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Influence of Germination with Different Selenium Solutions on Nutritional Value and Cytotoxicity of Lupin Seeds

JUANA FRIAS,[†] PIOTR GULEWICZ,[†] CRISTINA MARTÍNEZ-VILLALUENGA,[†] RADOSŁAW PILARSKI,[§] ENRIQUE BLAZQUEZ,[#] BEGOÑA JIMÉNEZ,[#] KRZYSZTOF GULEWICZ,[§] AND CONCEPCIÓN VIDAL-VALVERDE*,[†]

Instituto de Fermentaciones Industriales and Instituto de Química Orgánica General (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain; and Institute of Bioorganic Chemistry, Polish Academy of Sciences, str. Noskowskiego 12, 61-704 Poznań, Poland

The effect of different selenium solutions during germination of lupin seeds (*Lupinus angustifolius* L. cv. Zapaton) on the content of total selenium, protein, amino acids, soluble carbohydrates, total antioxidant activity, and cytotoxicity on HL-60 human leukemic cell line has been studied. Seeds were germinated in the presence of selenite (Na_2SeO_3) or selenate (Na_2SeO_4) solutions at different concentrations (0, 2, 4, 6, and 8 mg/L) for 5 days at either 20 or 25 °C. The addition of inorganic Se forms significantly increased Se content in lupin sprouts in a dose-dependent manner. The highest Se content in lupin sprouts was observed when germination was carried out with selenate solutions at 20 °C (11 μg/g of dw) or 25 °C (14 μg/g of dw). The Se-enriched sprouts presented an improvement in antioxidant activity (up to 117.8 and 103.5 μmol of Trolox/g of dw) as well as in essential amino acid content, and no cytotoxicity was observed on HL-60 human leukemic cells. Lupin seeds germinated with 8 mg/L selenate solutions for 5 days at 20 °C exhibited a higher germination rate (>90%) and a higher concentration of some essential amino acids than those obtained in selenite solutions in the same germination conditions. Therefore, the employment of selenate solutions at a concentration of 8 mg/L and germination for 5 days at 20 °C may be suggested for the production of Se-enriched lupin sprouts.

KEYWORDS: Lupins; germination; selenium; protein; amino acids; carbohydrates; TEAC; cytotoxicity

INTRODUCTION

Selenium is essential in the diet at trace levels and is incorporated into the amino acids Cys and Met to form selenocysteine and selenomethionine, which are integrated into proteins to form selenoproteins. Selenoproteins include antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase, which is important for DNA synthesis, and iodothyronine deiodinase, which is important for the synthesis of thyroid hormones (*1*, *2*).

Selenium enters the food chain through plants, and the Se concentration of plants varies according to available soil Se concentration, its bioavailability for uptake into plant roots, and plant species (*3*). Selenium deficiency is more prevalent in regions where the soil selenium content is low such as northeastern/western United States, northeastern China, Russia, and Finland. Se deficiency is rare, and low selenium status has been associated with Keshan disease (myocardial necrosis) and Kashim−Beck disease (endemic osteoarthritis). Moreover, the selenium supply in almost all European countries is below the recommended daily intake (*4*, *5*). The criterion of maximization of plasma selenoproteins has been chosen by different countries to give the reference nutrient intake that has been recommended by the WHO as 40 μg/day for men and 30 μg/day for woman and by the German Nutrition Society as 30−70 μg/day for both sexes (*2*).

Besides nutritional roles, Se is associated with the prevention of several diseases such as cancer, heart disease, viral diseases, and other conditions that involve increased levels of oxidative stress (*7*). Therefore, efforts to increase Se concentration in the diet are urgent for both current and future generations. A strategy to achieve this goal includes increased consumption of higher Se foods through sprouting seeds in Se-enrich media. Sprouts are one of the most complete and nutritionally beneficial of all foods, providing proteins, carbohydrates, fiber, minerals, and vitamins (*7*, *8*). Additionally, sprouts are known to be selenium accumulators (*9*), and it is known that plants can accumulate selenium from different inorganic sources (e.g., selenite, sel-

* Author to whom correspondence should be addressed (e-mail ificv12@ifi.csic.es; telephone 34 91 5622900, ext. 241).
[†] Instituto de Fermentaciones Industriales (CSIC).
[§] Polish Academy of Sciences.
[#] Instituto de Química Orgánica General (CSIC).

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enate). During the growth of sprouts, Se is incorporated in the newly synthesized proteins. Rye grain treated with selenite during germination has been shown to accumulate 55 mg/kg Se, which was all converted to organic forms, mostly Se-Met, and the Se-rich rye was then blended with ordinary rye to make bread with a final Se concentration of 4 mg/kg (*10*). Other applications of Se-rich germinated seeds may be considered, such as the use of fresh legume sprouts, which can be mixed into various food products.

Lupins are a valuable source of protein that can partially replace traditional proteins of animal origin and are being used as an alternative to soybeans in the preparation of infant formulas, human foods, and protein products due to their low cost, nutritional quality, and technological properties (*11, 12*). Selenium toxicity has been reported at high levels, and the maximum total safe upper dietary Se intake level was set at 400 μg/day (*13*). The toxicity of selenium is closely correlated to the chemical form and levels ingested, so an assessment of the cytotoxicity of Se-enriched foods is required.

The aim of this work was to study the influence of the germination of lupin seeds (*Lupinus angustifolius* L. cv. Zapaton) in different sodium selenium solutions (Na_2SeO_3 and Na_2SeO_4) for 5 days at 20 or 25 °C on the content of selenium, protein, amino acids, soluble carbohydrate, total antioxidant activity (TEAC), and cytotoxicity in an HL-60 human leukemic cell line.

MATERIALS AND METHODS

Materials. *Seeds.* Lupin seeds of *L. angustifolius* L. cv. Zapaton were provided by the Agrarian Research and Technology Development Service of the Agriculture and Commerce Council of the Junta de Extremadura (Spain). Seeds were cleaned and stored in polyethylene containers at 4 °C until their use for germination experiments.

Germination. Ten grams of seeds was soaked in 50 mL of 0.07% sodium hypochlorite for 30 min. These seeds were drained and washed with distilled water until neutral pH. Afterward, seeds were soaked in distilled water (50 mL) for 5.5 h, with shaking every 30 min. The imbibed seeds were germinated on a pilot-scale germinator G-120 model (ASL Snijders International S.L.) by layering them over a moist filter paper in a germination tray. Several germination batches were carried out using different Se solutions of sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) at different concentrations (2, 4, 6, and 8 mg/L). Germination was performed in triplicate for each batch in the dark for 5 days at 20 or 25 °C. A control group of lupin seeds germinated in distilled water was also prepared. After harvesting, all sprouts were washed carefully with distilled water (1:20 w/v) to remove the selenium on the sprout surface, and germination rates and seedling development were measured for each experimental group. The germination rate was determined by the following formula: (number of germinated seeds/number of total seeds submitted to the germination process) × 100. To obtain the embryonic axis length, 20 measurements of each germination were done and the mean value was calculated. Afterward, sprouts were stored at −20 °C, freeze-dried, and milled. Powdered samples were put into plastic bags under vacuum and stored at 4 °C until analysis.

Chemical Analysis. *Total Selenium Determination.* For element determination, 0.1 g of lupin sprouts was digested in hermetic Teflon vessels. Acid digestion was carried out with 0.5 mL of HNO_3 and five drops of H_2O_2. The mix was maintained in the furnace at 100 °C during 5 h. Once the digestion was completed, the solution was dissolved to 10 mL with Milli-Q water. Se analyses were performed using a Perkin-Elmer (PE) longitudinal AC Zeeman (AAnalyst 600) AAS equipped with a transversely heated graphite atomizer and a built-in fully computer-controlled AS-800 autosampler (Perkin-Elmer Hispania, S.A., Madrid, Spain). PE pyrolitic graphite-coated tubes with an L'vov platform were used. The detection limit (LOD) for Se was 0.001 μg/g. Instrument calibration was performed every day with a minimum quality value accepted for $r^2 > 0.990$. Three replicates were performed for every sample. Relative standard deviations (RSD) in replicates were always below 10%.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. The TEAC assay was based on reduction of the $ABTS^{\bullet+}$ radical cation by the antioxidants present in PBS extracts. The $ABTS^{\bullet+}$ radical cation was prepared by mixing ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate. This mixture was allowed to stand overnight at room temperature until the reaction was completed and the absorbance was stable. TEAC was determined following the procedure described by Re et al. (*14*) with minor modifications. For measurements, the $ABTS^{\bullet+}$ solution was diluted with PBS until absorbance readings reached 0.700 ± 0.020 at 734 nm. For the photometric assay, 1.48 mL of the $ABTS^{\bullet+}$ solution and 20 μL of the lupin seed and sprout extracts or Trolox standards were mixed and measured immediately at 30 °C after 10 min at 734 nm using a spectrophotometer (Beckman DU-70, model 150, series 1574). Appropriate solvent blanks were run in each assay. The TEAC of PBS extracts was expressed as percentage of inhibition of absorbance at 734 nm using the Trolox standard curve.

Total Protein Determination. Protein content was determined according to the Kjeldahl method (*15*). The nitrogen data were converted into protein values by applying a conversion factor of 6.25.

Amino Acid Determination. Determination of protein amino acids was carried out by acid hydrolysis, derivatization, and HPLC quantification using the method described by Martínez-Villaluenga et al. (*16*). Tryptophan was determined according to the method described in the *Official Methods of Analysis of the Association of Analytical Chemists*, 988.15 (*17*).

Soluble Carbohydrate Determination. Monosaccharides and disaccharides were determined by HPLC according to the method of Martínez-Villaluenga et al. (*18*). The HPLC chromatograph (Waters Associates, Milford, MA) consisted of a Waters model 510 pump, a Rheodyne model 7000 sample injector, and a reflection type differential refractometer detector model R410 (Waters). The HPLC system was controlled by a PC running Maxima software (Waters). Chromatography was performed on a Carbohydrate Analysis column (3.9 i.d × 300 mm) (Waters). Acetonitrile/distilled water (75:25 v/v, HPLC grade) was used as the mobile phase at the flow rate of 2.0 mL/min. Solvents were filtered through a 0.45 μm Millipore FH membrane (Bedford, MA) and degassed under helium.

Different amounts of sucrose, glucose, and fructose standards (Merck, Germany) and samples were dissolved in distilled water. Acetonitrile of HPLC grade (Sharlau) was added to each solution to obtain a composition similar to that of the mobile phase. Samples and standard solutions were filtered through a 0.45 μm Millipore FH membrane before analysis, and 100 μL was injected. Quantification of each sugar was performed by comparing the peak areas with those of the standard solutions. Calibration curves were plotted for each sugar and adjusted by using the method of least-squares. The regression coefficients of the curves for di- and monosaccharides were always greater than 0.990.

Cytotoxicity Evaluation. Extracts from lupin seeds and sprouts were obtained as in Frias et al. (*19*). The human HL-60 leukemic cell line was obtained from American Type Culture Collection (Rockville, MD). The culture was maintained on RPMI medium containing 10% fetal calf serum and 1% penicillin G (Sigma, St. Louis, MO) and streptomycin (Sigma), at 37 °C in a humidity-controlled incubator at 90% relative humidity and 5% CO_2. After a few passages, cells were centrifuged, resuspended in fresh medium at the concentration of 0.30 × 10^6 cell/mL, and transferred onto several plates in 2 mL volumes. The cells were exposed to 100 μg/mL of raw and germinated seed extracts and control (sterilized water) during 48 h. Then, trypan blue dye (Sigma) was added to cell cultures in a ratio of 1:1 for 10 min. Twenty microliters of cell suspension was loaded by micropipet into Bürker chambers. The cells were counted under a microscope at 100× magnification. The number of viable cells was determined for cell proliferation approach.

The tetrazolium reduction assay (MTT) was performed in duplicate following the method of Denizot and Lang (*20*). Briefly, 100 μL of the cultured medium was transferred into microcentrifuge tubes and centrifuged for 5 min at 1600 rpm. The supernatants were removed and cells resuspended in 100 μL of fresh medium. The cells were

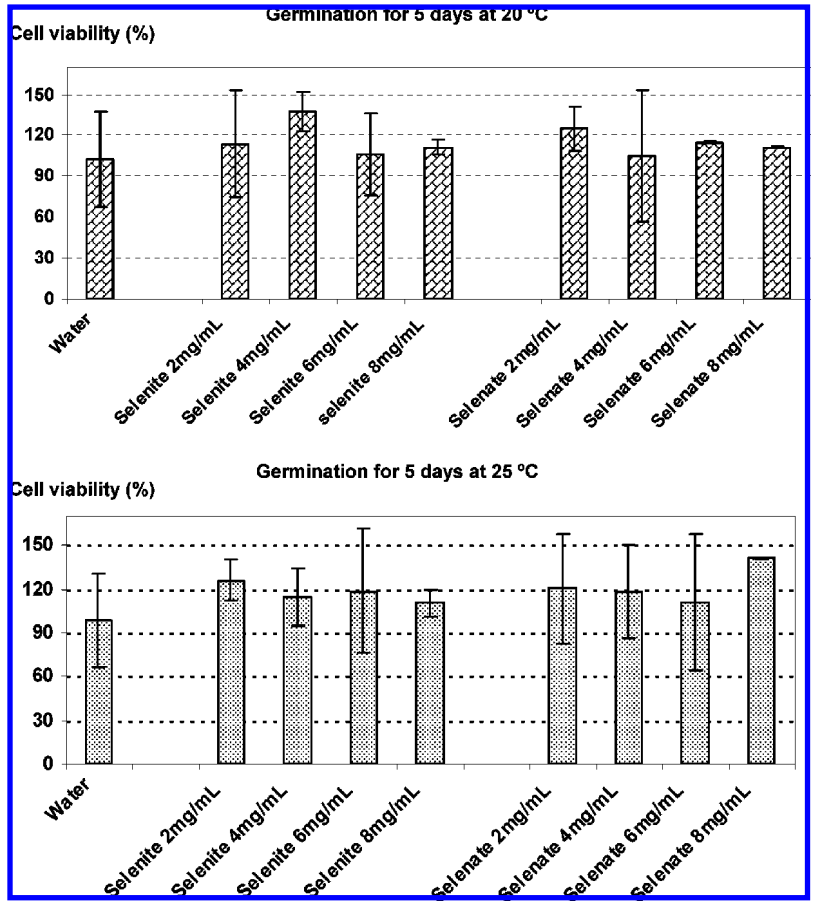

Figure 1. MTT assay performed on HL-60 exposed to extracts from Se-enriched lupin sprouts.

exposed to 100 μg/mL of raw and germinated seed extracts and control (sterilized water) for 24 h. After this time, 20 μL of MTT (Sigma) at a concentration of 5 mg/mL in PBS was added to each sample. All plates were incubated for 4 h at 37 °C in a humidity-controlled incubator at 90% relative humidity, 5% CO_2, and 120 μL of DMSO (Sigma) was added. After 3 h of incubation at 37 °C, samples were centrifuged, and 25 μL of Sorensen buffer (0.1 M glycine, 0.1 M NaCl, pH 10.5) was added to the supernatants. Absorbance of the formazan product was measured at 570 nm, and the results were expressed as percent cell viability.

Statistical Analysis. Data were expressed as means ± standard deviation and subjected to multifactor analysis of variance by applying the least significance difference test using the Statgraphic 4.0 program (Statistical Graphics Corp., Rockville, MD) for Windows.

RESULTS AND DISCUSSION

Effect of Selenium on Germination Efficiency of Lupin Seeds. Table 1 shows the effect of selenite (Na_2SeO_3) and selenate (Na_2SeO_4) salts on the germination rate of lupin seeds after 5 days of germination at 20 and 25 °C. When selenite solution was used, a decrease in the germination rate was observed, which was dose-dependent for lupin sprouts germinated at 20 and 25 °C. The concentration of selenate solution had no effect on the germination rate of lupin seeds when the temperature used was 20 °C. However, a dose-dependent reduction in germination rate values was found in lupin seeds sprouted at 25 °C. The germination rate of lupin seeds with both Se solutions was significantly lower at 25 °C than at 20 °C ($P \leq 0.05$).

Table 2 compiles the effect of selenite (Na_2SeO_3) and selenate (Na_2SeO_4) salts on embryonic axis length after 5 days of germination at 20 and 25 °C. Similarly to germination rates, germination at 20 °C also produced a longer embryonic axis than at 25 °C. A dose-dependent reduction in the sprout length was observed in lupin seeds after 4 days of germination with either selenite or selenate solutions at 20 and 25 °C. No information has been found previously about the effect of Se enrichment on the germination rate or embryonic axis length of sprouts during germination of lupins or other legume seeds.

Table 1. Effect of Selenite (Na_2SeO_3) and Selenate (Na_2SeO_4) Solutions on Germination Rate of Lupin (*L. angustifolius* Cv. Zapaton) Seeds after 5 Days of Germination at 20 and 25 °C[a]

	germination rate (%)	
selenium salt (mg/L)	at 20 °C	at 25 °C
0	94.16 ± 3.03 b	88.51 ± 2.85 c
Na_2SeO_3 solution		
2	89.27 ± 2.78 ab2	81.31 ± 2.62 ab1
4	86.64 ± 2.79 a2	79.64 ± 2.56 ab1
6	85.94 ± 2.76 a2	78.54 ± 2.53 a1
8	85.54 ± 2.75 a2	77.88 ± 2.51 a1
Na_2SeO_4 solution		
2	93.54 ± 3.01 b2	83.82 ± 2.70 b1
4	93.34 ± 3.01 b2	82.17 ± 2.64 ab1
6	93.31 ± 3.01 b2	80.41 ± 2.59 ab1
8	93.29 ± 3.01 b2	77.77 ± 2.50 a1

[a] Mean value ± SD. Different letters/numbers in the same column/row indicate significant differences ($P \leq 0.05$).

Effect of Temperature, Selenium Source, and Concentration on Selenium Uptake during Lupin Seed Germination. The effects of temperature, selenium source, and concentration on total selenium content in lupin sprouts are shown in **Table 3**. Sprouts germinated in water exhibited a Se concentration of 0.14 μg/g of dw. The addition of inorganic Se forms to water significantly increased the Se content in lupin sprouts in a dose-

Table 2. Effect of Germination with Different Selenium Solutions on Embryonic Axis Length (Centimeters) of Lupin (*L. angustifolius* Cv. Zapaton) Seeds after 5 Days of Germination at 20 and 25 °C[a]

selenium salt (mg/L)	sprouts germinated at 20 °C	sprouts germinated at 25 °C
0	7.62 ± 0.25 e	7.14 ± 0.23 d
Na_2SeO_3 solution		
2	7.33 ± 0.24 de2	5.78 ± 0.19 d1
4	6.44 ± 0.21 c2	5.40 ± 0.17 c1
6	6.00 ± 0.19 ab2	4.96 ± 0.16 ab1
8	5.91 ± 0.19 a2	4.79 ± 0.15 a1
Na_2SeO_4 solution		
2	7.36 ± 0.24 de2	5.29 ± 0.17 c1
4	7.06 ± 0.23 d2	5.16 ± 0.17 bc1
6	6.31 ± 0.20 bc2	4.83 ± 0.16 a1
8	5.94 ± 0.19 a2	4.89 ± 0.16 ab1

[a] Mean value ± SD. Different letters/numbers in the same column/row indicate significant differences ($P \leq 0.05$).

Table 3. Selenium Content (Micrograms per Gram of Dry Weight) of Lupin (*Lupin angustifolius* Cv. Zapaton) Seeds Germinated in Selenite and Selenate Solutions for 5 Days at 20 and 25 °C[a]

	temperature of germination	
selenium salt (mg/L)	20 °C	25 °C
0	0.14 ± 0.01 a	0.14 ± 0.01 a
Na_2SeO_3 solution		
2	1.50 ± 0.01 b2	1.13 ± 0.02 b1
4	2.75 ± 0.11 d	2.64 ± 0.22 c
6	4.74 ± 0.05 f	4.89 ± 0.26 e
8	4.79 ± 0.10 f	4.92 ± 0.02 e
Na_2SeO_4 solution		
2	2.21 ± 0.09 c	2.61 ± 0.16 c
4	3.26 ± 0.22 e1	4.50 ± 0.12 d2
6	8.82 ± 0.40 g	8.64 ± 0.02 f
8	10.97 ± 0.08 h1	13.51 ± 0.04 g2

[a] Mean value ± SD. Different letters/numbers in the same column/row indicate significant differences ($P \leq 0.05$).

Table 4. Total Antioxidant Capacity TEAC (Micromoles of Trolox per Gram of Dry Weight) of Lupin Seeds Germinated in Selenite and Selenate Solutions for 5 Days at 20 and 25 °C[a]

	temperature of germination	
selenium solution (mg/L)	20 °C	25 °C
0	68.40 ± 1.79 a	68.11 ± 1.07 a
Na_2SeO_3 solution		
2	76.20 ± 4.10 b	73.35 ± 1.22 b
4	76.62 ± 3.19 bc1	88.41 ± 0.59 d2
6	79.97 ± 1.44 bcd1	89.55 ± 0.77 d2
8	117.81 ± 2.43 f2	105.18 ± 3.36 e1
Na_2SeO_4 solution		
2	75.64 ± 0.39 bc	69.05 ± 2.69 a
4	81.05 ± 1.70 cd2	73.10 ± 1.36 b1
6	84.56 ± 1.55 d2	77.77 ± 0.59 c1
8	103.51 ± 2.67 e2	80.36 ± 2.66 c1

[a] Mean value ± SD. Different letters/numbers in the same column/row indicate significant differences ($P \leq 0.05$).

dependent manner. Significantly higher Se uptake was observed when selenate solution was used, and Se concentrations reached 11 and 14 μg/g of dw, respectively. Germination temperature had an effect on Se uptake when the process was carried out in the presence of 4 and 8 mg/L of selenate solutions in which the Se content was significantly ($P \leq 0.05$) higher at 20 °C than at 25 °C.

Selenate is known to be more readily taken up by plants than selenite, as observed by Ximenez-Embun et al. (*21*) in Indian mustard, sunflower, and lupin, which agrees with the results presented in this work. Pedrero et al. (*22*) observed that Se-enriched broccoli obtained by hydroponic culture and exposed to selenite solutions for up to 40 days showed an increase in the total Se content in the plant and was higher in roots than in stems.

Plant species may vary widely in Se uptake and accumulation. Se enrichment of lupin sprouts in the present work was lower than in other sprouts reported in the literature. Rye grain treated with selenite during germination has been shown to accumulate 55 μg of Se/g of dw (*10*). Wheat and alfalfa germination enriched Se up to concentrations of 100 and 150 μg/g of dw, respectively (*9*).

Plants convert Se mainly into selenomethionine (Se-Met) and incorporate it into protein instead of methionine. Se-Met is the major selenocompound in cereal grains, grassland legumes, and soybeans, whereas selenomethylcysteine (SeMCys) is the major selenocompound in Se-enriched plants such as garlic, onions, sprouts, and wild leeks (*3*). Pedrero et al. (*22*) observed that in Se-enriched broccoli, Se-Met was the major species found in roots, whereas selenomethylselenocysteine was the main species found in fruit. However, plants may accumulate selenium in other forms. Whanger (*23*) identified selenocompounds in plants as selenate, selenite, SeCys, Se-Met, selenohomocysteine, SeMCys, Se-Met selenoxide, Se-methyl-Se-Met, selenocystathione, dimethyl diselenide, selenosinigrin, selenopeptide, and senowax. In this preliminary work we do not study the identification of selenocompounds accumulated during germination of lupin seeds, and it has been focused only on the total Se content of sprouts.

Taking into consideration that consumption of about 30 g of germinated lupin (in dry weight basis) is equivalent to 300 g of fresh product, Se-enriched sprouts obtained by germination with the addition of selenate solutions at 8 mg/L covers the recommended daily intake of Se established by the WHO as 40 μg/day for men and 30 μg/day for women and is below the maximum total safe dietary intake of Se level (400 μg/day).

Total Antioxidant Activity of Se-Enriched Lupin Sprouts. Total antioxidant activities measured as TEAC of Se-enriched sprouts obtained by germination in selenite and selenate solutions for 5 days at 20 and 25 °C are shown in **Table 4**. TEAC values represent the cumulative ability of hydrophilic antioxidants from sprouted lupin extracts to scavenge $ABTS^{\bullet+}$ cation radicals compared to Trolox. Germination has been shown as a process that improves the antioxidant activity of legumes (*24*, *25*). The Se-enriched lupin sprouts presented higher TEAC than lupins germinated with water. When the process was conducted at 20 °C, the total antioxidant activity was similar ($P \leq 0.05$) for both selenium solutions, except with 8 mg/L selenite solution, which produced the highest TEAC level (117 μmol of Trolox/g of dw). Lupin sprouts obtained with selenite solutions at 25 °C showed higher TEAC values in comparison with selenate solutions (**Table 4**). Se content was positively correlated with TEAC values in lupin sprouts using selenite solutions either at 20 or at 25 °C ($r = 0.59$ and 0.90, respectively) and selenate solutions either at 20 or at 25 °C ($r = 0.84$ and 0.89, respectively).

All of these results show that the higher antioxidant capacity found in Se-enriched lupin sprouts may be related to the biotransformation of inorganic Se forms in compounds able to scavenge $ABTS^{\bullet+}$ cation radicals. Because selenate cannot be oxidized during the scavenging reaction of $ABTS^{\bullet+}$, our results agree with Flohe (*26*), who reported that plants accumulate selenium as analogues of methylcysteine, γ-glutamylocysteine, cystatonine, and adenozylomethionine, some of which may exhibit free radical scavenging activity.

Table 5. Soluble Carbohydrate Content (Grams per 100 g of Dry Weight) in Lupin Sprouts Obtained by Germination in Selenite and Selenate Solutions for 5 Days at 20 and 25 °C[a]

	sprouts germinated at 20 °C			sprouts germinated at 25 °C		
selenium solution (mg/L)	sucrose	glucose	fructose	sucrose	glucose	fructose
0	8.64 ± 0.49 a	2.85 ± 0.20 a	3.75 ± 0.25 a	8.73 ± 0.42 a	2.74 ± 0.20 a	3.97 ± 0.34 b
Na_2SeO_3solution						
2	8.55 ± 0.32 a	2.81 ± 0.26 a	3.78 ± 0.28 a	9.19 ± 0.38 abc	2.58 ± 0.12 a	3.62 ± 0.14 ab
4	8.74 ± 0.35 a	2.79 ± 0.26 a	3.69 ± 0.29 a	9.12 ± 0.31 abc	2.63 ± 0.21 a	3.65 ± 0.16 ab
6	8.67 ± 0.41 a	2.80 ± 0.20 a	3.72 ± 0.27 a	9.02 ± 0.35 abc	2.59 ± 0.27 a	3.67 ± 0.31 ab
8	8.62 ± 0.34 a	2.78 ± 0.21 a	3.80 ± 0.24 a	9.51 ± 0.63 bc	2.91 ± 0.35 a	3.72 ± 0.21 ab
Na_2SeO_4solution						
2	8.75 ± 0.28 a1	2.67 ± 0.15 a	3.66 ± 0.33 a	9.54 ± 0.40 bc2	2.80 ± 0.32 a	3.85 ± 0.10 ab
4	8.74 ± 0.36 a	2.85 ± 0.17 a	3.58 ± 0.32 a	9.69 ± 0.61 c	2.72 ± 0.18 a	3.59 ± 0.25 ab
6	8.95 ± 0.35 a	2.79 ± 0.14 a	3.53 ± 0.19 a	9.61 ± 0.51 bc	2.76 ± 0.30 a	3.68 ± 0.19 ab
8	8.94 ± 0.24 a	2.74 ± 0.16 a	3.68 ± 0.28 a	9.69 ± 0.70 c	2.80 ± 0.20 a	3.85 ± 0.15 ab

[a] Mean values ± SD. Different letters/numbers in the same column/row for each carbohydrate indicate statistical differences ($P \leq 0.05$).

Table 6. Amino Acid and Protein Content in Lupin Sprouts Obtained by Germination in Selenite and Selenate Solutions for 5 Days at 20 °C[a]

	Na_2SeO_3 solutions					Na_2SeO_4 solutions			
amino acid	0 mg/L	2 mg/L	4 mg/L	6 mg/L	8 mg/L	2 mg/L	4 mg/L	6 mg/L	8 mg/L
Nonessential Amino Acids (Grams per 100 g of Dry Weight)									
Asp	3.76 a	3.72 a	3.73 a2	3.79 a2	3.76 a2	3.73 a2	3.78 a2	3.74 a2	3.82 a2
Glu	8.52 bc	8.61 c	8.12 a	8.13 a	8.08 a	8.58 c	8.30 ab	8.28 ab	8.26 a
Ser	1.36 d	1.27 bcd	1.21 abc	1.16 ab	1.12 a	1.32 cd	1.23 abcd	1.36 d	1.22 abc
Gly	2.10 ab	2.15 ab	2.10 ab	2.15 ab	2.19 b	2.08 a	2.10 ab	2.15 ab	2.19 b
Arg	3.44 c	3.42 c	3.10 a1	3.08 a	3.04 a	3.41 c	3.12 a	3.33 bc	3.21 ab
Ala	1.47 ab	1.37 a	1.56 bc	1.59 bc	1.55 bc	1.34 a	1.56 bc2	1.67 c	1.59 bc2
Pro	1.27 a	1.17 a	1.22 a	1.26 a	1.28 a	1.19 a	1.19 a	1.23 a	1.24 a
Essential Amino Acids (Grams per 100 g of Dry Weight)									
His	0.41 a	0.42 a	0.50 bc	0.53 c	0.52 bc	0.45 ab	0.54 cd	0.61 de2	0.65 e2
Val	1.76 b	1.80 b	1.62 a	1.60 a	1.59 a	1.76 b	1.76 b	1.73 b	1.76 b
Met + Cys	0.21 ab	0.21 ab	0.23 bc	0.24 c	0.22 abc	0.20 a	0.21 a	0.23 bc	0.22 abc
Ile	0.91 a2	1.20 b	1.26 bc	1.28 bc	1.24 bc	1.21 b	1.24 bc	1.35 cd	1.46 d2
Leu	2.64 ab2	2.66 ab2	2.56 ab	2.52 ab	2.53 ab	2.68 ab	2.48 a	2.55 ab	2.70 b2
Phe	2.02 c	1.95 abc	1.93 ab	1.96 abc	1.89 a	1.98 abc	1.96 abc	1.95 abc	2.06 c
Tyr	1.31 a	1.32 a	1.46 b2	1.45 b	1.42 ab2	1.31 a	1.34 ab	1.41 ab	1.39 ab
Lys	1.87 ab	1.86 ab	2.04 c	1.99 bc	2.01 c2	1.84 a	2.03 c2	2.01 bc2	2.11 c
Thr	1.26 a	1.36 ab	1.36 ab	1.38 b	1.39 b	1.31 ab	1.34 ab	1.36 ab	1.50 c2
Trp	0.57 a	0.66 b	0.71 bc	0.67 b	0.69 bc	0.77 bc2	0.79 bc1	0.82 bc2	0.83 c2
protein (g/100 g of dm)	32.23 a	32.57 a	32.88 a	32.18 a	31.72 a	32.32 a	32.36 a	32.34 a	32.20 a

[a] Mean values. Different letters in the same row indicate statistical differences ($P \leq 0.05$). Different numbers in the same row for each amino acid between Tables 6 and 7 indicate statistical differences ($P \leq 0.05$).

The antioxidant defense includes enzymes such as superoxide dismutase (SOD), catalase (Cat), glutathione peroxidase (GSH-Px), and low molecular weight antioxidants (vitamins A, E, and C, carotenoids, glutathione, uric acid) (*27*). Initially, it was assumed that the antioxidant activity of Se was related to its presence in GSH-Px enzymes. Further studies showed that Se was also present in many other proteins such as thioredoxin reductases (TR, TrxR), which catalyze the reduction of protein thiosulfide bridges, although other functions of selenoproteins remain unclear (*28*).

Soluble Carbohydrate Content in Se-Enriched Lupin Sprouts. Table 5 compiles soluble carbohydrate content in lupin germinated in water and in selenium solutions for 5 days at 20 and 25 °C. Lupin sprouts provided fructose and glucose (3 and 4% dw, respectively), and sucrose was present in a large amount (8.6% dw). Martínez-Villaluenga et al. (*18*) reported the absence of these monosaccharides in raw lupin seeds while they exhibited 3% dw of sucrose and raffinose (0.6% dw), stachyose (4.5% dw), and verbascose (1.4% dw) were also present. When the germination process was carried out at 20 °C, no significant ($P \leq 0.05$) effect on the soluble carbohydrate content was found with the addition of selenite or selenate solutions. However, when germination was conducted at 25 °C, significant differences ($P \leq 0.05$) in sucrose content were found with the highest selenite concentration (8 mg/L) or selenate solutions. In these cases, lupin sprouts exhibited up to 10% more sucrose than control. Germination is a complex metabolic process by which higher carbohydrates are hydrolyzed to disaccharides and monosaccharides. As is well-known, α-galactosides (raffinose, stachyose, and verbascose) present in legumes disappear or decrease as a consequence of germination (*8*, *29*).

Amino Acid Content of Se-Enriched Lupin Sprouts. The amino acid compositions in lupin sprouts obtained with water, selenite, and selenate solutions at 20 and 25 °C are compiled in **Tables 6** and **7**, respectively. In lupin seeds germinated in water (control), nonessential amino acids (NEAA) Asp, Glu, and Arg were present in high amounts. Among the essential amino acids (EAA), high contents of Val, Leu, Phe, and Lys and low amounts of His, Met + Cys, and Trp were found. Germination of lupin seeds in the presence of different inorganic Se solutions did not significantly ($P \leq 0.05$) modify or slightly reduced the content of NEAA, regardless of the Se source, concentration, and germination temperature (**Tables 6** and **7**). However, concerning EAA, Se enrichment during germination caused a significant ($P \leq 0.05$) and dose-dependent increase in His, Ile, and Trp, and this increase was higher at 20 °C when

Table 7. Amino Acid and Protein Content in Lupin Sprouts Obtained by Germination in Selenite and Selenate Solutions for 5 Days at 25 °C[a]

	Na_2SeO_3 solutions					Na_2SeO_4 solutions			
amino acid	0 mg/L	2 mg/L	4 mg/L	6 mg/L	8 mg/L	2 mg/L	4 mg/L	6 mg/L	8 mg/L
				Nonessential Amino Acids (Grams per 100 g of Dry Weight)					
Asp	3.48 a	3.48 a	3.46 a1	3.48 a1	3.50 a1	3.45 a1	3.50 a1	3.53 a1	3.58 a1
Glu	8.56 d	8.62 d	8.36 c	8.20 ab	8.17 a	8.51 d	8.34 bc	8.26 abc	8.20 ab
Ser	1.31 bc	1. 25 abc	1.28 abc	1.20 ab	1.15 a	1.34 bc	1.27 abc	1.35 c	1.26 abc
Gly	2.02 ab	1.99 a	2.06 ab	2.06 ab	2.10 ab	2.11 ab	2.15 ab	2.14 ab	2.18 b
Arg	3.31 a	3.30 a	3.26 a2	3.23 a	3.17 a	3.32 a	3.20 a	3.21 a	3.21 a
Ala	1.42 a	1.35 a	1.44 a	1.50 a	1.50 a	1.37 a	1.40 a1	1.52 a	1.44 a1
Pro	1.22 a	1.18 a	1.16 a	1.18 a	1.25 a	1.21 a	1.22 a	1.22 a	1.24 a
				Essential Amino Acids (Grams per 100 g of Dry Weight)					
His	0.39 a	0.41 ab	0.45 abc	0.50 cd	0.50 cd	0.46 abc	0.52 d	0.48 cd1	0.47 bcd1
Val	1.73 a	1.72 a	1.80 ab	1.80 ab	1.84 b	1.78 ab	1.72 a	1.72 a	1.72 a
Met + Cys	0.21 a	0.21 ab	0.22 abc	0.24 c	0.22 abc	0.21 a	0.21 a	0.23 abc	0.23 bc
Ile	0.87 a1	1.18 ab	1.16 b	1.19 bc	1.20 bc	1.22 bc	1.27 c	1.25 bc	1.26 bc1
Leu	2.54 a1	2.25 a1	2.54 a	2.55 a	2.50 a	2.52 a	2.54 a	2.52 a	2.50 a1
Phe	1.95 a	1.91 a	1.94 a	1.93 a	1.95 a	1.93 a	1.91 a	1.95 a	1.99 a
Tyr	1.26 ab	1.23 a	1.32 abc1	1.36 abc	1.37 bc1	1.34 abc	1.37 abc	1.38 bc	1.44 c
Lys	1.84 a	1.82 a	1.86 a	1.87 a	1.89 a1	1.87 a	1.84 a1	1.87 a1	1.91 a
Thr	1.26 a	1.30 a	1.28 a	1.30 a	1.34 a	1.33 a	1.30 a	1.35 a	1.36 a1
Trp	0.56 a	0.63 b	0.70 c	0.69 c	0.72 c	0.64 b1	0.70 c2	0.72 c1	0.72 c1
protein (g/100 g of dw)	32.15 a	32.63 a	32.53 a	32.18 a	32.65 a	32.00 a	31.83 a	32.06 a	31.88 a

[a] Mean values. Different letters in the same row indicate statistical differences ($P \leq 0.05$). Different numbers in the same row for each amino acid between Tables 6 and 7 indicate statistical differences ($P \leq 0.05$).

selenate solutions were used. Lupin sprouts obtained at 20 °C also brought about a significant rise in Lys and Thr with both Se solutions. Our results agree with Hu et al. (*30*), who found an increment in total and essential amino acid content of green tea sprayed with sodium selenite solutions.

The protein content of lupin sprouts is compiled in **Tables 6** and **7**, and no significant ($P \leq 0.05$) effect of Se uptake was observed during germination.

Cytotoxicity of Se-Enriched Lupin Sprouts on Human HL-60 Leukemia Cells. Figure 1 shows the cell viability performed on HL-60 exposed to extracts from Se-enriched lupin sprouts. Extracts of lupin sprouts obtained in water were used as control. Cell viability after treatment with different extracts of Se-enriched lupin sprouts showed no significant ($P \leq 0.05$) differences compared to control. Information on the cytotoxicity of legume sprouts is very scarce. Extracts from broccoli, radish, alfalfa, and fenugreek seeds germinated in water had no cytotoxic effect on HL-60 leukemic cells (*19*, *31*). It has been reported that Se exhibits a dose-dependent biological−toxicological response, which is strongly dependent upon the chemical form and metabolic activity of each Se compound (*32*). At high concentrations, Se compounds can be either cytotoxic or possibly carcinogenic (*13*). Our results show that Se level uptake in lupin sprouts obtained in the used conditions did not present cytotoxic effects on HL-60 cells.

In conclusion, significant accumulation of selenium was possible in lupin seeds during sprouting in solutions containing selenite and selenate. Se uptake depended on factors such as Se source, dose, and temperature of germination. The highest Se content in lupin sprouts was observed with selenate solutions at 20 °C (11 μg/g of dw) or 25 °C (14 μg/g of dw). Enriched Se sprouts resulted in an improved antioxidant activity, reaching values of 117.8 and 103.5 μmol of Trolox/g of dw, and no cytotoxicity was observed. Lupin seeds germinated with 8 mg/L selenate solutions for 5 days at 20 °C exhibited a higher germination rate (>90%) and higher concentration of some essential amino acids than those obtained in selenite solutions in the same conditions. Therefore, the results provided in this study indicate the possibility of safely enhancing the Se daily intake in humans with a new functional food with high antioxidant activity.

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