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Changes in Nutritional Value and Cytotoxicity of Garden Cress Germinated with Different Selenium Solutions

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The selenium supply in almost all European countries is below the recommended daily intake, and different strategies are followed to fortify foods. In the present work, the influence of germination of garden cress (*Lepidium sativum* cv. Ogrodowa) in different selenium solutions (Na_2SeO_3 and Na_2SeO_4) on Se uptake, total antioxidant capacity, glucosinolates, protein, and amino acids was studied. Cytotoxicity in HL-60 human leukemic cell line was also assessed. The addition of selenite (Na_2SeO_3) or selenate (Na_2SeO_4) led to a significant increment in Se uptake in garden cress sprouts, and the highest Se content was observed at 8 mg/L in both inorganic Se solutions (36−38 μg/g of dm). The Se-enriched sprouts presented a large total antioxidant capacity (142−157 μmol of Trolox/g of dm), total glucosinolate content (99−124 μg/g of dm), protein (36−37% dm), and total essential amino acid content (40−41 g/100 g of protein), and no cytotoxicity on HL-60 human leukemic cells was observed. Garden cress sprouts obtained with selenite solution at 8 mg/L presented the best nutritional qualities and might provide a substantial proportion of Se in European diets. Bearing in mind the high nutritional value of sprouts, these may serve for the production of functional foods.

KEYWORDS: *Lepidium sativum*; garden cress; selenium; antioxidant capacity; glucosinolates; protein; amino acids; cytotoxicity

INTRODUCTION

There is a wealth of information that shows the health-promoting properties of selenium (Se). Although its deficiency is considered to be an important factor in the development of various serious illnesses such as Keshan disease (myocardial necrosis) and Kashim−Beck disease (endemic osteoarthritis) in some regions with low soil concentrations of this element, most research has focused on the possible protective properties of Se against several types of cancer and the role of low Se status in the pathogenesis of cardiovascular disease (CVD), the immune system, viral infections, and human fertility (*1*). The function of Se as a bioactive compound is mediated by antioxidant selenoproteins, such as glutathione peroxidase and thioredoxin reductase, enzymes that play an important role in reducing biological oxidative stress (*2*).

The amount of total Se intake depends to a great extent on its concentration in food, and the Se supply in almost all European countries is below the daily intake recommended by the WHO (*3*). Therefore, efforts to increase Se concentration in the diet are of great interest. It is known that sprout seeds are Se accumulators from different inorganic sources (*4*, *5*) and that Se is incorporated in the newly synthesized proteins during sprout growth (*6*). The consumption of Se-enriched sprouts either as fresh vegetables or as an ingredient of improved staple foods could increase the Se status of consumers. Liu et al. (*7*) has suggested that enrichment of vegetables with bioactive compounds, including selenium, may enhance efficacy in the prevention of prostate cancer. Finley et al. (*8*) demonstrated the ability of high-Se broccoli or high-Se broccoli sprouts to protect against chemically induced mammalian or colon cancer in animal studies.

Sprouts are one of the most complete and nutritional foods and provide proteins, carbohydrates, fiber, minerals, and vitamins (*9*). In addition, the consumption of garden cress (*Lepidium sativum*) sprouts is increasing today because, although traditionally they are considered to be antiscorbutic, depurative, and a stimulant (*10*), they are also attractive foods with connotations of freshness and lightness and are nutritious and easy to digest. They can be consumed as components of salads, soups, and sandwiches, adding texture, a pleasant appetizing flavor, and visual interest to the dishes (*11*). Garden cress sprout flours can also be used as valuable ingredients in functional food products (*12*), contributing to the health status of consumers.

Additionally, garden cress, like other cruciferous plants, contains glucosinolates (GLS). These compounds are attracting considerable interest in research because they can be hydrolyzed

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by the myrosinase enzyme and form isothiocyanates (ITC), an important group of compounds associated with cancer chemopreventive activity (*13*, *14*). The main GLS in garden cress seeds is glucotropaeolin (GTPL), an aromatic GLS that breaks down to form benzyl isothiocyanate (BITC), the major metabolite found in human urine after the consumption of garden cress (*15*). Nakamura et al. (*16*) demonstrated inducing effects on phase 2 enzymes and apoptosis by BITC, which exhibited biological activities similar to sulfurophane. Garden cress is also an important source of protein, fat, carbohydrates, dietary fiber, minerals, and vitamins and, like other *Brassica* seeds, also contains other bioactive compounds such as flavonoids, polyphenols, and vitamin C that can help to protect cells from damage by free radicals (*6*, *10*).

Due to the consumer's interest in fresh and nutritious vegetables, fortification of garden cress sprouts with selenium can be an exceptional way to provide health-promoting characteristics to an attractive food, beyond its basic nutrition. The aim of this work was to study the influence of germination of garden cress (*L. sativum* cv. Ogrodowa) in different selenium solutions (Na_2SeO_3 and Na_2SeO_4) on Se uptake, total antioxidant capacity, glucosinolates, protein, and amino acids. Cytotoxicity in HL-60 human leukemic cell line was also studied.

MATERIALS AND METHODS

Material. *Seeds*. Garden cress seeds (*L. sativum* cv. Ogrodowa) were supplied by CNOS, Poland.

Germination. Germination of garden cress seeds was carried out according to the method of Frias et al. (*5*). Briefly, 10 g of raw seeds was soaked in 50 mL of 0.07% sodium hypochlorite for 30 min. These seeds were drained and washed with distilled water until they reached a neutral pH. Afterward, seeds were soaked in 50 mL of distilled water for 5.5 h and shaken every 30 min. The imbibed seeds were placed in individual trays to germinate in a pilot scale germinator G-120 model (ASL Snijders International S.L., The Netherlands). Three independent germination batches were carried out for either sodium selenite (Na_2SeO_3) or sodium selenate (Na_2SeO_4) (Sigma Aldrich, Steinheim, Germany) at 4 and 8 mg/L on the basis of our previous experience (*5*). Germination was performed in the dark for 5 days at 20 °C and 95% humidity. A control experiment of cress seeds germinated in distilled water was also performed. After 5 days, all sprouts were washed carefully with distilled water (1:20 w/v) to remove the selenium solutions on the sprout surface. Afterward, sprouts were freeze-dried and milled. Powdered samples were put into plastic bags under vacuum atmosphere and stored at 4 °C until analysis.

Chemical Analysis. *Total Selenium Determination*. Half gram samples of garden cress seeds and sprouts were placed in Teflon crucibles, and 13 mL of concentrated nitric acid was added. Mineralization was performed in the microwave oven CEM MARS 5 (CEM Corp.) at 210 °C for 10 min. After mineralization, the samples were transferred to volumetric flasks and the volume was adjusted to 15 mL with distilled water.

The selenium contents in raw cress seeds and water sprout cress were determined by inductively coupled plasma mass spectrometry (ICP-MS; Varian Inc., Victoria, Australia). For garden cress sprouted under selenium solutions, total selenium content was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Vista MPX instrument with charge coupled devices (CCD) (Varian Inc.).

Determination of Protein. Protein content was determined by using Kjeldahl's method (*17*). The nitrogen data were converted into protein values by applying a conversion factor of 6.25.

Determination of Amino Acids. Determination of protein amino acids was carried out by acid hydrolysis, derivatization, and HPLC quantification using the method described by Martínez-Villaluenga et al. (*18*). Tryptophan was determined according to the method described in the *Official Methods of Analysis of the Association of Analytical Chemists* (*19*).

Chemical Score. The lowest percentage of the content of each amino acid in a sample was expressed as a percentage of the requirements of the same amino acid for preschool children, according to the following formula:

$$\text{chemical score} = (\text{essential amino acid in food protein}/\text{essential amino acid requirement in preschool children}) \times 100$$

The suggested pattern of requirements for preschool children was used: His, 1.9; Ile, 2.5; Leu, 6.6; Lys, 5.8; Met + Cys, 2.5; Phe + Tyr, 6.3; Thr, 3.4; Trp, 1.1; and Val, 3.5; expressed in grams per 100 g of protein (*20*).

The amino acid with the lowest percentage is called the limiting amino acid, and this percentage is the chemical score.

Determination of Glucosinolates. Total GLS were extracted following enzymatic desulfatation according to the method given in the *Official Journal of European Communities* (*21*). Desulfo-GLS were separated and quantified by HPLC, as described previously (*22*).

Determination of Trolox Equivalent Antioxidant Capacity (*TEAC*). Total antioxidant capacity in raw and sprouted garden cress was determined according to the method of Frias et al. (*5*).

Cytotoxicity Evaluation. Extracts from garden cress seeds and sprouts were obtained as in Frias et al. (*5*). Briefly, powdered samples from garden cress seeds and sprouts (50 mg) were suspended in 2.5 mL of deionized water and sonicated for 30 min (Sonorex AK103H). The extracts were centrifuged for 15 min at 12000 rpm. The supernatant was filtered through a 0.22 μm membrane into sterile test tubes. Aliquots of filtered supernatant (1 mL) were dried, and residues were dissolved in sterilized water to a final concentration of 1 mg/mL.

The human leukemic cell line (HL-60) was obtained from the American Type Culture Collection (ATCC; Manassas, VA). For cell cytotoxicity determination, cells were seeded at 3×10^5 cells/mL in six-well plates and cultured on RPMI medium containing 10% fetal bovine serum (Sigma, St. Louis, MO) and 1% penicillin G and streptomycin (Sigma). Cells were treated with 100 μg/mL of raw and Se-enriched garden cress sprouts (Se concentrations ranged from 0 to 3 μg/mL) and incubated at 37 °C in a humidity-controlled incubator at 5% CO_2 for 24, 48, and 72 h. Cells treated with sterile distilled water were used as negative control. MTT assay was carried out to determine the number of viable cells as described in Frias et al. (*5*).

Statistical Analysis. Data were expressed as the mean ± standard deviation of three independent replicates. Data were subjected to multifactor analysis of variance (ANOVA) using the least-squares differences (LSD) test with the Statgraphic 5.0 Program (Statistical Graphic, Rockville, MD).

Table 1. Selenium Content and Total Antioxidant Capacity of Raw Seeds and Sprouts of *Lepidium sativum* Cv. Ogrodowa Obtained with Water or Selenite or Selenate Solutions[a]

seed	total selenium (μg/g of dm)	TEAC (μmol of Trolox/g of dm)
raw seeds	<0.1 a	128.14 ± 0.53 a
germination in water	<0.1 a	134.97 ± 1.32 b
germination in Na_2SeO_3 solution		
4 mg/L	20.48 ± 0.38 b	156.91 ± 1.47 e
8 mg/L	36.21 ± 2.17 d	152.61 ± 1.92 de
germination in Na_2SeO_4 solution		
4 mg/L	27.43 ± 0.07 c	150.93 ± 2.72 d
8 mg/L	38.55 ± 1.35 d	142.19 ± 3.14 c

[a] Mean value ± SD. Different letters in the same column indicate significant difference ($P \leq 0.05$, LSD test).

RESULTS AND DISCUSSION

Effect of Selenium Source and Concentration on Selenium Uptake during Garden Cress Germination. Table 1 shows the total Se content in raw seeds and sprouts of garden cress germinated in water or different Se sources (Na_2SeO_3 or Na_2SeO_4) and concentrations (4 and 8 mg/L). The content of Se in raw seeds and garden cress germinated in water exhibited very low Se concentration (< 0.1 μg/g of dm) in accordance with the amount of Se found in a composition database for other vegetable sprouts (*23*, *24*). The addition of inorganic Se forms to water during germination sharply

Table 2. Glucosinolate Content (Micromoles per Gram of Dry Matter) of Raw Seeds and Sprouts of *Lepidium sativum* Cv. Ogrodowa Obtained with Water or Selenite or Selenate Solutions[a]

	aliphatic				arylic	indole			
	PROG	GRAP	NAP	GTPL	4-OH-GB	GB	4-met-GB	neo-GB	total GLS
raw	tr a	0.07 ± 0.01 a	0.40 ± 0.01 a	130.98 ± 0.06 f	tr a	tr a	tr a	tr a	131.46 f
germination in water	0.35 ± 0.01 d	0.42 ± 0.01 c	0.40 ± 0.00 a	88.60 ± 0.48 a	tr a	0.14 ± 0.01 b	0.74 ± 0.00 c	tr a	90.95 a
germination in Na_2SeO_3 solutions									
4 mg/L	0.32 ± 0.02 c	0.39 ± 0.00 c	0.52 ± 0.02 c	119.52 ± 0.05 d	tr a	0.18 ± 0.01 c	0.79 ± 0.03 d	tr a	121.72 d
8 mg/L	0.26 ± 0.01 b	0.33 ± 0.00 b	0.47 ± 0.01 c	121.66 ± 0.37 e	tr a	0.17 ± 0.01 c	0.94 ± 0.03 e	tr a	123.83 e
germination in Na_2SeO_4 solutions									
4 mg/L	0.31 ± 0.01 c	0.41 ± 0.01 c	0.42 ± 0.03 a	103.85 ± 0.20 c	tr a	0.21 ± 0.01 d	0.93 ± 0.00 e	tr a	106.13 c
8 mg/L	0.31 ± 0.01 c	0.53 ± 0.03 d	0.39 ± 0.01 a	97.28 ± 0.08 b	tr a	0.16 ± 0.00 bc	0.61 ± 0.01 b	tr a	99.26 b

[a] Mean value ± SD. Columns with different letters indicate statistical differences for each cultivar ($P \leq 0.05$, LSD test). PROG, progoitrin; GRAP, glucoraphanin; NAP, napoleiferin; GTPL, glucotropaeolin; 4-OH-GB, 4-hydroxyglucobrassicin; GB, glucobrassicin; 4-met-GB, 4-methoxyglucobrassicin; neo-GB, neo-glucobrassicin; tr, trace (≤0.05 μmol/g).

increased Se uptake by the seeds, in a dose-dependent manner because 8 mg/L led to higher Se content than 4 mg/L. Significantly ($P \leq 0.05$) higher Se contents were obtained when 4 mg/L selenate solutions were used, whereas no significant differences between Se sources were found at a concentration of 8 mg/L.

Plant species can vary widely in Se uptake and accumulation depending on the Se source and the seed variety under study. Indian mustard, sunflowers, and lupin accumulated higher Se contents in the presence of selenate than in selenite solutions (*5*, *25*). In addition, garden cress germinated with 8 mg/L selenate solution accumulated higher Se content (39 μg/g of dm) than lupin sprouts grown with the same selenate dose (11 μg/g of dm) (*5*). Se uptake by the plant is incorporated into the amino acids Cys and Met to form selenocysteine (Se-Cys) and selenomethionine (Se-Met), which are integrated into proteins to form selenoproteins. Previous studies demonstrated that cruciferous plants (radish, broccoli) grown in selenate transformed <40% of total Se content as selenoamino acids; however, plants incorporated >95% of the total Se content as selenoamino acids when they were grown in selenite, from which selenomethylselenocysteine (SeMetSeCys) was the primary specie (*26*, *27*). This compound is converted quickly to methylselenol by cleavage of the Se-methyl group, considered to be the critical metabolite for protection against certain cancers (*28*). On the basis of previous literature, Se-enriched garden cress sprouts germinated in selenite solutions are likely to contain higher amounts of chemopreventive selenium forms compared to sprouts grown in selenate.

Taking into consideration that the amount of garden cress sprouts consumed daily in our dishes might be around 20−30 g of fresh weight, equivalent to 3−4 g of dry product, Se-enriched sprouts grown with the highest Se content (8 mg/mL selenate solution) will provide ∼100 μg of Se/day, below the tolerable upper intake level for adults (400 μg of Se/day) (*29*). Simonoff and Simonoff (*30*) recommended Se as a nutritional prophylaxis against cancer at a dose of 50−100 μg/day. According to these results consumption of Se-enriched garden cress sprouts would contribute to increase human Se status and may prevent certain types of cancer.

Total Antioxidant Activity of Se-Enriched Garden Cress Sprouts. Total antioxidant capacity, measured as Trolox equivalent antioxidant capacity (TEAC), of raw and Se-enriched garden cress sprouts obtained under selenite and selenate solutions is shown in **Table 1**. The antioxidant capacity of raw garden cress was higher (128 μmol of Trolox/g of dm) than values found in other cruciferous seeds, such as broccoli (63−90 Trolox/g of dm) (*31*). Germination in water for 5 days at 20 °C led to a slight but significant ($P \leq 0.05$) TEAC increase (5%). Germination of cress seeds in these conditions under inorganic Se solutions increased antioxidant capacity (11−22%) and, for the same concentration, the sodium selenite solutions presented the highest TEAC levels.

Total antioxidant capacity measured as TEAC accounts for the ability of hydrophilic antioxidants from the sample to scavenge $ABTS^{\bullet+}$ cation radicals, compared to the hydrophilic vitamin E equivalent compound Trolox. Due to the implications of Se in antioxidant status, TEAC was expected to increase with Se uptake in sprout cress. However, in this study TEAC values did not increase in parallel to Se accumulation. These results suggest that other bioactive compounds such as polyphenols, vitamins C and E, carotenoids, and glutathione, among others, could also play an important role in the total antioxidant capacity (*32*).

There is very little information about the antioxidant capacity of garden cress. Souri et al. (*33*) reported that the antioxidant activity of garden cress leaves against linoleic acid peroxidation seems to be comparable with those of α-tocopherol and quercitin. Conforti et al. (*10*) observed that garden cress showed the highest anti-inflammatory effect among other Mediterranean dietary plants, possibly due to its high sitosterol contents and antioxidants that could exert anti-inflamatory effects (*34*). No information has been found in relation to the effect of Se enrichment during germination on the antioxidant capacity of garden cress.

Glucosinolates in Se-Enriched Garden Cress Sprouts. The content of individual and total GLS in seeds of raw and germinated garden cress in water and under Se solutions is presented in **Table 2**. Total GLS content in raw cress was 131.5 μmol/g of dm. The Arylic GLS glucotropaeolin was the most abundant GLS in unprocessed seeds, accounting for 99% of the total GLS. Aliphatic GLS were found in very small amounts (gluconapin, 0.4 μmol/g of dm; glucoraphanin, 0.07 μmol/g of dm; and progoitrin in traces). Indole GLS were detected only in trace amounts.

Water germination of garden cress produced a noticeable decrease in total GLS (30%) mainly due to the reduced glucotropaeolin content observed. However, the aliphatic GLS glucoraphanin increased sharply after 5 days of germination, and napoleiferin did not change significantly ($P \leq 0.05$). On the other hand, progoitrin, glucobrassicin, and 4-methoxyglucobrassicin GLS that were present in trace amounts in ungerminated garden cress seeds appeared in the sprouts, but always in amounts of <1 μmol/g of dm. Napoleiferin did not change significantly ($P \leq 0.05$) during germination (**Table 2**).

Germination of garden cress seeds in the presence of Na_2SeO_3 solutions reduced the contents of total GLS by only 6−7%, whereas larger losses were found when Na_2SeO_4 solutions were used during germination (20 and 25% for 4 and 8 mg/L concentrations, respectively). Reduction in total GLS content during cruciferous growth could be explained by either a dilution

Table 3. Protein and Amino Acid Contents and Chemical Score of Raw and Germinated *Lepidium sativum* Cv. Ogrodowa Seeds[a]

		seeds germinated in				
	raw seeds	water	4 mg/L Na_2SeO_3	8 mg/L Na_2SeO_3	4 mg/L Na_2SeO_4	8 mg/L Na_2SeO_4
protein (g/100 g of dm)	32.04 a	35.03 b	36.45 b	37.17 b	35.91 b	35.86 b
NEAA (g/100 g of protein)						
Glu	21.92 c	20.81 d	17.35 a	18.34 b	17.81 ab	17.91 ab
Asp	10.92 ab	10.79 ab	10.82 ab	11.53b	10.76 a	10.85 ab
Gly	5.61 b	5.35 b	4.51 a	4.51 a	4.25 a	4.39 a
Ser	5.36 b	5.12 b	4.01 a	4.20 a	4.24 a	4.15 a
Pro	5.01 b	4.82 b	3.90 a	4.18 a	3.83 a	4.07 a
Ala	4.03 a	3.79 a	4.02 a	4.49 a	4.29 a	4.19 a
Arg	3.98 a	4.02 a	4.79 b	5.12 bc	5.28 c	5.17 bc
EAA (g/100 g of protein)						
Leu	6.97 a	7.28 a	6.80 a	7.11 a	6.86 a	6.89 a
Lys	6.16 a	6.92 b	6.74 b	6.83 b	6.62 b	6.76 b
Val	5.91 b	5.50 ab	5.39 a	5.64 ab	5.45 ab	5.47 ab
Phe	5.24 a	4.91 a	4.86 a	4.73 a	4.84 a	4.68 a
Ile	4.62 a	4.53 a	4.46 a	4.57 a	4.51 a	4.58 a
Thr	4.15 a	3.71 a	3.73 a	3.91 a	3.83 a	3.88 a
His	3.41 b	3.02 a	2.92 a	2.97 a	2.97 a	2.93 a
Tyr	3.02 b	2.62 a	2.65 a	2.66 a	2.64 a	2.70 a
Trp	1.12 a	1.48 b	1.86 d	1.86 d	1.62 c	1.59 c
Met	0.64 b	0.56 a	0.58 ab	0.62 ab	0.62 ab	0.58 ab
Cys	0.37 b	0.28 a	0.27 a	0.27 a	0.24 a	0.28 a
total EAA	41.6 a	40.8 a	40.3 a	41.2 a	40.2 a	40.3 a
chemical score	40.2	33.6	35	35.6	34.4	34.4

[a] Mean values of three independent determinations. Different letters in the same row indicate statistical difference ($P \leq 0.05$; LSD test). Chemical score (Met + Cys limitant amino acids).

effect or the influence of the Se salts. Charron et al. (*35*) suggested that Se is metabolized by the sulfur assimilation pathway. Because GLS are sulfur-containing compounds, the synthesis of selenoamino acids and incorporation of these amino acids into proteins may adversely affect GLS synthesis and metabolism.

Total GLS decrease was mainly caused by changes in glucotropaeolin of garden cress sprouts as consequence of germination with inorganic Se solutions, which suffered reductions to those of total GLS. The aliphatic GLS progoitrin glucoraphanin and the indole GLS glucobrassicin and 4-methoxyglucobrassicin were also present in Se-enriched garden cress sprouts in levels similar to those found in water-germinated seeds. The napoleiferin contents in garden cress sprouts obtained under selenite solutions were significantly ($P \leq 0.05$) higher than those under selenate solutions, but these values were always < 1 μmol/g of dw. Our results agree with those reported by O'Hare et al. (*36*), who found that glucotropaeolin was the predominant GLS in garden cress sprouts in amounts of 12.3 μmol/g of fresh weight. It is important to highlight the high glucotropaeolin content in garden cress seeds, because it is the precursor of its corresponding benzyl ITC (BITC). BITC has been recognized as an effective chemoprotective agent against carcinogenesis (*37*). The protective mechanism of action of garden cress has been linked to the attenuation of 2-amino-3-methyl-imidazol[4,5-*f*]quinoline (IQ)-induced DNA damage and induction of uridine-diphospho-glucuronosyl transferase (UDPGT) (*38*).

Amino Acids and Protein Content in Se-Enriched Garden Cress Sprouts. The protein contents and amino acid compositions in raw and garden cress sprouts are compiled in **Table 3**. Raw seeds presented a high protein content (32% dm), and germination for 5 days at 20 °C in darkness led to a significant ($P \leq 0.05$) increase (9−14%). Se uptake during sprouting did not significantly affect ($P \leq 0.05$) the protein content, and these results match those observed in Se-enriched lupin seeds (*5*). In garden cress seeds, nonessential amino acids (NEAA) Glu and Asp were predominant, followed by Gly and Ser. Among the essential amino acids (EAA) high contents of Leu, Lys, and Val and low amounts of Trp, Met, and Cys were found in raw garden cress. These results are in accordance with amino acid values for garden cress seeds presented by Gocavi et al. (*12*), although these authors did not report amounts of Trp and Cys.

Germination of garden cress in water did not significantly ($P \leq 0.05$) modify the content of most NEAA, and only a decrease of Glu by 5% was observed. Among the EAA, increases in Lys and Trp (12 and 32%, respectively) and losses in Tyr, Met, and Cys (13, 12, and 24%, respectively) were found (**Table 3**). The presence of inorganic Se solutions during germination led to a significant ($P \leq 0.05$) reduction in the contents of Glu, Gly, Ser, and Pro (between 16 and 25%), whereas the amount of Arg increased (20−33%) and no significant ($P \leq 0.05$) differences in the contents of Asp and Ala were found (**Table 3**). With regard to EAA, Se enrichment during germination caused a significant ($P \leq 0.05$) increase in Lys (7−12%), irrespective of the Se source, and Trp was higher in cress germinated under selenite (66%) than in selenate solutions (42−45%). However, His, Tyr, and Cys decreased during garden cress germination (12, 10, and 35%, respectively), regardless of the inorganic source of Se administered. No information has been found for the effect of germination, either in water or in Se solutions, on the amino acid contents of garden cress. In lupin seedlings, the contents of His, Ile, Trp, Lys, and Thr increased slightly during germination at 20 °C for 5 days in organic Se solutions (*5*). Total essential amino acid contents did not change after germination of garden cress in water or under Se solution (**Table 3**). These results differ from those recorded in green tea sprayed with sodium selenite solutions, whereas an increase in total amino acid contents was found (*39*). Se-enriched broccoli florets grown on soil fertilized with sodium selenate were shown to contain 2.7-fold the free amino acid content of those grown on control soil (*40*).

Because garden cress and seedlings seem to be a rich source of protein, we considered it important to know the quality and chemical scores in relation to levels recommended for preschool

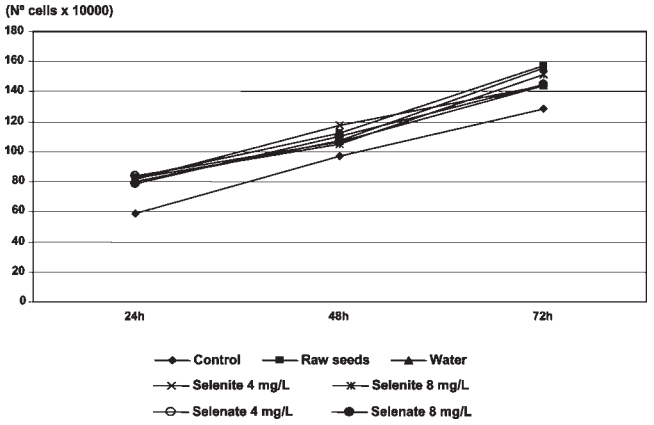

Figure 1. Proliferation of HL-60 cells exposed to extracts of raw and sprouted garden cress (*Lepidium sativum* cv. Ogrodowa) in water or selenite or selenate solutions.

children (**Table 3**). The chemical score of raw seeds was 40.2, which decreased after germination, as a consequence of decreases in Met and Cys. The sprouts grown in water have a chemical score of 33.6, and Se fortification of garden cress sprouts leads to levels between 34.4 for selenate and 35−35.6 for selenite solutions. Despite the reduction in the chemical scores of germinated seeds, the values are quite similar to those of other vegetables (*41*). No data have been found in the literature about nutritional indices of garden cress, and results show that this vegetable not only provides bioactive compounds but also contributes to consumers' nutritional status.

Cytotoxicity of Se-Enriched Garden Cress Sprouts on Human HL-60 Leukemic Cells. Figure 1 shows the proliferation of HL-60 cells exposed for 24, 48, and 72 h to extracts of raw garden cress and water and Se-enriched sprouts. The control assay was carried out in sterilized water. Cell proliferation reached levels of 78−84 (× 10000) cells after the first 24 h of exposure to raw and sprouted garden cress extracts, the number of cells increased to 105−117 (× 10000) after 48 h, and cell counts reached levels of 144−157 (× 10000) after 72 h of exposure. No significant ($P \leq 0.05$) differences between extracts of raw garden cress and seeds germinated in water or germinated in solutions enriched with inorganic Se were found, and numbers of cells recorded were slightly higher than those obtained for sterilized water, whereas cell proliferations of 58, 97, and 128 (× 10000) cells after 24, 48, and 72 h, respectively, were found (**Figure 1**).

These results suggest that extracts of garden cress promote HL-60 proliferation, and the addition of inorganic Se did not lead to any apparent toxic effect on cell growth. No information has yet been found about the cytotoxicity of garden cress or about the effect of Se accumulation during seed sprouting of these vegetables. In extracts from Se-enriched lupin sprouts, no cytotoxic effects on HL-60 cells were observed (*5*). It has been reported that Se exhibits a dose-dependent biological−toxicological response. However, the largest amount of Se in the sprouts of garden cress was found in seeds germinated with 8 mg/L Na_2SeO_4 (38.5 μg/g of dm). Bearing in mind that these vegetables are usually consumed to accompany salads, a portion of 100 g of fresh garden cress (90% humidity) will provide the recommended daily intake for Se and does not show toxic effects on HL-60 cells.

In conclusion, the addition of inorganic Se can significantly improve the Se uptake in garden cress sprouts, and the highest Se content was observed at 8 mg/L in both Se solutions (36−38 μg/g of dm). The Se-enriched sprouts also presented a high level of total antioxidant capacity (142−157 μmol of Trolox/g of dm), glucosinolates (99−124 μg/g of dm), protein (36−37% of dm), and total EAA (40−41 g/100 g of protein), and no cytotoxicity on HL-60 human leukemic cells was observed. Garden cress sprouts obtained with sodium selenite solution at 8 mg/L presented the best nutritional quality and may be suggested for the production of Se-enriched garden cress sprouts to increase the Se status of European consumers.

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