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Does selenium fortification of kale and kohlrabi sprouts change significantly their biochemical and cytotoxic properties?

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Highlights

- Biochemical and cytotoxic properties of Brassica Se-fortified sprouts were examined.
- About 10 % of selenium was incorporated into the sprouts as selenomethionine.
- Selenium differently stimulated the production of phenolic acids in the sprouts.
- Cytotoxic potency of sprouts regardless the Se fortification was observed.
- Se-enriched sprouts are attractive candidates for functional food.

Abstract

Background

The sprouts of Brassica vegetables are known from their nutritional and chemopreventive values. Moreover, sprouts fortification with some trace elements, like selenium, may increase their importance in human diet. Thus, the aim of our study was to examine if selenium enrichment of kale and kohlrabi sprouts may

influence their biochemical properties (phenolic acids and L-tryptophan content, antioxidant potential) or cytotoxic activity. Additional aim of the study was to evaluate the profile of selenium compounds and to describe the multidimensional interactions between the mentioned parameters.

Methods

Selenium content in the sprouts was evaluated by double-channel atomic fluorescence spectrometer AFS-230 with the flow hydride-generation system. Separation of selenium species in water soluble fraction was performed by size-exclusion LC-ICP-MS. The identification and quantification of phenolic acids and L-tryptophan was performed by HPLC. For antioxidant activity DPPH and FRAP methods were used. Cytotoxic activity of the sprouts extracts on a panel of human metastatic carcinoma cells was evaluated by MTT test.

Results

Selenium content in the fortified sprouts was several orders of magnitude higher than in the unfortified ones. Only small percentage of supplemented selenium (ca. 10 %) was incorporated into the sprouts as seleno-L-methionine, while the other detected selenium species remained unidentified. Selenium fortification differently stimulated the production of phenolic acids (sinapic, chlorogenic, isochlorogenic and caffeic acid) in the tested sprouts, depending on the particular species, selenium dose and the investigated compound. PCA analysis revealed strong correlation between antioxidant parameters and phenolic acids and L-tryptophan, while Se correlated only with caffeic acid. The sprouts extracts (≥ 1 mg/mL) showed cytotoxic potency to all the studied cancer cell lines (SW480, SW620, HepG2, SiHa), regardless the selenium supplementation.

Conclusion

Se-fortified kale and kohlrabi sprouts are good candidates for functional food ingredients. Moreover, these results indicate that the sprouts enriched with sodium selenite show higher nutritional value, without significant changes in their cytotoxic activity.

- **Previous** article in issue
- **Next** article in issue

Keywords

Selenium

Sprouts

Phenolic acids

Speciation analysis

Cytotoxic activity

1. Introduction

The sprouts of various vegetables from Brassica family have not only appreciable nutritional values, but are also cheap and easy to grow. They are rich in polyphenols, glucosinolates and vitamins, but also a number of trace elements, which support plant growth rate and exert beneficial influence on human health, when consumed [1]. Kale (*Brassica oleracea* L. var. *sabellica* L.), together with its sprouts, are known from their positive effect on cancer and cardiovascular diseases prevention [2,3]. Kohlrabi (*Brassica oleracea* convar. *acephala* var. *gongylodes*) is one of the underestimated but perspective Brassica vegetable, with only few data on the properties of its sprouts [4].

The sprouts, as extra source of trace elements, may play advantageous role for people, particularly those suffering from malnutrition or living in the areas with some elements deficiency (e.g. iodine, zinc). The problem concerns also the deficiency of selenium (Se), an important trace element [5]. Selenium fortified sprouts may deliver some benefits. Except from being the additional source of selenium, the enriched sprouts have greater antioxidant, anti-inflammatory and anti-tumour properties [1]. The selenium accumulation in plant organism can cause its growth by stimulating the seeds germination and thus promoting the production of secondary metabolites, which trigger multiple biological effects [1,6]. Avila et al. [7] indicated Brassica crops as a special group of plants with high ability to synthesize and accumulate monomethylated forms of selenium, such as Se-methylselenocysteine (SeMSCys). It is also suggested that selenium fortification of Brassica seeds may increase their chemopreventive activity, besides being a dietary source of a number of other bioactive compounds [8,9]. In addition, it is worth to mention that the extra amount of selenium protects plant organisms against the influence of drought [10], cold [11], UV radiation [12], heavy metals and salts [13].

Although the methods for enhancing Se levels in animal products (eggs, milk, meat) are well documented, the information on Se fortification of plants is still incomplete. Thus, the aim of our work was to examine biochemical properties of kale and kohlrabi sprouts – new example of plant functional foods, supplemented with different doses of selenium and to investigate the multidimensional interactions between the parameters studied. The different biochemical properties of Se-fortified new Brassica sprouts, being the subject of the present study, have not been investigated previously. Another objective of the study was to determine and compare cytotoxic activity of Se-fortified or unfortified sprouts. As the therapeutic window of selenium is rather narrow [14] we also tried to carefully select the level of supplementation in new sprouts, which may be implemented to the daily menu in the future. Additionally, speciation analysis was applied to evaluate the profile of selenium compounds.

2. Material and methods

2.1. Plant material and growth conditions for sprouts

Kale seeds (*Brassica oleracea* L. var. *sabellica* L.) were collected from the plants harvested in north-west Poland in 2016. Voucher specimen was deposited in the Department of Food Chemistry and Nutrition, Faculty of Pharmacy, Jagiellonian University Medical College (No#BOS/PP/PL1044). Kohlrabi seeds (*Brassica oleracea* convar. *acephala* var. *gongylodes*) were collected from the plants harvested in north-west Poland in 2012. Voucher specimen was deposited in the Legutko Seed Testing Laboratory (LE11/1135/12) and also in the Department of Food Chemistry and Nutrition, Faculty of Pharmacy, Jagiellonian University Medical College (No#BOAG/PP/PL 1043).

The seeds were immersed in commercially available mineral water (controls) or in the same water containing three different selenium concentrations (10 mg/L, 15 mg/L, 30 mg/L; selenium as sodium selenite) for 3 h. Then the seeds were grown for 6 days after seeding in EQMM Easy Green Microfarm in temperature of 24 ± 2 °C, at natural light conditions, being watered 3 times a day with mineral water or Se-supplemented water (selenium concentrations as above).

The extracts for the evaluation of phenolic acids content, antioxidant parameters and for cellular studies were prepared according to the protocols described in details in our previous paper [15].

2.2. Selenium content and speciation analysis

Selenium content in the sprouts was evaluated by means of the double-channel atomic fluorescence spectrometer AFS-230 with the flow hydride-generation system (Haiguang Instrument, Beijing, China), as described earlier [15,16].

Speciation analysis was applied to kale and kohlrabi sprouts grown for 6 days and watered either with mineral water or with selenium solution in mineral water (intermediate concentration, i.e. 15 mg/L). Digi-Prep system from SCP Science (Quebec, Canada) was used for digestion of samples. Two types of centrifuge: Jouan CR3i Model (Thermo Fisher Scientific, Waltham, MS) and a model 5415R 72 (Eppendorf, Hamburg, Germany) were used for various sample volumes. Separation of selenium species present in water soluble fraction was performed by means of size-exclusion LC-ICP-MS [17]. For incubation of the samples an ultrasonic bath Branson 2510 from Emerson (Danbury, CT), and a thermostatic shaking water bath OLS 200 from Grant (Cambridge, UK) were used. pH was adjusted with pH-213 pH meter from Hanna Instruments (Woonsocket, RI). The HPLC system used was: Agilent 1200 HPLC from Agilent Technologies (Tokyo, Japan), with the chromatographic columns size-exclusion Superdex-75 (10 × 300 mm x 13 µm) from GE Healthcare (Little Chalfont, UK) and anion exchange PRP x100 (4.1 × 250 mm x 5 µm) from Hamilton Robotics (Reno, NV).

2.3. Water extraction and size-exclusion HPLC fractionation of selenium

5 mL of water was added to 0.2 g of each sample; the mixture was incubated in ultrasonic bath (1 h) and centrifuged. The supernatant was eluted from a size exclusion HPLC Superdex-75 column (exclusion limit of 100 kDa and the optimal separation range of 3–70 kDa) with 100 mM ammonium acetate buffer (pH 7.5) at a flow rate of 0.7 mL/min. Selenium isotopes: ⁷⁶Se, ⁷⁷Se, ⁷⁸Se, ⁸⁰Se and ⁸²Se were monitored by ICP-MS. The ICP mass spectrometer used was an Agilent 7700x (Agilent, Tokyo, Japan) equipped with a collision cell; hydrogen was used as a collision gas to remove interferences; the correctness of the Se isotopic pattern was verified daily before the analysis. The instrumental LOD was 10 ppt. The observed isotopic pattern matched the theoretical one which indicated the absence of interference.

2.4. Selenomethionine and Se-methylselenocysteine determination

0.2 g of each sample was incubated with 5 mL of a protease solution (20 mg protease in 30 mM TRIS buffer, pH 7.5). Three consecutive incubations (17 h at 37 °C) with fresh portions of the enzyme solution were carried out. After each incubation, the sample was centrifuged (10 min at 2500 rpm) and the supernatant was transferred to a separate vial to which 5 μ L of β -mercaptoethanol was added. The 3 supernatants were pooled together and analysed by anion-exchange chromatography (Hamilton PRP-X100 column) coupled to ICP-MS. Separation was performed using gradient elution with buffers A (20 mM acetic acid/10 mM triethylamine) and B (200 mM acetic acid/100 mM triethylamine) according to the following program: 0–5 min: 0 % B, 5–30min: 0–100 % B, 30–40 min: 100 % B, 40–41: 100–0 % B, 41–45 min: 0 % B. The quantification was carried out using standard additions of SeMet and SeMetSeCys at three levels. The samples were analysed in duplicate. Analytical blanks and SELM-1 standard reference material certified for SeMet content (National Research Council Canada) were analysed in parallel. The observed isotopic pattern matched the theoretical one which indicated the absence of interference.

Analytical reagent grade chemicals and LC–MS grade solvents were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France) unless stated otherwise. Ultra-pure water (18 M Ω cm) obtained with a MiliQ system (Millipore, Bedford, MA) was used throughout. Hydrogen peroxide from Fisher Scientific (Hampton, NH) and nitric acid (INSTRA-ANALYZED) from Baker (Central Valley, PA) were used for sample digestion. Protease used was of proteomics grade. A standard solution of 1000 mg/L selenium was purchased from Plasma CAL standards (Teddington, UK). Standard stock solutions of SeMet and MetSeCys were prepared by dissolving powders obtained from Sigma Aldrich, and appropriately diluting with water.

2.5. Identification and quantification of phenolic acids

The identification and quantification of phenolic acids was performed by means of HPLC, as described previously [18], with Dionex HPLC system with PDA 100 UV-VIS detector, Hypersil Gold (C-18) column (5 μ m, 250 \times 4.6 mm, Thermo EC) mobile phase 1 % formic acid in water (A) and acetonitrile (B) in gradient mode 5–60 % B in 60 min, at a flow-rate of 1 mL/min. Identification of the compounds was done by comparing their retention time with those of the standards. Quantification of the predominant compounds was carried out by measuring the peak area with regards

to the appropriate standard curve prepared for the range of standards concentrations from 0.0625 to 1 mg/mL. All analyses were performed in triplicate and the mean value was expressed in mg/100 g dry weight (d.w.).

2.6. L-tryptophan determination

For L-tryptophan determination, the samples (0.5 g each) of air dried material were extracted with methanol as described previously [19]. The quantitative analysis of L-tryptophan were performed using a modified HPLC method [20]. Details of the parameters and conditions of chromatographic separation were reported previously [21]. Reference materials were from Sigma-Aldrich Co. Quantification was carried out based on the method of calibration curve.

2.7. Antioxidant activity and total phenolic compounds determination

In order to determine the antioxidant activity of the tested materials, the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH assay) was done according to Brand-Williams et al. method [22] with some modifications [23], while ferric reducing antioxidant power assay (FRAP) was conducted according to Benzie and Strain [24], with further modifications [23]. Total extractable polyphenols concentrations (TPC, considered also as Total Phenolic Compounds) were measured using Folin-Ciocalteu method with some modifications [23]. For DPPH, FRAP, and TPC assays Synergy-2 (BioTek, USA) reader with syringe rapid dispensers was used.

2.8. Cell cultures

Cytotoxic activity was evaluated using a panel of human metastatic carcinoma cells of various tissue origin: SW 480 Duke's type B primary colorectal adenocarcinoma (ATCC, CCL-228); SW 620 Duke's type C colorectal adenocarcinoma, derived from lymph node as metastatic site (ATCC, CCL-227); HepG2 hepatocellular carcinoma (ATCC, HB-8065) and SiHa cervical carcinoma cells (ATCC, HTB-35). SW 480 and SW 620 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Lonza, USA), HepG2 and SiHa cell lines were maintained in Eagle's Minimum Essential Medium (EMEM; Lonza, USA) as described earlier [25]. All media were supplemented with 10 % v/v FBS (Eurex Sp. z o.o., Poland) and antibiotic solution. The cells were grown in standard conditions (37 °C in a humidified atmosphere of 5 % CO₂). The morphology of cell culture was investigated by an inverted light

microscope (Olympus IX-70 microscope with fluorescence, Olympus, Hamburg, Germany).

2.9. Viability assay

The effect of the sprouts towards human carcinoma cells was determined using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay (Sigma-Aldrich, Germany). Briefly, 200 μ L of cell suspension was placed in 96-well microtiter plate for 24 h. Then the medium in each well was replaced with a new one, containing also the adequate volume of a diluted stock solution of the tested extracts or the solvent, dimethylsulfoxide (DMSO). The cells cultured in the medium alone were positive control (100 % of viability). The range of the tested concentrations of the extracts was: 0.1 mg/mL–5 mg/mL. After 24 h of incubation the medium was removed, MTT solution was added, the obtained formazan was dissolved in DMSO and the absorbance was measured at 550 nm (the reference wavelength was 690 nm) using microplate reader [26].

2.10. Statistical approach

ANOVA analysis with post-hoc Tukey or least significant difference tests were applied. A probability level of $p < 0.05$ was considered to be statistically significant. Principal Component Analysis model (PCA) was used in order to describe the correlation structure between the parameters and to reveal possible differences between the individual samples of the sprouts. Correlation weights (which express the strength of bivariate associations) for the pairs of the parameters in PCA model were calculated using the software delivered by MP System Co. (Chrzanów, Poland). The parameters with large weights (>0.3) in PCA model were assumed to be correlated. The Pearson correlation coefficients for pairs of the parameters were also calculated to compare the results obtained by different statistical methodologies. Statistical analyses were carried out using packages: STATISTICA v. 12 (Statsoft, Tulsa, OK, USA) and SIMCA-P v.9 (Umetrics, Umeå, Sweden).

3. Results

Selenium exposure did not cause any apparent sprouts growth inhibition even at exposure levels of 30 mg Se/L. Selenium content in the sprouts was shown in [Fig. 1](#). Water soluble selenium fraction was 35.1 % and 22.2 % of total selenium in kale and

kohlrabi fortified sprouts, respectively. Selenium as selenomethionine (SeMet) was present in both kale (7.3 ± 0.6 % of total Se) and kohlrabi (11.6 ± 0.6 % of total Se) sprouts. The results of the separation of selenium species present in water soluble fraction in kohlrabi and kale sprouts were illustrated in Fig. 2. The additional experiment carried out by the HPLC technique with higher resolution power (hypercarb HPLC column) confirmed the presence of additional three low molecular weight (<1000 Da) selenium species (one major and two minor) in the investigated samples (Fig. 2C and D), which were neither SeMet, nor Se (IV) and Se (VI).

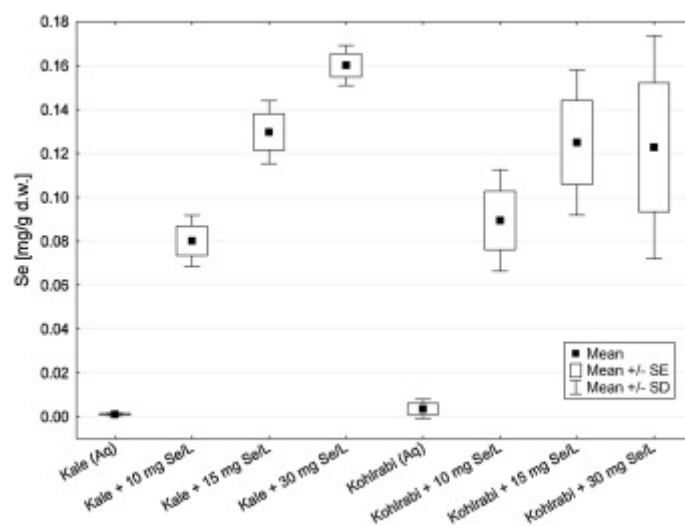


Fig. 1. Selenium concentration in kale and kohlrabi sprouts.

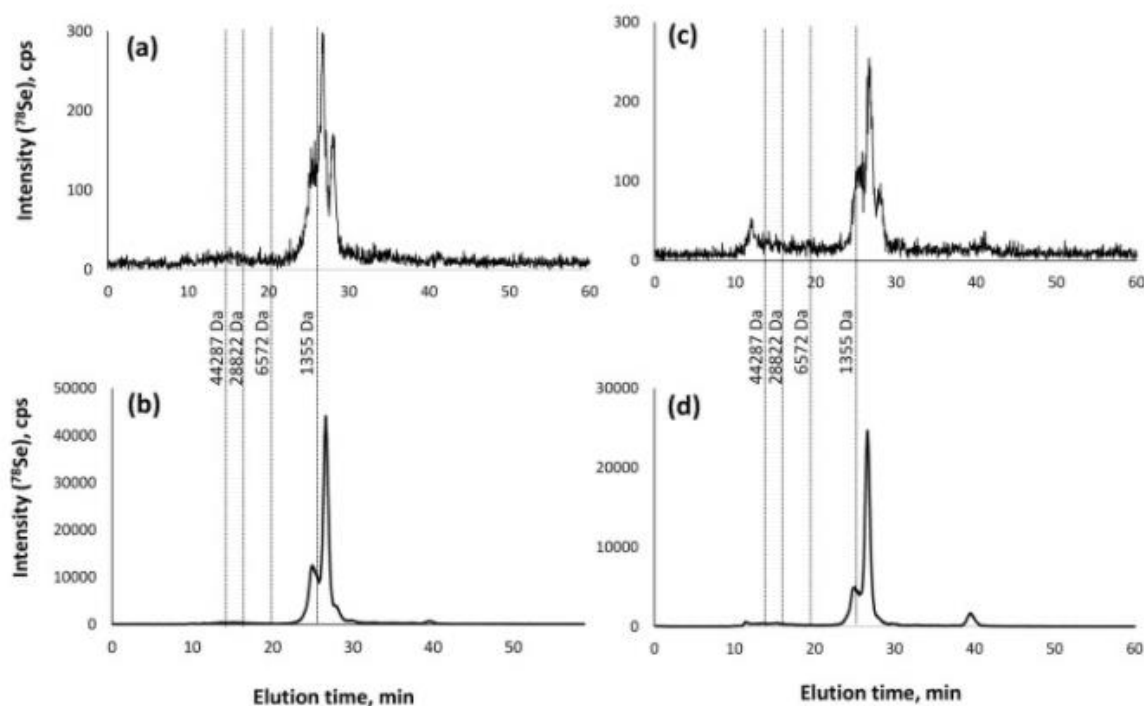


Fig. 2. Size-exclusion-ICP-MS chromatograms of selenium species present in the water soluble fraction of sprouts obtained using a Superdex-75 column with 100 mM ammonium acetate buffer (pH 7.5) elution at a flow rate of 0.7 mL/min, ^{78}Se ICP MS signal is shown; upper part – unfortified (a) kale and (b) kohlrabi sprouts, lower part – Se fortified (15 mg/L) (c) kale and (d) kohlrabi sprouts. Dashed lines show elution times of molecular weight standards used for the calibration of the column used.

Anion-exchange HPLC-ICP MS chromatograms obtained for aqueous extracts and proteolytic digests of kale and kohlrabi fortified sprouts were shown on [Fig. 3](#). Selenomethionine concentrations in aqueous extracts of both plants were on the same level of $0.80 \pm 0.10 \mu\text{g/g}$, while in proteolytic digests they were 9.8 ± 1.2 and $9.5 \pm 1.0 \mu\text{g/g}$ for kale and kohlrabi, respectively. The Se-methylselenocysteine concentrations in aqueous extracts were equal $0.60 \pm 0.10 \mu\text{g/g}$ and $0.83 \pm 0.20 \mu\text{g/g}$ for kale and kohlrabi sprouts, respectively. The corresponding values of SeMetSeCys concentrations in proteolytic digests were 0.72 ± 0.09 and 1.1 ± 0.2 , respectively. The content of phenolic acids, namely sinapic, chlorogenic, isochlorogenic, caffeic acid, and L-tryptophan were shown in [Fig. 4](#), [Fig. 5](#), respectively, while total phenolic content and antioxidant parameters in the sprouts (DPPH, FRAP) were summarized in [Fig. 6](#).

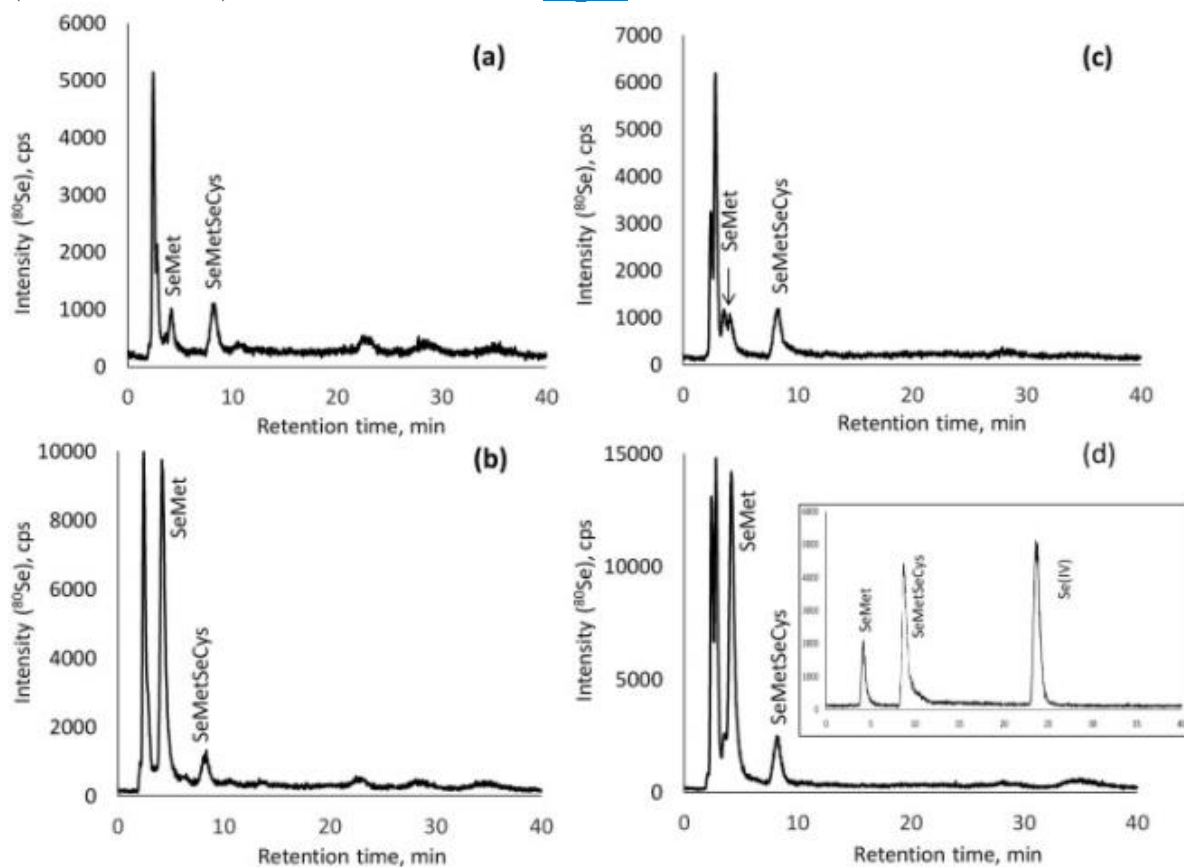


Fig. 3. Anion-exchange HPLC-ICP MS chromatograms obtained for aqueous extracts (a, c) and proteolytic digestates (b, d) of kale (a, b) and kohlrabi (c, d) sprouts. A chromatogram of standards is presented in the inset.

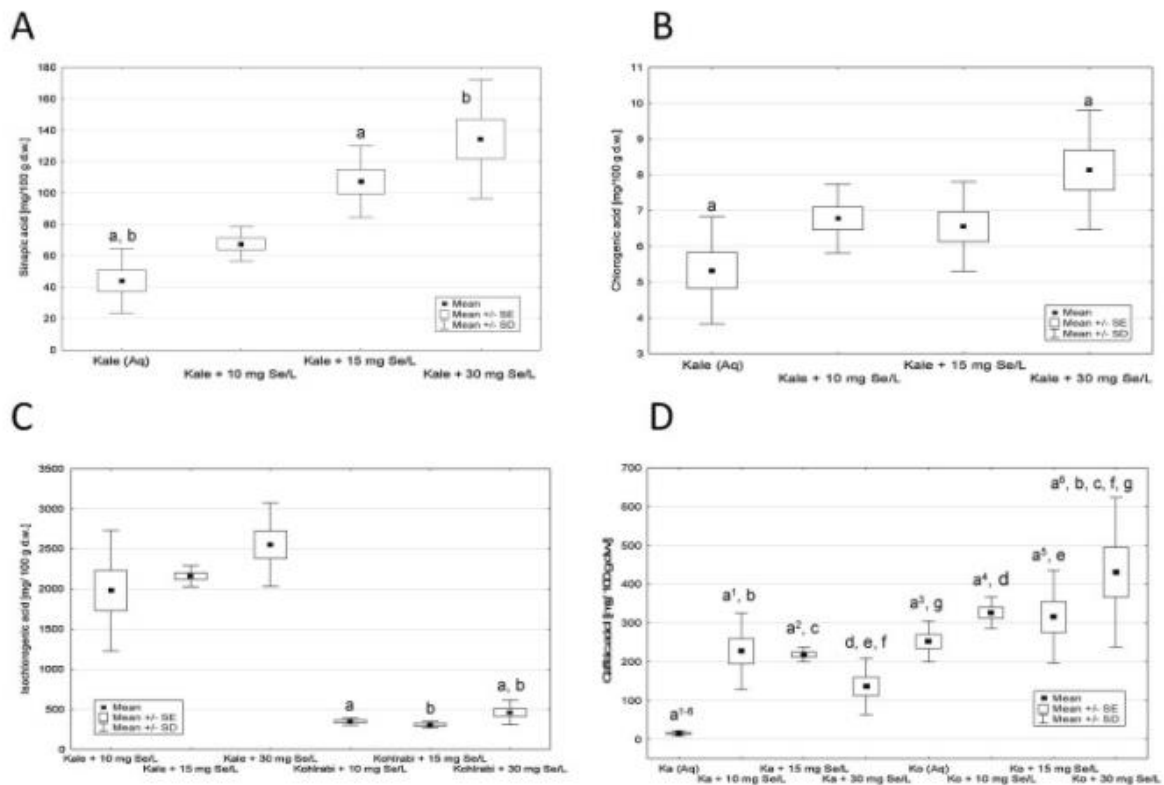


Fig. 4. Phenolic acids concentrations in kale and kohlrabi sprouts (the groups marked with the same index differed significantly). A: Sinapic acid; B: Chlorogenic acid; C: Isochlorogenic acid (because of apparent difference between kale and kohlrabi sprouts, statistical tests were applied separately for this acid); D: Caffeic acid (meaning of abbreviations Ka – kale, Ko – kohlrabi).

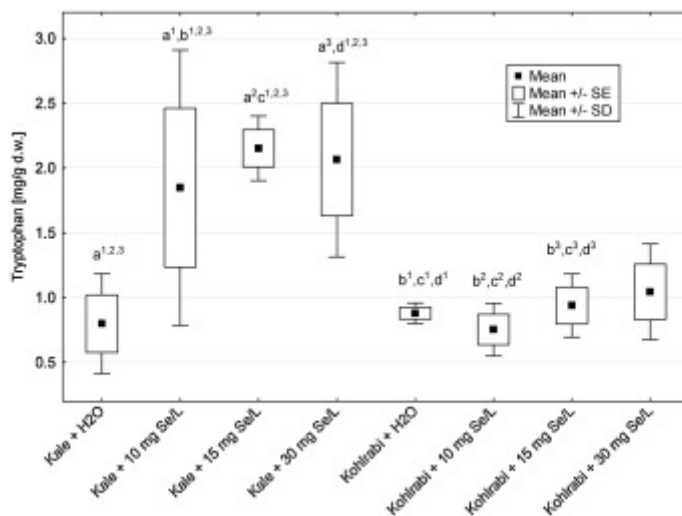


Fig. 5. L-Tryptophan concentrations in kale and kohlrabi sprouts (the groups marked with the same index differed significantly).

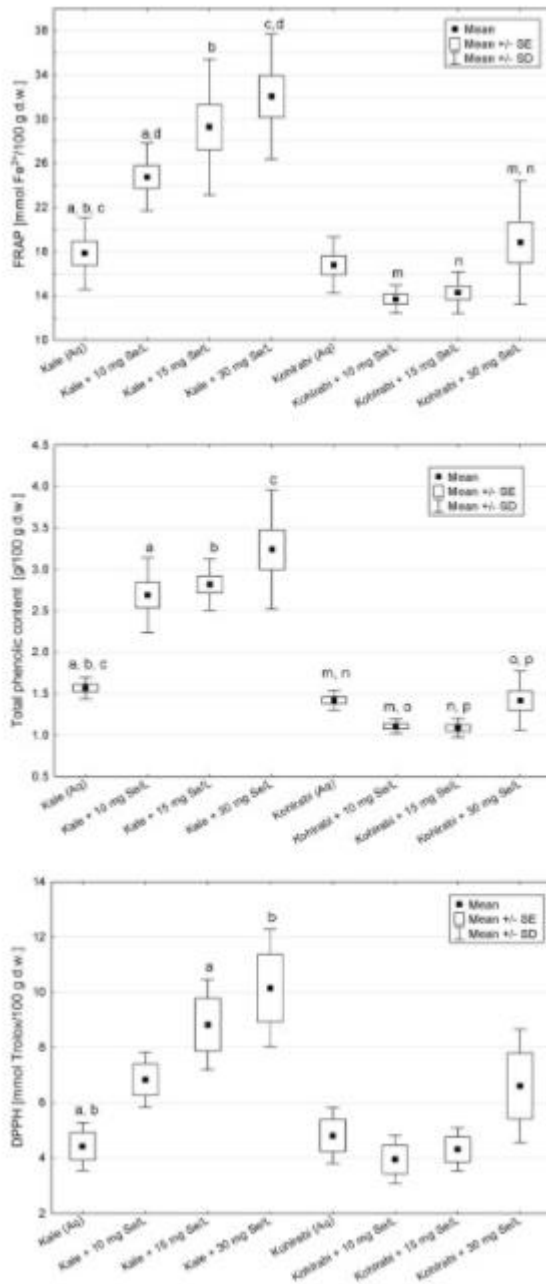


Fig. 6. Antioxidant parameters in kale and kohlrabi sprouts (the groups marked with the same index differed significantly; because of apparent difference between kale and kohlrabi sprouts, statistical tests were applied separately to both groups).

The highest content of sinapic acid was observed in kale sprouts fortified with 15 or 30 mg/L selenium (120.0 ± 1.8 and 175.7 ± 1.4 mg/100 g d.w., respectively), while in most samples of kohlrabi sprouts the compound was not detected (Fig. 4A). Chlorogenic acid occurred solely in kale sprouts (for most of the samples being in the range between 4 and 9 mg/100 g d.w., respectively), and its highest concentration (10.3 ± 0.1 mg/100 g d.w.) was noted for the sample fortified with 30 mg/L selenium

([Fig. 4B](#)). Isochlorogenic acid was quantitatively determined only in Se-fortified sprouts of both tested species. Its content in kale sprouts was one order of magnitude higher, as compared with kohlrabi sprouts (2349.8 ± 448.1 vs. 383.1 ± 116.9 mg/100 g d.w.). Moreover, isochlorogenic acid showed apparent dose dependent positive relationship with selenium in kale samples, however, without statistical significance ([Fig. 4C](#)). Caffeic acid content in almost all tested sprouts was of the same order of magnitude (mean values in the range 140–430 mg/100 d.w.), although with many detailed differences, and one exceptionally low amount in the case of unfortified kale sprouts (15.7 ± 7.1 mg/100 g d.w.) ([Fig. 4D](#)). L-tryptophan content was one order of magnitude higher in Se-fortified kale than kohlrabi sprouts, among which no significant differences in the content of the compound was observed ([Fig. 5](#)). [Fig. 6](#) shows the changes of the antioxidant parameters associated with the growing amounts of selenium used for the sprouts fortification.

PCA model constructed for all the above mentioned parameters had two significant components, according to cross-validation, and explained 87.5 % of the variance in the original parameters, with eigenvalues equalled 6.36 and 1.52 for the first and the second component, respectively. The first principal component in this model had large positive weights for all parameters apart from selenium and caffeic acid. Other phenolic acids, antioxidant parameters and L-tryptophan formed cluster of – in most cases – highly correlated parameters. The correlation structure between the parameters in the PCA model was shown in [Fig. 7A](#). The highest positive correlation weights based on the first principal component were revealed between TPC and both sinapic acid and FRAP, as well as between FRAP and isochlorogenic acid ([Table 1](#)). The second principal component was loaded positively by caffeic acid and selenium, and negatively by chlorogenic acid ([Fig. 7A](#)). Therefore caffeic acid had high, but opposed correlation weights with selenium and chlorogenic acid ([Table 1](#)). The correlations revealed by PCA method were confirmed also by Pearson correlation analysis, with an exception for the correlation between caffeic acid and selenium ([Table 1](#)). [Fig. 7B](#) shows the projection of the sprouts on the plane defined by the first two principal components of the PCA model. The sprouts formed four distinct clusters. The kohlrabi sprouts fortified with the highest dose of selenium (30 mg Se/L) deviated most from all the other sprouts.

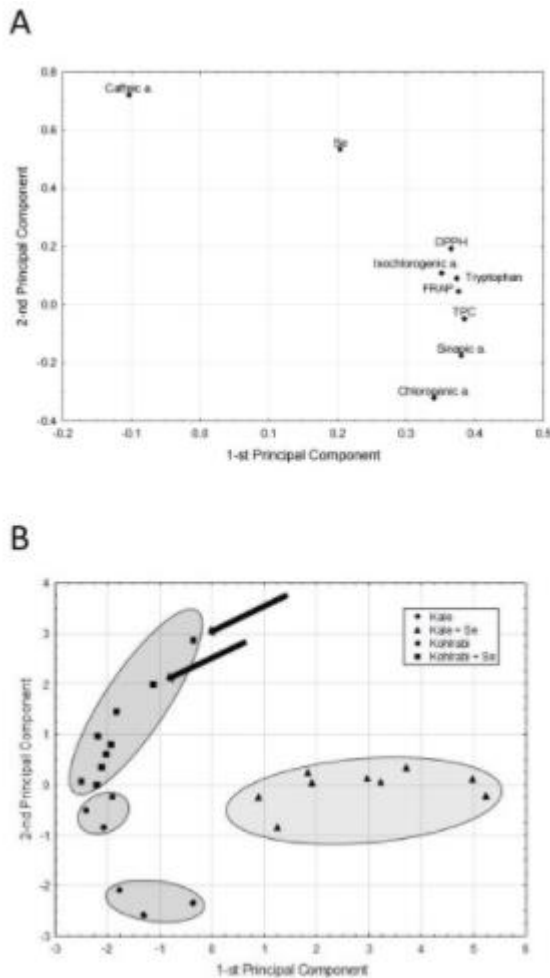


Fig. 7. PCA model evaluation. A: The weights of the first two components of PLS model; B: The projection of the sprouts on the plane defined by the first two principal components of the PCA model. The boundary lines of each cluster were depicted. The arrows indicate kohlrabi sprouts supplemented with 30 mg Se/L.

Table 1. Correlation weights (based on PCA model), and Pearson correlation coefficients for most significant bivariate associations (only correlation weights with absolute values bigger than 0.100 were shown).

Pairs of correlated parameters	Correlation weights (PCA)	Pearson correlation coefficients and level of significance
Se and caffeic a.	0.376	NS
Sinapic a. and TPC	0.140	$r = 0.926, p < 0.001$
FRAP and TPC	0.140	$r = 0.926, p < 0.001$
Isochlorogenic a. and FRAP	0.140	$r = 0.852, p < 0.001$
Isochlorogenic a. and TPC	0.134	$r = 0.914, p < 0.001$
Isochlorogenic a. and DPPH	0.133	$r = 0.837, p < 0.002$
Isochlorogenic a. and tryptophan	0.131	$r = 0.816, p < 0.05$
FRAP and tryptophan	0.130	$r = 0.808, p < 0.001$

Pairs of correlated parameters	Correlation weights (PCA)	Pearson correlation coefficients and level of significance
FRAP and DPPH	0.129	$r = 0.958, p < 0.001$
DPPH and tryptophan	0.126	$r = 0.817, p < 0.005$
TPC and tryptophan	0.123	$r = 0.848, p < 0.005$
Sinapic a. and chlorogenic a.	0.123	$r = 0.897, p < 0.001$
Sinapic a. and FRAP	0.122	$r = 0.897, p < 0.001$
TPC and DPPH	0.115	$r = 0.890, p < 0.001$
Sinapic a. and isochlorogenic a.	0.112	$r = 0.871, p < 0.05$
Chlorogenic a. and TPC	0.106	$r = 0.831, p < 0.005$
Chlorogenic a. and caffeic a.	-0.133	$r = -0.529, p < 0.001$

The results of the study on cytotoxic activity of the sprouts extracts were shown on [Fig. 8](#). Either none (colorectal adenocarcinoma SW 480 cells, [Fig. 8A](#)) or only single significant differences ([Fig. 8B–D](#)) were observed in the activity of the extracts from Se-fortified or unfortified sprouts. However, interesting differences in the impact on the tested cancer cells were observed between the unfortified kale and kohlrabi sprouts, more pronounced in the lower tested concentrations (0.1–0.5 mg/mL). Unfortified kale sprouts were more active to SW480 and SiHa cells ([Fig. 8A](#) and [D](#), respectively), while cytotoxic effect of kohlrabi sprouts was more profound in SW620 and HepG2 cells ([Fig. 8B](#) and [C](#), respectively). Phase contrast microphotographs of cervical carcinoma SiHa cells after 24 h of incubation with the sprout extracts were shown on [Fig. 8E](#). The microscopic observations of these cells after 24 h of the incubation with the sprout extracts revealed the disruption of typical monolayer growth of the cells and their tendency to detach and float in the medium ([Fig. 8E](#)) in comparison to the control conditions.

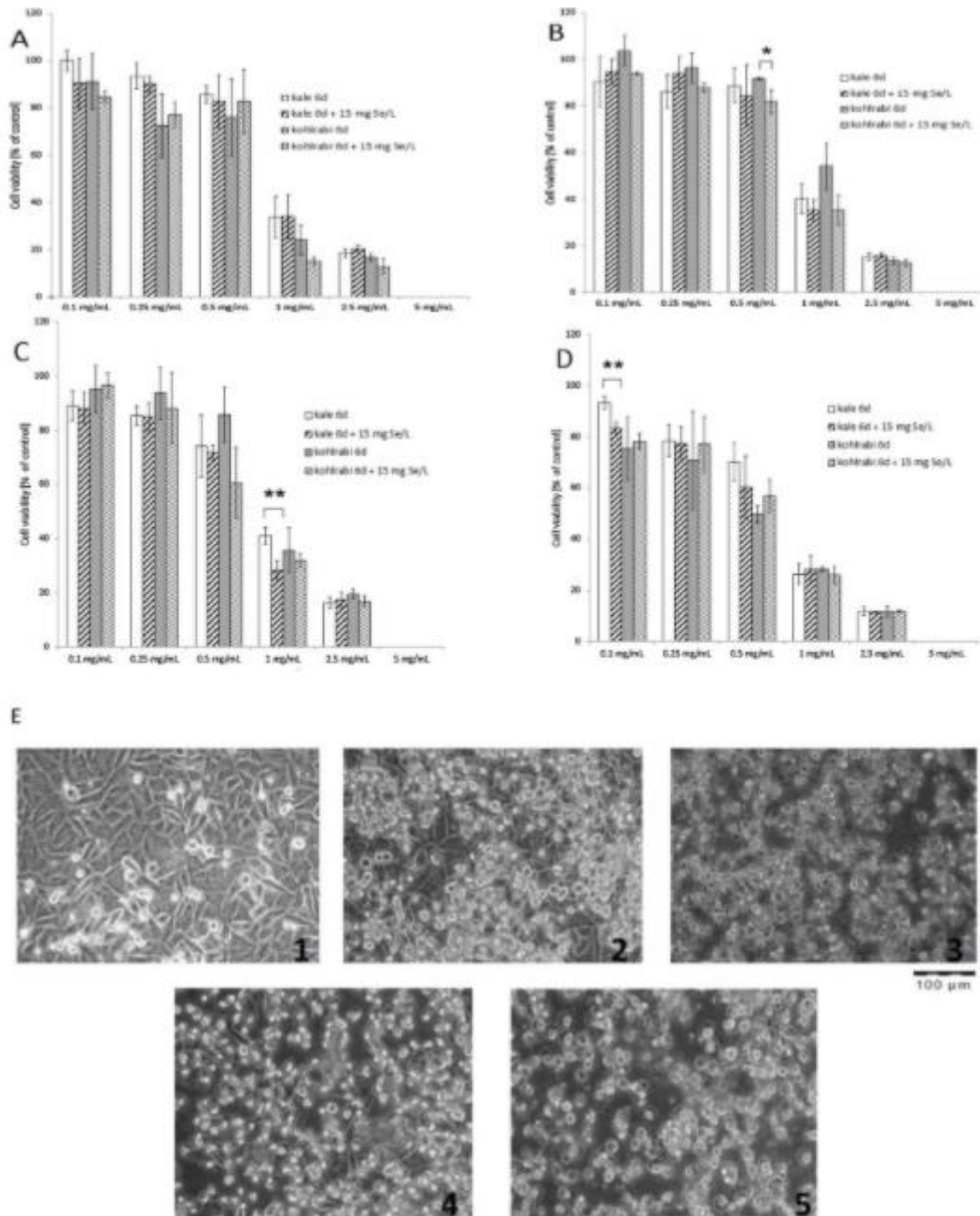


Fig. 8. Cytotoxic activity kale and kohlrabi sprouts extracts (*difference statistically significant) in colorectal adenocarcinoma SW480 (A), colorectal adenocarcinoma SW620 (B), hepatocellular carcinoma HepG2 (C), cervical carcinoma SiHa (D); (E): Phase contrast microphotographs of cervical carcinoma SiHa cell cultures. (1): untreated cells; (2): cells exposed to the extracts of kale; (3): cells exposed to the extracts of kale + 15 mg Se/L; (4): cells exposed to the extracts of kohlrabi; (5): cells exposed to the extracts of kohlrabi + 15 mg Se/L (all the tested sprouts were grown for 6 days; the concentration of all the extracts was 1 mg/mL).

4. Discussion

Selenium food fortification with i.e. SeMet, selenite and Se-yeast is a one of the possibilities to increase the amount of this trace element in daily nutrition [27]. Implementation of functional foods (also rich in Se) and searching for their new examples may offer health benefits and can get strong acceptance of consumers, hopefully leading to the change of their dietary habits [27,28]. Brassica crops and their sprouts are well-documented and often consumed example of chemopreventive food, but among them kohlrabi sprouts are not fully examined yet [7,28]. Brassica plants are also known from their high ability to accumulate some forms of Se, but the information on the influence of this trace element on the synthesis of secondary metabolites is still incomplete [7,23]. Thus, our study was designed to better comprehend the optimization of selenium plant treatment to obtain the products of increased nutritional and chemopreventive values and high safety. This aim was supported by the novel approach of PCA model-based comparison of the relationships between the evaluated parameters.

As we expected, Se-enriched sprouts proved to be more rich in this trace element when compared to the unfortified material, with the difference even several orders of magnitude higher (Fig. 1). The kale or kohlrabi sprouts fortified with the lowest dose of selenium can be classified as secondary Se accumulators (Se content 0.025–0.100 mg/g d.w.), while the sprouts enriched with higher Se doses as primary accumulators (Se content 0.100–1.000 mg/g d.w.) [1]. Thus, apart from so far known sprouts of wheat or alfalfa that can accumulate selenium [29], the kale and kohlrabi sprouts also have the high ability to easily incorporate Se, which is an important and novel information. Different Brassica sprouts have varied capacity to accumulate selenium, with the lowest observed for Brussels sprouts, and higher for green cabbage, cauliflower and broccoli. The Se accumulation also depends on the cultivars, as significant variation among the sprouts of cauliflower, Chinese cabbage and kale cultivars was observed, ranging from 25 % in cauliflower to 2.3-fold difference in kale [7].

Several plants have been reported to accumulate and transform the inorganic forms of selenium into its bioactive organic compounds, present in commercially available products [30]. The SEC-ICP-MS profiles obtained for kale and kohlrabi sprouts were very similar (Fig. 2), with the presence of relatively low molecular weight (MW < 2000 Da) Se containing species, but no water soluble high molecular weight seleno-species. The selenium distribution seems to be the same in extracts of the control (untreated) and enriched samples, which suggests that no new species of Se were

induced after the supplementation. In addition, the relatively low content of SeMet in the fortified material shows that Se may be incorporated into species other than proteins. However, future speciation studies are necessary to evaluate the exact composition of bioaccessible and bioavailable fractions of selenium in the tested sprouts. The analysis should also consider selenized species not extractable to the water fraction which, as it was mentioned before, can be expected in plant organisms [31,32]. Although the aqueous extracts of both plants contained only traces of selenomethionine, its concentration was significantly higher after the proteolysis, which liberated this selenoaminoacid from the Se-containing proteins, while the intensity of Se-methylselenocysteine peaks was not affected by the proteolysis, and the content of this species remained at the same level in aqueous extracts and proteolytic digests of both plants (Fig. 3).

The retention times of the peaks eluting at the beginning of the chromatograms do not match those of commercially available standard species (Fig. 3). A dedicated study, beyond the scope of the current work, would be required to carry out comprehensive *de novo* identification of selenium species present in the studied material.

Avila et al. [7] noted that the sprouts of green cabbage cultivars and Brussels had low SeMSCys content (average 50–70 µg/g d.w.) in comparison to the sprouts of the other Brassica crops, while Chinese cabbage accumulated relatively high (100–150 µg/g d.w.) SeMSCys. In the sprouts of broccoli, green cabbage and cauliflower cultivars, similar level of SeMSCys was detected, while for the sprouts of Chinese cabbage and kale cultivars significant variation of SeMSCys level was noted [7]. SeMSCys was also the main selenium species in 28 sprouts species harvested in a high selenium environment. Moreover, in several of the evaluated sprouts, selenomethionine, non-metabolized selenite, γ -glutamyl-Se-methylselenocysteine and an unknown selenium compound were found [33]. In our experiment we obtained much lower concentrations of this selenoaminoacid. However, Seppänen et al. proved recently, that its concentrations depended strongly on the treatment of sprouts during their growth and might differ even by a few dozen factors [34].

Although selenium is not essential for plants, a number of beneficial effects of this element on plant physiology have been observed in many plant species [35]. Se can up-regulate plant enzymes (peroxidases and reductases) responsible for antioxidant protection [36]. Moreover, the plants supplemented with Se are also more resistant to other environmental stressors like ultraviolet radiation or heavy metal

contamination [37]. However, the role Se plays in plant physiology, strongly depends on both its concentration and plant tolerance [35]. This implies the question about the interaction between Se and other, potentially beneficial compounds (secondary metabolites) present in plant organisms. To answer this question, in our experiment we focused on the quantitative analysis of phenolic acids and L-tryptophan. Sinapic and chlorogenic acids were detected only in kale sprouts, both untreated and Se-fortified. While sinapic acid amount increased proportional to the selenium doses used for the fortification (Fig. 4A), chlorogenic acid content differed only between the untreated samples and the ones fortified with the highest Se dose (Fig. 4B). Isochlorogenic acid was present only in Se-fortified kale and kohlrabi sprouts, but in much lower amount in the latter species (Fig. 4C). The tendency of increasing caffeic acid content in the samples treated with higher doses of selenium was observed only for kohlrabi sprouts (Fig. 4D). Thus, selenium differentially affected the production of the analysed phenolic acids, depending on the particular sprout species and Se dose but generally caused the increase in the content of these compounds, with subsequent progression of antioxidant activities in kale sprouts (Fig. 6). The general routes of possible selenium influence on plant phenolic compounds were recently reviewed by Chomchan et al. [38] and our results seem to be consistent with the conclusions of this review. We have proved that low selenium concentrations generally stimulated phenolic acids production in the tested sprouts, apparently acting similar to light stressor, however the exact mechanisms should be clarified in the future.

Apart from polyphenolic compounds, Brassica vegetables and their sprouts can be also an important source of indole derivatives [4]. Tryptophan as a serotonin precursor may affect depression and mood scores of healthy participants [4]. In our experiment we have observed that selenium fortification induced significant, but not dose-dependent, increase in L-tryptophan content in kale sprouts (Fig. 5), with no effect for kohlrabi sprouts. Our experiment is probably the first to demonstrate the impact of Se fortification of L-tryptophan synthesis in any Brassica sprouts, but further studies are needed to explain the observed differences between the species.

As Brassica vegetables and sprouts are known from their antioxidant properties [18], in the next step of our experiment we determined if Se fortification may influence the total phenolic content (TPC) and antioxidant parameters (FRAP, DPPH) of the tested sprouts. The obtained results followed the same pattern of changes as we have noted for phenolic acids analysis. Steady increase with the increasing Se doses for

kale sprouts, and no such trend, or even lower results for intermediate Se doses, were observed for kohlrabi sprouts (Fig. 6). It suggests that kale sprouts are more vulnerable to Se-stimulated higher antioxidant activity, in comparison to kohlrabi sprouts. On the other hand, it can be interpreted as greater ability of kohlrabi sprouts to equilibrate changing Se status as homeostatic phenomena [1]. Similar positive impact of low selenium dose (10 mg/L) on TPC content was found for *A. cruentus* and *A. tricolor* sprouts, with the opposite effect for *A. caudatus* sprouts. A higher selenium dose (15 mg Se/L) caused insignificant increase in TPC in amaranth sprouts, in comparison to the sprouts treated with 10 mg Se/L. Moreover, significant increase in antioxidant activity was observed for selenium fortified *A. cruentus* sprouts. For the other two evaluated amaranth cultivars, the antioxidant activity either did not change, or decreased strongly, in response to 15 mg Se/L water dose (*A. tricolor*) [23]. No clear relation between the selenium concentration and antioxidant activity (FRAP, DPPH) of kale and kohlrabi sprouts was found, however Djanaguiraman et al. noted that the increase in the antioxidant activity was positively correlated with the Se content in the plants [39].

To better interpret the relationships between the obtained results we involved PCA, an approach which has not been applied to the analysis of kale or kohlrabi sprouts so far. The applied PCA model revealed correlation structure between the investigated parameters. Among phenolic acids only caffeic acid was not strongly correlated with other secondary metabolites, but it correlated with selenium (Fig. 7A, Table 1). This may suggest particular selenium dependence of the enzymes catalyzing metabolic changes of caffeic acid, and should be clarified in future studies.

Four distinct clusters formed by the sprouts on the plane defined by the first two principal components of the PCA model reflected different species and treatment (fortification of Se or its lack). This means that PCA model was effective enough to differentiate between the examined groups of the sprouts (Fig. 7B) and to indicate the sprouts deviating most from the others. In our previous papers PCA model was also applied [23,40] and proved to be an useful tool in relevant description of the relationships among parameters being investigated.

Finally, we examined the cytotoxic properties of selenium treated and untreated kale and kohlrabi sprouts, to answer the question on their possible chemopreventive potential. In our experiments we used a panel of four human cancer cell lines (SW480, SW620, HepG2, SiHa) of different tissue origin, to determine multidirectional effect of the tested extracts. Moreover, two of the chosen colorectal adenocarcinomas also

differed in the stage of tumorigenesis and the degree of invasion. The tested sprout extracts exerted varied cytotoxicity towards the cancer cells at concentration 1.0 mg/mL and higher (Fig. 8). Interesting differences in the impact of untreated kale and kohlrabi sprouts on primary (SW480) and metastatic (SW620) colorectal adenocarcinoma cells were also noted, with more profound effect of kale sprouts on the latter cell line. Other Brassica sprouts (broccoli, rutabaga) revealed selective cytotoxic activity against different human cancer cell lines (HepG2, SW480) with no significant effect on normal cells [40,41]. In comparison to these results, the tested kale and kohlrabi sprouts at the concentration of 1 mg/mL had significantly higher cytotoxic activity against HepG2 and SW480 cells. In our previous study on selenium treated (15 mg/mL) amaranth sprouts (*A. cruentus*, *A. caudatus*, *A. tricolor*) no significant difference in the cytotoxic activity between the treated and controlled sprouts on RAW 264.7 cells was observed, which is consisted with the presented results [23].

5. Conclusions

Our results indicate that Se enrichment is an interesting and perspective idea for plant functional foods industry in the future. The addition of selenium stimulated the production of some phytochemical compounds in different ways, depending on the particular sprout species, selenium dose and the investigated compound. As selenium correlated only with caffeic acid, and other parameters (DPPH, FRAP, TPC, phenolic acids and L-tryptophan) were strongly intercorrelated, the detailed mechanisms of selenium action should be clarified in future studies. The obtained results also proved that the tested sprouts induced significant decrease in the viability of SW480 and SW620 colorectal adenocarcinoma cells, differing in metastatic potential. This may have some future implications for the use of selenium-fortified or unfortified kale and kohlrabi sprouts in the chemoprevention of colon cancer. Moreover, we did not observed any negative influence of Se on the content of the investigated active compounds. Thus, Se-fortified kale and kohlrabi sprouts may become potentially attractive and safe ingredients of functional food.

Author's statement

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acquisition **Paweł Paśko**: Conceptualization, Methodology, Resources, Writing- Original draft preparation, Visualization, Writing- Reviewing and Editing, Investigation, Project administration **Agnieszka Galanty**: Methodology, Writing- Reviewing and Editing, Data Curation, Investigation **Malgorzata Tyszka-Czochara**: Methodology, Investigation **Renata Wietecha-Posłuszny**: Methodology, Investigation **Pol Salvans Rubió**: Investigation **Henryk Bartoń**: Methodology **Ewelina Prochownik**: Investigation **Bożena Muszyńska**: Methodology, Resources **Katarzyna Sułkowska-Ziaja**: Investigation **Katarzyna Bierła**: Investigation **Ryszard Łobiński**: Resources, Supervision **Joanna Szpunar**: Resources, Methodology, Writing- Reviewing and Editing, Data Curation, Investigation **Shela Gorinstein**: Supervision.

Declaration of Competing Interest

Not exist.

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Appendix A. Supplementary data

The following is Supplementary data to this article: [Download : Download zip file](#)

[\(341B\)](#)

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