.1. Reagents

All the commercial standards of selenium, namely sodium selenite (Se(IV)), selenomethionine (SeMet), and Se-methylselenocysteine (MeSeCys), as well as dithiotreitol (DTT) and β-mercaptoethanol (βME), were purchased from Merck-Sigma (Steinheim, Germany). Selenomethionine oxide (SeMetO) was achieved by the addition of 1 mL of 30% (v/v) H_2O_2 to 10 mL of a 0.1 mol L^{-1} HCl solution with selenomethionine (1 mg Se L^{-1}) which was left overnight in the dark [19]. Ultrapure water was obtained using a MILI-Q system (Millipore Burlington, MA, USA). Methanol of the HPLC grade used as a mobile phase component in HPLC analysis was also purchased from Merck (Darmstadt, Germany).

2.2. Samples

Organic beetroot juice (Biofood) was purchased in the local grocery store and, according to the information available on the juice label, it was produced from organic beets cultivated in Ciechocin (Kuyavia, Poland). The beets needed to prepare the squeezed juice

were purchased in the same store and also came from organic farming. The procedure for obtaining the juice included washing and peeling 500 g of beets and then blending them in a juicer. Then, the juice was filtered through an 8-folded cheese cloth to eliminate particulates. No extra spices were added to the juice, and no extraction method was applied in order to simulate real conditions of juice consumption. The samples are stored at different temperatures (20 °C, 4 °C, and −19 °C) at various periods of time without light. Before HPLC analysis, all of the samples were filtered using a 0.22 µm PTFE filter (Millipore).

Determination of total selenium content in these samples was carried out by microwave digestion in the mixture of concentrated HNO_3 and $HClO_4$ acids (4:1, v/v), similar to that used in our previous studies [18].

2.3. Chromatographic Analysis

Chromatographic analysis of selenium species was performed using a Shimadzu LC system coupled to an 8030 triple quadrupole mass spectrometer (Kyoto, Japan). The ESI ionization was used, operating in positive (organic selenium species) or negative (inorganic selenium species) mode. For chromatographic separation of selenium species, a sulfobetaine stationary phase was used, commercially available as ZIC-HILIC column (100 × 2.1, 3 µm) from Merck. The separation was carried out using isocratic elution mode, with 85% content of methanol (MeOH) and water and delivered at 0.2 mL min^{-1} [22]. The same stationary phase was used for the separation of polyphenolic compounds, however, in this case a gradient elution was used, as follows: 0–4 min 98% B, 6–7 min 90% B, 8–8.4 min 80% B, 8.4–12 min 50% B, and 13–10 min 98% B, where B is acetonitrile (ACN) and A is water. Both chromatographic separations were conducted at 30 °C. Selenium compounds, as well as polyphenols, were identified based of the knowledge of their fragmentation patterns and retention times obtained using commercial standards. Quantification of compounds was carried out based on the calibration curves obtained in SRM mode.

2.4. Antioxidant Activity by DPPH Assay

The radical scavenging activity was examined in vitro using DPPH assay. A total of 0.1 mL of a sample was added to 2.4 mL of DPPH solution (9×10^{-5} mol L^{-1} in methanol). After 30 min, the decrease in the absorbance was measured at 518 nm. Each sample was analyzed in triplicate and the results are expressed as a Trolox equivalent (TRE) in µM. Spectrophotometric measurements were performed using a Parkin Elmer (Waltham, MA, USA) Lambda 20 UV–VIS spectrophotometer. Data were processed with WinLab software version 2.85.04.

2.5. Statistical Analysis

Results are expressed as mean ± standard deviation (at least three replicates). Analysis of variance and significant differences among means and correlation analysis were performed with one-way ANOVA. The significance level was based on a confidence level of 95.0%. The experimental data were analyzed using the Analiza Statystyczna program (available at http://beta.chem.uw.edu.pl/stat/ (accessed on 19 September 2022)).

3. Results and Discussion

3.1. Total Se Content and Its Species in Beetroot Juices

The concentration of total selenium and its individual species in the studied beetroot juices are presented in Table 1.

Table 1. Selenium species in beetroot juices (in mg L^{-1}).

Juice	Total Se	MeSeCys	SeMet	SeMetO	Se(IV)
Purchased organic	1.85 ± 0.003 [a]	0.29 ± 0.004 [a]	0.11 ± 0.005 [a]	0.94 ± 0.008 [a]	0.45 ± 0.007 [a]
Homemade squeezed	3.60 ± 0.004 [b]	0.28 ± 0.003 [a]	0.08 ± 0.001 [b]	1.44 ± 0.006 [b]	1.73 ± 0.008 [b]

Results are expressed as mean ± standard deviation (n = 3). Different letters in each column indicate a difference at a significance level of p = 0.05.

Homemade freshly squeezed beetroot juice contains much more selenium compared to the sold organic juice. Inorganic Se(IV), followed by selenomethionine oxide, are the main Se species in this homemade juice from raw beets. Additionally, SeMetO also dominates, after Se(IV), in purchased beetroot juice, while the concentrations of SeMet and MeSeCys did not differ significantly from each other in the studied juices. The root vegetable non-accumulators (which accumulate less than 100 mg Se/kg of their dry weight) are capable of absorbing inorganic selenium species present in the soil, converting at least a portion of Se into other compounds and storing selenium in their tissues both as inorganic and organic forms [31,32]. For organic juice, the sum of determined selenium species obtained by chromatographic analysis amounted to 97% and 98% of total selenium for organic and homemade beetroot juices, respectively.

The literature data proposed different mechanisms as well as various final products, which may be involved in the SeMet transformation cycle [33–38]. Selenomethionine oxide and methyloselenone are the possible reaction products according to Larsen et al. [33]. In another work, the first oxidation product of SeMet in the initial phase of the reaction was described as selenomethionine selenoxide, which was then converted via the deaminated selenoxide and further oxidized to Se(IV) and methaneseleninic acid [36]. Other studies showed the presence of selenium heterocycles, called selenuranes, [37] as well as the formation of SeMetO and then S-(methylseleno) cysteine as the next product of SeMet degradation [23]. The formed selenomethionine selenoxide can be reduced back by the addition of thiols and ascorbic acid [38] or glutathione [39], while Bierla et al. reported that the SeMetO, originally present in a sample, cannot be converted back to SeMet [40]. The wide range of SeMet to SeMetO ratios observed during selenium speciation analysis in different samples probably depends on storage time and conditions, as well as on their matrices.

3.2. Stability of Selenium Species in Beetroot Juices

Two series of both beetroot juices under study were prepared, without and with the addition of reductive thiols, DDT and βME, respectively. They were stored at different temperatures (20 °C, 4 °C, and −19 °C) for various periods of time, and then the concentrations of selenium species were determined. The obtained results as a function of time storage for samples without thiol addition are illustrated in Figure 1. Detailed concentrations of selenium species in these samples are presented in Table S1 in the Supplementary Materials.

The concentration of SeMet in homemade prepared juice without thiol addition was rapidly decreased, regardless of the storage temperature and, after 2 days of storage, its concentration was below the LOD value. In contrast, the concentrations of SeMetO were increased in this juice samples stored at all temperatures in the following order: 20 °C < −19 °C < 4 °C after 8 days. In similar samples of organic beetroot juice, a fast decrease of SeMet concentration was also observed, although after 6 days of storage at 20 °C still retains about 45% of its initial concentration.

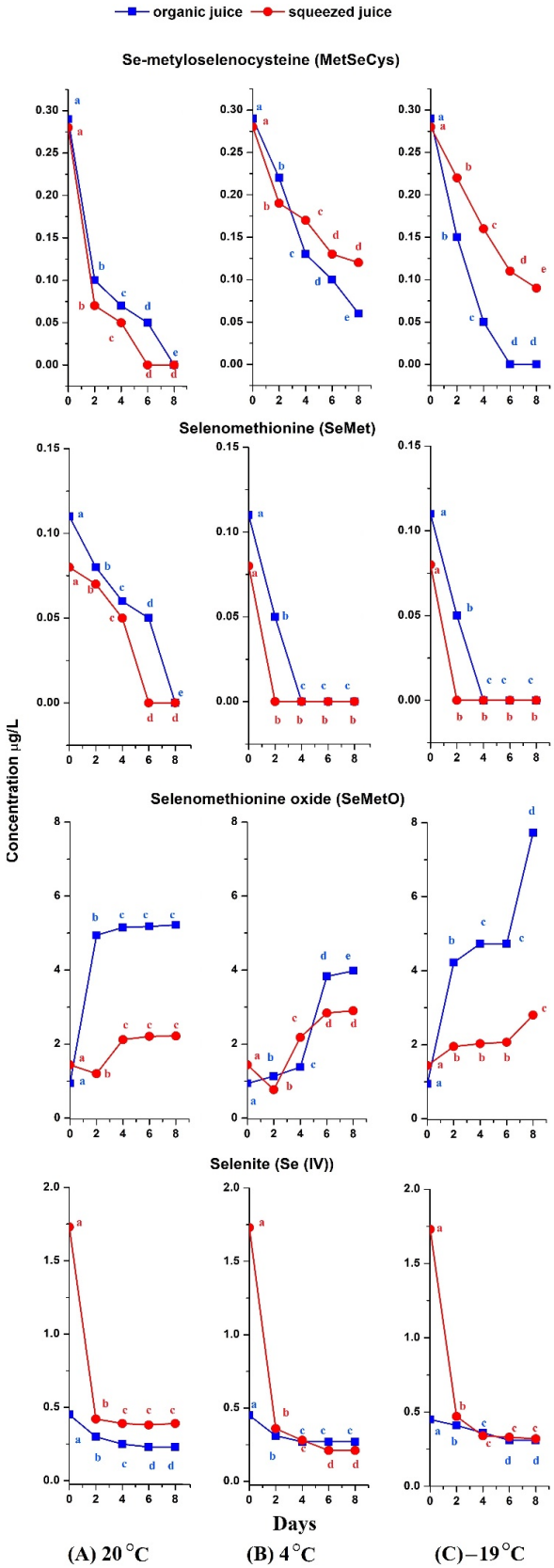
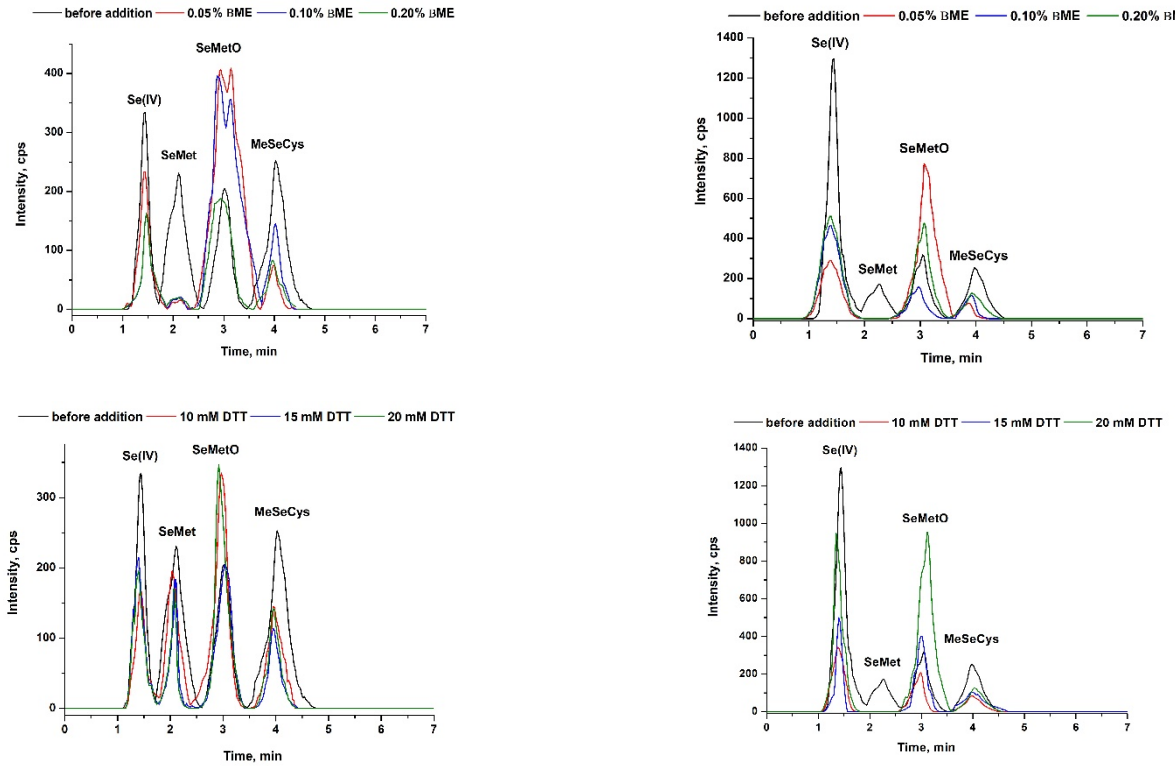


Figure 1. The changes in concentrations of selenium species in purchased organic and homemade squeezed beetroot juice without the addition of thiols stored at (**A**) 20 °C, (**B**) 4 °C, and (**C**) −19 °C as a function of storage time. Different letters in each point of the graph indicate a difference at a significance level of $p = 0.05$, showed a decrease in Se(IV) response after storage and that only 7% of this selenium form was present in the water extract after 4 days at 4 °C [18].

The MetSeCys was also highly unstable, and the biggest losses of this Se compound were observed for squeezed juice, particularly when stored at room temperature. The concentrations of Se(IV) in both juices under study increased with time storage, especially when they were stored in the fridge and freezer for longer than 4 days. This form of selenium may be one of the postulated products of the oxidation of SeMet (as well as other selenoamino acids) [36,41]. Moreover, it has been shown that SeMet oxidation was more favourable as the temperatures decreased, which might affect the stability of Se-enriched food on storage [17,35]. From the other side, speciation analysis of Se in buckwheat showed a decrease in Se(IV) response after storage and that only 7% of this selenium form was present in the water extract after 4 days at 4 °C [18].

Figure 2 shows the chromatograms of selenium species in organic and squeezed beetroot juices after the addition of different concentrations of DTT or βME at 20 °C. The results for other temperatures of storage are included in Table S2. The presence of reductive thiols, according to the literature data [21,39], should decrease the concentrations of SeMetO reversing the oxidation process of selenomethionine. However, in both juice samples, SeMet concentrations decreased below the limit of detection (0.05 μg L^{-1}) after the addition of thiols, regardless of their concentration. On the contrary, SeMetO concentration increased, as if the oxidation process of selenomethionine occurs after the addition of thiols. The only exception is the result for organic beetroot juice with DTT addition, where this loss of SeMet was only small and not significantly different in Tukey's test ($p < 0.05$) for all used thiol additions. The increase in SeMetO peaks was also observed in the samples with thiols; however, there are no clear trends regarding the type of thiol and its concentration. In the chromatogram for organic juice in the presence of two βME concentrations, peak splitting for SeMetO can be observed (Figure 2).



(**a**) Organic beetroot juice (**b**) Squeezed beetroot juice

Figure 2. Chromatograms of selenium species in (**a**) organic and (**b**) squeezed beetroot juices after the addition of different concentration of DTT or βME thiols. Storage temperature 20 °C. The ZIC-HILIC column with eluent—MeOH/H_2O (85/15) (v/v). The data and the relevant statistical analysis are presented in Table S1.

This bimodal phenomenon, while other peaks in the samples did not show similar distortion, may be due to the presence of two SeMetO diastereomers [37,42]. The MeSeCys peaks were also decreased after the addition of thiols, but not so much as in the case of selenomethionine. Its losses were bigger in organic beetroot juices than in squeezed juice samples. The degradation of MeSeCys was also observed in the standard solutions [15]. A significant decrease in Se(IV) concentration was most likely caused by their reduction by thiols to elemental selenium. Among various approaches for the synthesis of selenium nanoparticles, the methods involving naturally occurring substances in plant extracts are preferred, as they could act both as reducing agents and stabilizers [43]. Thus, quantification of Se(IV) and SeMet/SeMetO in the presence of the recommended thiols (added for the reduction of SeMetO) is not advisable.

Generally, the addition of DDT or βME does not increase the concentration of selenomethionine with a possible decrease in SeMetO concentration, as was expected. Moreover, the interpretation of the chromatographic profiles becomes more complicated. To the best of our knowledge, there is no exact explanation regarding the effect of the used thiols, especially in the presence of a sample matrix. Betalains and polyphenols, the main compounds present in beetroots, also exhibit reducing properties [44]. However, their mutual interactions with selenium species require further research. The interactions of polyphenols with other food components are of great interest considering the beneficial effects on human health [45,46]. These interactions, which may be synergistic or antagonistic, can take place during the processing and storage of food, affecting their stability, enzymatic activity, and nutritional value.

3.3. Antioxidant Activity of Beetroot Juices

The antioxidant activities of the studied beetroot juices during storage at different temperatures were determined based on their scavenging effect on the 1,1-diphenyl- 2-picryl-hydrazyl (DPPH) radical. The obtained results, expressed as the Trolox equivalent, are presented in Figure 3. The purchased organic beetroot juice showed a higher ability to scavenge free radicals when stored at 4 °C. Homemade juice prepared from raw vegetables remarkably lost its antioxidant properties regardless of the storage conditions. It is worth mentioning that the presence of DDT or βME did not affect the results in DPPH assay for both beetroot juices just after the preparation of these samples. The specific antioxidant activity values of beetroot juices after the addition of DTT or βME and storage at different temperatures are shown in Table S3. During the prolonged time of storage, as various reactions may take place with numerous final products, the antioxidant activities of juices were changed, but in ambiguous ways. Pavlović et al. also observed a slow decrease in antioxidative activity in beetroot juice (obtained by maceration of roots in a centrifugal juicer, and then pasteurised for 15 min at 90 °C), which was stored in dark at 4 °C [47].

The antioxidative capacity of beet juices depends mainly on the concentration of phenolic compounds, most of which belong to the betalain family (several betacyanins and betaxanthins) that serve as colour pigments and possess high antioxidant potential in-vitro [29,48,49]. Beet juice is also rich in vitamin C, a strong antioxidant, that is often used as a natural preservative [50]. It was reported that organic cropping of red beetroot plants increased the content of ascorbic acid by 23.3% and antioxidant activity by 30.3% in comparison with conventional cultivation [51].

Table 2 shows the main compounds determined in the studied beetroot juices. The higher concentrations of betanin, a major red pigment, as well as vulgaxantin I, the predominant yellow pigment from a group of betaxanthins, were found in purchased organic beetroot juice. However, there is no information on what amount of vegetable that juice was prepared from. The concentrations of these pigments decreased during storage of juices, and their faster loss was observed in homemade juice and at a higher temperature of storage. The highest percentage reduction in betanin content was measured in the squeezed juice sample (27%) after storage at 20 °C, which could be linked to the low thermal stability of betalains [52]. The action of endogenous enzymes (β-glucosidases, polyphenol oxidases,

peroxidases), present in red beets, may also account for betalain degradation and colour losses [44,52].

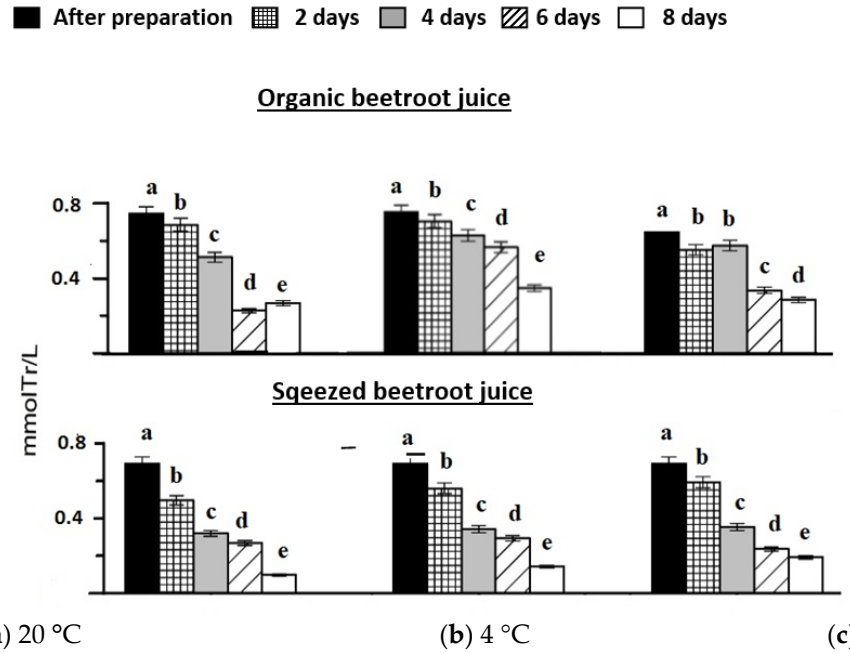


Figure 3. The antioxidant activities of beetroot juices stored at different temperatures, (**a**) 20 °C; (**b**) 4 °C, and (**c**) −19 °C, using DPPH assay. Different letters indicate a difference at a significance level of $p = 0.05$.

Table 2. The concentrations of the main compounds in the studied beetroot juices during their storage at different temperatures (in mg L^{-1}).

Organic Beetroot Juice							
Compound		**−19 °C**		**4 °C**		**20 °C**	
	Initial	**4 Days**	**8 Days**	**4 Days**	**8 Days**	**4 Days**	**8 Days**
Betanin	763 ± 25 ^{a}	740 ± 32 ^{a}	675 ± 31 ^{b}	725 ± 30 ^{c}	650 ± 25 ^{c}	690 ± 27 ^{b}	656 ± 19 ^{c}
Vulgaxantin I	413 ± 17 ^{a}	393 ± 17 ^{b}	360 ± 15 ^{c}	371 ± 18 ^{c}	341 ± 13 ^{d}	350 ± 15 ^{d}	312 ± 12 ^{e}
pHBA	9.00 ± 0.347 ^{a}	4.33 ± 0.210 ^{b}	4.10 ± 0.181 ^{b}	3.91 ± 0.150 ^{b}	<LOD	3.92 ± 0.111 ^{b}	<LOD
Gallic acid	0.20 ± 0.01 ^{a}	0.11 ± 0.01 ^{b}	0.12 ± 0.01 ^{b}	0.10 ± 0.02 ^{b}	0.060 ± 0.01 ^{c}	0.10 ± 0.02 ^{b}	0.05 ± 0.01 ^{c}
Ferulic acid	16.2 ± 0.60 ^{a}	0.27 ± 0.02 ^{b}	0.23 ± 0.01 ^{c}	0.30 ± 0.03 ^{d}	<LOD	0.25 ± 0.02 ^{c}	0.21 ± 0.01 ^{d}
EGCG	26.1 ± 0.904 ^{a}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Epicatechin	<LOD	<LOD	0.12 ± 0.01 ^{a}	<LOD	0.058 ± 0.002 ^{b}	<LOD	<LOD
Catechin	<LOD	<LOD	23.3 ± 0.902 ^{a}	<LOD	17.57 ± 0.731 ^{b}	<LOD	<LOD
Squeezed beetroot juice							
Compound		**−19 °C**		**4 °C**		**20 °C**	
	Initial	**4 days**	**8 days**	**4 days**	**8 days**	**4 days**	**8 days**
Betanin	563 ± 20 ^{a}	506 ± 23 ^{b}	473 ± 19 ^{c}	485 ± 21 ^{c}	455 ± 20 ^{d}	450 ± 20 ^{d}	410 ± 13 ^{e}
Vulgaxantin I	327 ± 14 ^{a}	300 ± 10 ^{b}	270 ± 12 ^{c}	273 ± 11 ^{c}	240 ± 10 ^{d}	230 ±12 ^{d}	195 ± 8 ^{e}
pHBA	<LOD	<LOD	<LOD	<LOD	<LOD	1.47 ± 0.060 ^{a}	<LOD
Gallic acid	0.14 ± 0.04 ^{a}	0.05 ± 0.002 ^{b}	<LOD	0.06 ± 0.001 ^{b}	<LOD	0.05 ± 0.002 ^{b}	<LOD
Ferulic acid	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
EGCG	20.4 ± 0.930 ^{a}	<LOD	<LOD	0.33 ± 0.02 ^{b}	0.13 ± 0.01 ^{c}	<LOD	<LOD
Epicatechin	<LOD	0.83 ± 0.03 ^{a}	0.93 ± 0.05 ^{b}	1.58 ± 0.08 ^{c}	1.67 ± 0.08 ^{d}	1.08 ± 0.05 ^{e}	1.23 ± 0.04 ^{f}
Catechin	<LOD	<LOD	<LOD	<LOD	<LOD	6.25 ± 0.20 ^{a}	6.30 ± 0.15 ^{a}

Results are expressed as mean ± standard deviation ($n = 3$). Different letters in each row indicate a difference at a significance level of $p = 0.05$.

Apart from betalains, small amounts of polyphenolic acids, such as *p*-hydroxybenzoic (pHA), gallic, and ferulic acids have been identified, particularly in organic beetroot juice.

From the group of flavonoids, various catechins were found. Epigallocatechin-3-gallate (EGCG) in beetroot juices was very unstable and completely disappeared after 2 days, regardless of the storage temperature. The EGCG is thermally sensitive and undergoes epimerization to generate gallic acid, catechin, or epicatechin during the processing of foods, and its stability is influenced by pH value and food components [53]. In contrast to other published data [54,55], we did not detect other flavonols (such as kaempferol, quercetin, or apigenin), most probably due to their low abundance in the studied juices or their occurrence in conjugated forms, which are not extracted just by the water. Generally, the observed decrease in antioxidant activity of studied beetroot juices may be caused by the instability and degradation of selenium species, as well as other compounds with antioxidant properties.

Regarding selenium species, their antioxidant activity in the DPPH assay decreases in the following order: MeSeCys > SeMet ≈ Se(VI) > Se(IV), and for all species, the values were lower than for α-tocopherol at the same concentration [56]. Selenomethionine and SeMetO exhibited similar antioxidant properties in DPPH assay. The reducing capacity determined by the CUPRAC method (cupric reducing antioxidant capacity) increases as follows: Se(IV) < SeMetCys < Se(VI) < SeMe, indicating that selenomethionine may be easily oxidized.

4. Conclusions

Beetroot juice, in addition to its health-beneficial compounds, such as betalains, inorganic nitrates, vitamins, and minerals, also contains selenium. This element is an essential part of several antioxidant enzymes and protects cells from oxidative stress. The knowledge of the content of individual Se species is important to evaluate their potential for human health, as various Se forms have different biological utilizations, toxicities, and nutrition values. However, analysis of selenium species in food samples and supplements creates difficulties due to the possible instability of its compounds during storage and extraction, which may result in misidentifications. Particularly, problems with the oxidation of selenomethionine during the preparation of various kinds of samples have been frequently reported. The results from our experiments with the addition of DDT or βME did not provide clear results. The expected decrease in SeMetO concentration with the simultaneous increase in SeMet signal did not take place in beetroot juice samples. Moreover, the applied reductive thiols affect the quantification of other selenium species, particularly Se(IV), which is not mentioned in the literature data.

Since the samples of the examined juices showed different selenium profiles during storage (with and without the addition of thiols), it appears that changes in speciation have occurred, either from lengthy storage under different conditions or by interaction with the sample matrix, as well as by linked processes. It is very likely that the formed selenoxide may be an equivalent to selenomethionine in biological action, and is metabolized in the same way. However, more studies need to be performed to clear this problem. Thus, the inclusion of SeMetO in the quantitative determination of SeMet content could be reasonable.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app122412634/s1, Table S1: The concentrations of selenium species in beetroot juices without addition of thiols during their storage at different temperatures (in mg L^{-1}); Table S2: The concentrations of selenium species in beetroot juices with the addition different concentrations of DTT and βME during their storage in different temperatures (in mg L^{-1}); Table S3: The antioxidant activity of beetroot juices in DPPH assay after addition of DTT and ME and stored at different temperatures.

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Conflicts of Interest: The authors declare no conflict of interest.

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