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# Assessing the level of key antioxidants in wheat seedlings consecutive sodium selenite treatment

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***Abstract***

The purpose of this study was to quantify the main antioxidant components of wheat seedlings, consecutive sodium selenite treatment at doses of 5 respectively 10 ppm selenium. Was evaluated as the content of total chlorophylls, chlorophylls a, chlorophylls b, carotenes and xanthophylls, vitamin C and antiradical activity of wheat seedlings. Sodium selenite had benefits on seedlings, manifested by a significant increase ($p<0.05$) of chlorophylls levels (until +173.03%), carotene and xanthophylls (until +318.71%), vitamin C (until +1728.33%) and improving of their antiradical capacity (until +600.77%).

***Keywords***: sodium selenite, wheat seedlings, chlorophyll, carotene and xanthophylls, vitamin C, antiradicalic activity

## 1. Introduction

Modern diets include more and more raw foods and functional food products. Germs and seedlings have high biological value through the quality and quantity of nutrients. Seedlings and germs consumption is part of prevention and therapeutic feeding.

Germs are young plants. At this stage of their development, have a higher concen-tration of protein, vitamins, minerals, enzymes, RNA, DNA, bioflavonoids, T cells, etc., than at any other time of their life - even compared with the mature plant [1].

Wheat germs are the embryos of seeds, rich in protein, fiber, polyunsaturated fats, vitamin E, vitamin B1, B2, B6, phosphorus, zinc, thiamine, magnesium and pantothenic acid. Considering the large number of essential nutrients from the cereals germs, they bring many benefits to our health. The germs contains a high amount of natural antioxidants.

Plants contain high concentrations of redox-active antioxidants, such as polyphenols, carotenoids, tocopherols, ascorbic acid and enzymes with antioxidant activity, which fight against hazardous oxidative damages of plant cell components [2-3]. Antioxidants have a significantly role to delay or prevent the oxidation of easily oxidable substrates [4-6]. Therefore, help prevent heart diseases, cancer and aging. Wheat germs is a rich source of natural fiber, essential to maintain the efficient functioning of the intestines [7-9].

Seedlings growing from germs. Sprouted grains are thought of as having exceptional nutritive value. Sprouting of grains causes increased enzyme activity, a loss of total dry matter, an increase in total proteins, a change in aminoacids composition, a decrease in starch, increases in sugars, a slight increase in crude fats and crude fibers, and slightly higher amounts of certain vitamins and minerals [10].

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Chlorophyll is one of strong antioxidant substances. So, chlorophyll adding by food ratio lead on significant decrease of oxidative effects induced by carcinogens. An important property of chlorophyll is the ability to form molecular compact complexes with some chemical substances which are incriminated to determine cancer.

Carotenoids are notable for their wide distribution, structural diversity and very important biological function. Carotenoids are terpenoids synthetized in the plants plastides as hidrocarbons (carotenes) and their oxigenated derivatives (xanthophylls). These compounds confer to the tissues a yellow, orange or red colour [10]. Carote-noids have been credited with other benefi-cial effects on human health: enhancement of the immune response and reduction of the degenerative diseases risk such as cancer, cardiovascular diseases, cataract and macular degeneration [10-11].

Beneficial effects of vitamin C consumption are compelling and familiar to all, but its combination with other antioxidant substances are still being studied. Scientists worldwide are concerned about finding new ways to enrich the level of vitamin C in plants. In the germination of cereals, vitamin C content of seedlings (sprout) increases by 300% against unsprouted grains [12-13]. In wheat sprout are about 2.6-3.0 mg vitamin C/100 g [12].

Along with vitamin C, another antioxidant is selenium. The importance of selenium for living organisms is being increasingly studied. Selenium meet strategic roles in animal bodies. Best known role of selenium in biological systems is the co-factor for GSH-Px, antioxidant enzyme.

## 2. Materials and methods

Seedlings were obtained by germinating of wheat seeds in the presence of sodium selenite. For germination were used textile germination beds. There were three groups, one witness (M) and two experimental (E):

- M (distilled water)
- $E_1$ (5 ppm Se)
- $E_2$ (10 ppm Se)

Application of sodium selenite solution and distilled water it was made 24 in 24 hours and equal volumes for all lots of the same species, seedlings benefit from the same environmental conditions (temperature, humidity, light).

For wheat seedlings it was evaluated the level of chlorophylls a, b and total concentration of vitamin C and antioxidant activity.

*2.1. Determination of chlorophylls* .Chlorophylls content of seedlings was studied by spectrophotometry. The plant material was triturated with acetone 80% in the presence of quartz sand. The homogenate obtained was then centrifuged at 3000 rpm. and the supernatant was collected in a glass containers. The precipitate was reextracted with acetone until the extract became colorless. Supernatants were collected and colori-metred at 646, that 663 nm UV-VIS Perkin Elmer spectrophotometer. Acetone extract obtained was dosed spectrophotometrically against a blank of acetone 80% for the two data wavelengths. Under McKinney-Arron relationship [14], chlorophyll content was quantified as:

$$\text{Chl a} = 12.21 \times (A_{663}) - 2.81 \times (A_{646})$$
$$\text{Chl b} = 20.13 \times (A_{646}) - 5.03 \times (A_{663})$$
$$\text{Chl total} = 17.32 \times (A_{646}) + 7.18 \times (A_{663})$$

where:

Chl a – chlorophyll a, in mg/kg
Chl b – chlorophyll b, in mg/kg
Chl total – total chlorophyll, in mg/kg
$A_{663}$ – sample absorbance at 663 nm
$A_{646}$ – sample absorbance at 646 nm.

*2.2. Determination of carotenes and xanthophylls.* The content of carotenes and xanthophylls (c + x) from seedlings acetone extracts was measured by spectrophotometric method [15-16], at a wavelength of 470, 646, respectively 663 nm and was used for calculating the relationship:

$$carotene + xanthophyll = [(1000 \times A_{470}) - (3.27 \times Chl\ a) - 1.04 \times Chl\ b] / 229$$

where: $A_{470}$ – sample absorbance at 470 nm

*2.3. Determination of vitamin C.* Determination of vitamin C was made with potassium dichromate in the presence of potassium iodide-starch. Initially, vitamin C (ascorbic acid) is oxidized to dehydro-ascorbic acid by iodine. Thereafter, the reaction occurs:

$$Cr_2O_7^{2-} + 6\,I^- + 14\,H^+ = 3\,I_2 + 2\,Cr^{3+} + 7\,H_20$$

Iodine released thus starch stained blue. They weighed a sample on analytical balance, then was brought quantitatively into a titration vessel, were added 10 ml of 2n hydrochloric acid, diluted to 50 ml with distilled water and then triturated, added 1 ml of 1% starch solution (freshly prepared) and 1 ml 0.1 N potassium iodide, after which the solution was titrated with 0.1 N potassium dichromate until persistent blue color. Quantification of vitamin C content was done according to the relation: 1 ml 0.1 N potassium dichromate is equivalent to 0.008806 g vitamin C [17].

*2.4. Assessment of antiradical activity.* The antioxidant activity was determined by spectrophotometrically DPPH method. Acetone extract from each analyzed samples were taken in a stoppered test tube provided over which introduced DPPH solution of acetone and then the mixture was introduced into the mold spectrophoto-meter, using acetone compensation. The absorbance at 517 nm was recorded and changed over time. Recordings were performed with Perkin Elmer spectrophoto-meter, Lambda 25. Based VIS absorption spectra of DPPH solutions of different concentrations to obtain calibration curve: Absorbance (517 nm) = f (c, mM). Thus were prepared DPPH solution of different concentrations that were colorimeters and then was prepared a calibration curve. With the aid of this curve it was calculated the average reaction speed of DPPH with each sample.

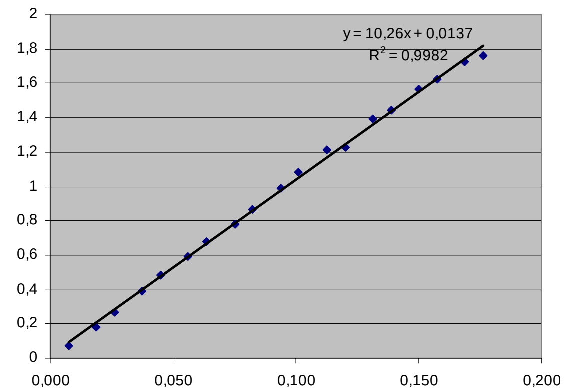


***Figure 1.*** Calibration curve of DPPH Abs = f (c, mM)

To quantify antiradical activity of the analyzed samples, the slope was calculated for solutions of DPPH and the reaction rate was established according to the relationship [18]: v = Δc/Δt.Data were statistically analysed by ANOVA.

## 3. Results and discussions

VIS overlapping curves of wheat acetone extracts are presented in figure 2.

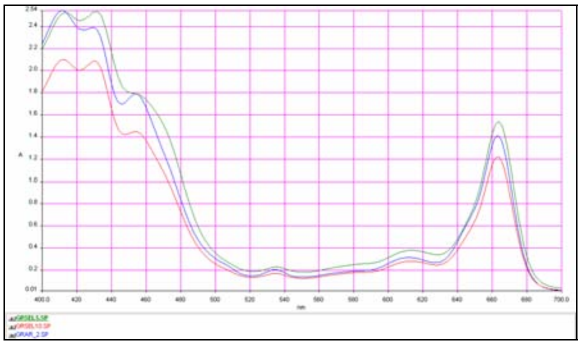

***Figure 2**.* VIS-curves of the wheat extracts after natrium selenite treatment

The results are presented in table 1. and figure 3-9.

***Table 1.*** Antioxidant compounds level and antiradical capacity of wheat sprout obtained from seeds germination in the presence of natrium selenite treatment

| | Specification / Group | M (distilled water) | $E_1$ (5 ppm Se) | $E_2$ (10 ppm Se) |
|---|---|---|---|---|
| X±Sx | Total chlorophyll* | 1,571±0,014 | 4,194±0,005 | 4,812±0,008 |
| | Chlorophyll *a** | 1,313±0,003 | 3,160±0,006 | 3,585±0,010 |
| | Chlorophyll *b** | 0,012±0,002 | 0,008±0,001 | 0,010±0,001 |
| | Carotene and xanthophyll* | 1,5685±0,511 | 2,4738±0,47 | 6,5675±0,66 |
| | Vitamin C** | 0,30±0,01 | 3,25±0,01 | 5,61±0,04 |
| | Antiradical capacity*** | 0,387±0,003 | 2,705±0,003 | 2,155±0,001 |

* mg/g; ** mg/100g; *** *µM/s.*

*3.1.Chlorophylls level.* Chlorophylls level in seedlings obtained from germinated wheat seeds in the presence of sodium selenite is shown in the figures 3-5.

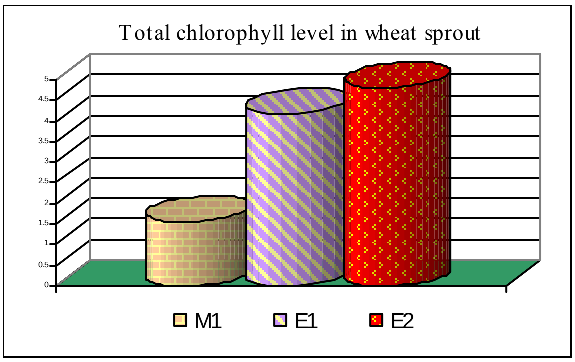


***Figure 3.*** Total chlorophyll level dynamics of sprout obtained by wheat seeds germination in the presence of natrium selenite ite (mg/g)

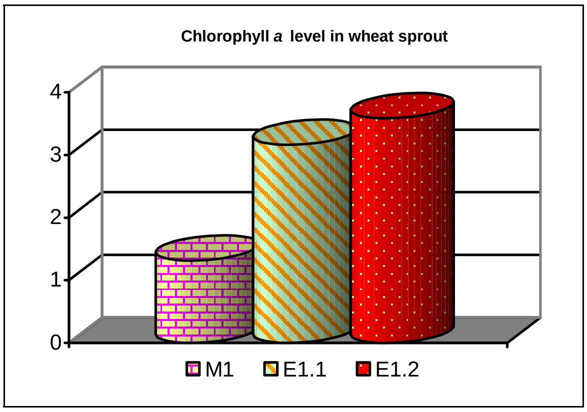


**Figure 4.** Chlorophyll a level of wheat sprout obtained from seeds germination in the presence of natrium selenite (mg/g)

*Total chlorophyll* wheat seedlings obtained by germinating seeds in the presence of sodium selenite (experimental) was significantly ($p<0.05$) higher than the control group ($E_1$/M: +166.96 %; $E_2$/M: +206.3%). Increasing concentrations of selenium, increased the total content of chlorophylls in wheat seedlings ($E_2/E_1$: 14.73%). The data obtained were similar to the results of [19].

*Chlorophyll a* in wheat seedlings obtained by germination after treatment with sodium selenite ($E_1$ şi $E_2$) values showed significant ($p<0.05$) higher than group M - treated with distilled water ($E_1$/M: 140.67%, $E_2$/M: 173.03%). The dose of sodium selenite directly influenced the level of chlorophyll in wheat seedlings ($E_2/E_1$: +13.44%).

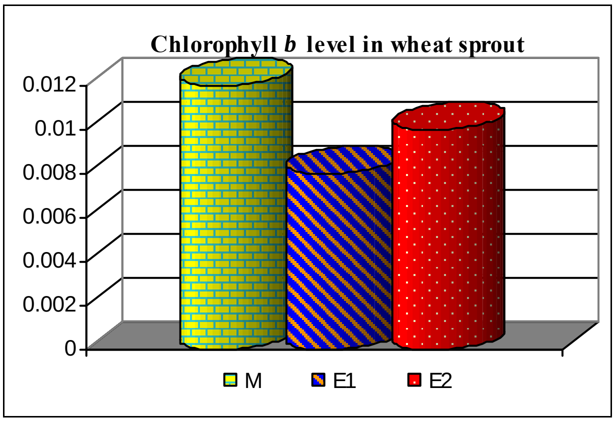


***Figure 5***. Chlorophyll b level of wheat sprout obtained from seed germination in the presence of natrium selenite (mg/g)

*Chlorophyll b*. Treatment with sodium selenite resulted in significant reduction ($p<0.05$) of chlorophyll b content in experimental ($E_1$/M: -33.33%; $E_2$/M: -16.66%).

Chlorophyll b levels were directly correlated with the dose of selenium applied ($E_2/E_1$: +25.0%). In the literature, [19] reported significant increases in levels of total chlorophylls, chlorophyll a and b in wheat seedlings treated with low doses of selenium (0.5 to 1.0 ppm Se).

Hawrylak et al. (2007) [20], after treatment with sodium selenite applied to lettuce (*Lactuca sativa L.),* have reported increased total chlorophylls content in treated plants compared to the control. Increased levels of chlorophylls is probably due to selenium intervention in chlorophyll biosynthesis pathways, particularly as a result of interaction with enzymes containing sulfhydryl group (5-aminolevuinic acid-dehydratase and deaminase porfobilinogen) (Padmaja et al., 1990, quoted by [20]).

### *3.2. The level of carotenes and xanthophylls*

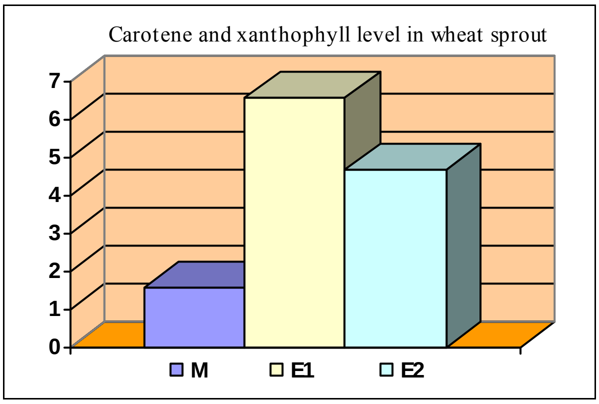


***Figure 6.*** Carotene and xanthophylls level dynamics in wheat shoots obtained by seed germination in the presence of natrium selenite

*Carotene and xanthophylls* in wheat seedlings germinated in the presence of sodium selenite have been significantly elevated ($p<0.05$) in experimental versus control groups. The increases were: $E_1$/M: +318.71%; $E_2$/M: +199.88%. The content of carotenes and xanthophylls was in inverse correlation with the applied selenium as sodium selenite ($E_2/E_1$: -28.37%). The results were correlated with data from literature. Thus, successive treatment with selenium, even in small doses (0.5 to 3.0 ppm), [19] found increased levels of carotenoids in wheat seedlings. A significant increase in carotene content of lettuce found after treatment with sodium selenite at doses of 5 ppm, but the dose of 20 ppm reduced level of carotenoids found in plants [20].

*3.3. The level of vitamin C.* Vitamin C levels in wheat seedlings is shown in the figure 7.

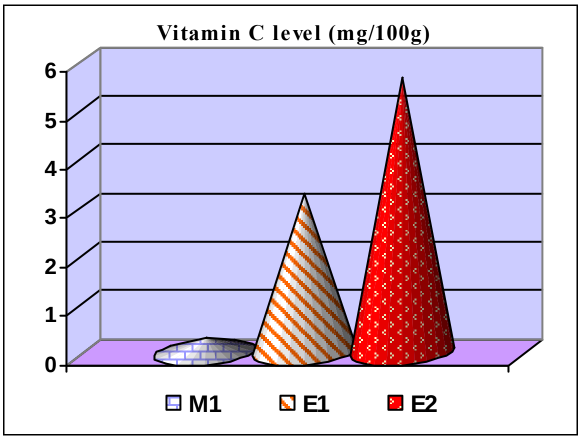


***Figure 7***. Vitamin C level dynamics in wheat shoots obtained by seed germination in the presence of natrium selenite

Selenium intake of wheat $E_1$ and $E_2$ groups led to a significant increase ($p<0.05$) levels of vitamin C in seedlings of these groups, compared with the control group. Increases were: $E_1$/M: +958.63%; $E_2$/M: +1728.33%. Vitamin C level was directly correlated with the dose of selenium administered ($E_2/E_1$: +72.49%).

The results were consistent with data reported by Qingmao et al. (1998) [21] in salad, Jung et al. (2000) [22] in coriander plant and Hu et al. (2001) [23], XU et al., (2003) [24] and Huang et al., (2005) [25], that the treatment with sodium selenite of plants green tea, causes a significant improvement in the level of vitamin C. The level of vitamin C in seedlings of wheat, barley and oats germinated in the presence of sodium selenite was comparable to the level of vitamin C in pears, lettuce, cucumber (3-4 mg/100g), beets, apples, pears, blackberries (5-6 mg/100g) [26].

*3.4. Antiradical activity.* VIS spectra of acetone extracts obtained from seedlings germinated grain are presented in figure 8. Antiradical capacity of wheat seedlings treated with sodium selenite is shown in figure 9.

Antiradical activity (antioxidant) of wheat seedlings germinated in the presence of sodium selenite significantly increased ($p<0.05$) compared to control groups ($E_1$/M: +600.77%; $E_2$/M: +458.29%). The highest antioxidant capacity was observed in groups treated with 5 ppm ($E_1$ - 2.705 μm / s) being treated group followed by 10 ppm ($E_2$ - 2.155 μm/s).

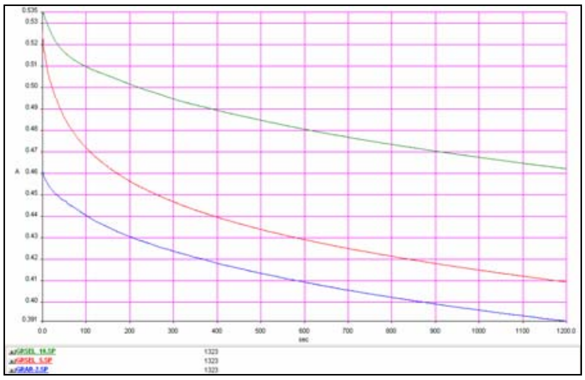

***Figure 8.*** VIS spectra of germinated wheat shoot extracts after selenium treatment

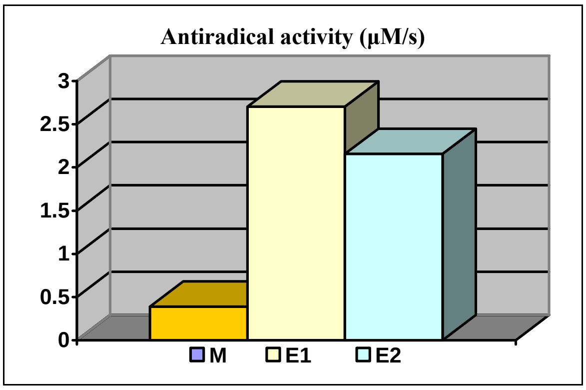


***Figure 9.*** Antiradical activity of wheat shoots obtained by seed germination in the presence of natrium selenite (μM/s)

The antioxidant activity was in inverse correlation with the dose of exposure to selenium ($E_2/E_1$: -20.33%). The results were consistent with those of [24, 27].

Evaluation of the antiradical was studied by Zielinski et al. [28] and de la Luz Mora [29] on seedlings germinated lupine and clover. Their results show an increase in antioxidant activity in the groups treated with selenium.

Antiradical activity of wheat seedlings germinated in the presence of sodium selenite significantly increased ($p<0.05$) compared to control groups ($E_1$/M: +600.77%; $E_2$/M: +458.29%). The highest antiradical capacity was observed in groups treated with 5 ppm ($E_1$ - 2.705 μm/s) being treated group followed by 10 ppm ($E_2$ - 2.155 μm/s).

The antioxidant activity was in inverse correlation with the dose of exposure to selenium ($E_2/E_1$: -20.33%). The results were consistent with those of [24,27]. The possible role of selenium in antioxidant activity increase is explained by its ability to retard plant senescence.

The young plants (seedlings) antioxidant effect of selenium is associated with increased activity of glutathione-peroxidase (GSH-Px). In mature plants, the addition of selenium compounds can improve the antioxidant capacity by preventing the reduction in the level of tocopherols (Hartikainen and Xue, 1999, quoted by [28]) and improve the work-superoxide dismutase (SOD) [29].

Following treatment with sodium selenite, antioxidant activity was positively correlated with vitamin C, correlation simple - Pearson (r) was 0.841. Antiradical activity in wheat seedlings was positively correlated with the experimental groups: vitamin C, carotene and xanthophylls, chlorophylls a and total, but negatively correlated with chlorophyll b. Correlation coefficients between antioxidant compounds in wheat shoots consecutive natrium selenite treatment is shown in table 2.

***Table 2.*** Correlation between mean antioxidant compounds of wheat shoots obtained by seed germination in the presence of natrium selenite

| | *AA* | *Vit C* | *C+X* | *chl tot* | *chl a* | *chl b* |
|---|---|---|---|---|---|---|
| AA | 1 | | | | | |
| Vit C | 0,841 | 1 | | | | |
| C+X | 0,969 | 0,732 | 1 | | | |
| chl tot | 0,617 | 0,691 | 0,683 | 1 | | |
| chl a | 0,621 | 0,693 | 0,687 | 0,999 | 1 | |
| chl b | -0,682 | -0,593 | -0,51 | 0,092 | 0,088 | 1 |

## 4. Conclusions

Research on the content of compounds with antioxidant activity and antiradical ability of wheat seedlings from seeds germinated in the presence of sodium selenite showed:

- higher concentrations of a, b and total chlorophylls at the seedlings germinated in the presence of sodium selenite compared with control groups and in direct correlation with the level of selenium;
- higher concentrations of carotenes and xanthophylls from seedlings germinated in the presence of sodium selenite compared with control groups, the inverse correlation with the applied selenium;
- beneficial effect of sodium selenite on the level of vitamin C in wheat seedlings directly correlated with the selenium dose;
- beneficial effect of sodium selenite on the antioxidant activity of wheat seedlings, inversely correlated with the dose of selenium;
- positive correlation between antioxidant activity of seedlings from seeds germinated in the presence of sodium selenite with vitamin C level, the main antioxidant in seedlings;

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