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THE DETERMINATION OF ANTIOXIDANT ACTIVITY OF SELENIUM-ENRICHED WHEAT AND PEA SPROUTS, AS WELL AS THEIR MICROBIOLOGICAL ANALYSIS

Éva BÓDI, Judit REMENYIK, Andrea NEMES, Judit HOMOKI, Ferenc PELES, István FEKETE, Béla KOVÁCS

University of Debrecen, Centre for Agricultural and Applied Economic Sciences, H-4032, Debrecen, Hungary

Corresponding author email: bodieva@agr.unideb.hu

Abstract

In this present study, we prepared selenium-enriched food sprouts, where the antioxidant capacity was analyzed, and we also determined their microbiological status. When we were about to decide which micronutrients to use during treatments, we took into account the fact that we can take in only a small amount of selenium by consuming food. During our research we wanted the following question to be answered: If sprouts are treated with increasing concentration of selenium, does it have any effect on the original antioxidant capacity of the sprouts, which is mainly due to high vitamin content of sprouts.

Furthermore, we think it is important to make microbiological analysis, because germination conditions, for example temperature, pH, all this will create an ideal environment for the growth of microorganisms. So our goal was to determine how the selenium concentration that affects the total plate count, coliform bacteria count and Staphylococcus aureus count of sprouts.

We determined the aboriginal antioxidant capacity of sprout with the PHOTOCHEM chemiluminometer and we applied pour plate technique for the mapping of the mycrobiological state of sprouts.

Experimental results are evaluated, that increasing concentrations of selenite or selenate treatment had an effect primarily on water-soluble antioxidant capacity of sprouts. The water-soluble antioxidant capacity of wheat sprout was much higher than the measured values in pea sprout, this may be linked to what we measured. That is much higher ascorbic acid content in the case of wheat sprout, which is well known as one of the most important compounds with antioxidant properties of wheat sprout. As a result of microbiological research we come to the conclusion, that the highest concentrations of selenite or selenate treatment have a relative significant anti-microbial effect in the case of wheat sprouts. Coliform and total plate count showed no clear decreasing tendency, although the values of treatments in both cases were below the control values.

Key words: *food sprouts, antioxidant capacity, selenium, microbiology.*

INTRODUCTION

The research of compounds with antioxidant properties has been the focus of medicine and food science in recent years (Veres et al., 2005). These are molecules based on specific definition, which are present in small concentration in the system compared to the oxidized substrate molecules and significantly slow down or completely inhibit their oxidation states (Stefanovits-Bányai, 2008; Halliwell and Gutteridge, 1984).

Biological importance of antioxidants lies in its ability to neutralize free radicals, which can arise as a result of variety of enzymatic reactions and adverse external influence. These reactive molecules are not only responsible for the ageing of our organism, but are responsible for various diseases, for example, immune system problems, cardiovascular diseases and cancer development (Veress and Fáry, 2004).

The most important components, which is in the human body's antioxidant defense system part, which can be according to records Cornetti (2009) lipophilic (vitamin E, ß-carotene, vitamin A), hydrophilic (Vitamin C, amino acids, polyphenols), cytosolic antioxidants (Coenzyme Q10) and structural antioxidants.

Several international literature data shows that the subject of our research in consumption of food sprouts play an important role in ensuring the body's antioxidant status, namely same sprouts contain the above-mentioned components in particularly high concentration.

Mainly vitamin C, E and polyphenols content give the significant part of antioxidants in sprouts, as Moriyama and Oba (2004) , Brajdes and Vizireanu (2012) and Yang et al. (2001) have shown during their research.
For example, Fernandez-Oroczo et al. (2006) reported that the germination of lupine seems to be a good way in aspect of increasing antioxidant capacity, because the vitamin C and polyphenols content was significantly increased during germination.
However some trace elements have vital role in the body's antioxidant defense, as component of enzyme which has important functions has in antioxidant'network (Prasad and Kucuk, 2002).
For example selenium, is the component of antioxidant effects glutathione peroxidase (GPx), which neutralize damaging free radicals and other reactive oxygen compounds with hydrogen peroxide and other harmful lipid and phospholipid hydroxides (Al-Kunania et al., 2001), as well as it inhibits DNA'impairment and formation of metabolically active carcinogens (Karag et al., 1998). However the presence of selenium is essential to the functioning of the enzyme, because the function of enzyme suffers disturbance in the absence of selenium (Meister and Anderson, 1983).
Since the above-mentioned fact points this out, that the selenium, than enzyme creator may contribute antioxidants'protection to our body, therefore our goal was to determine in our present experiment, if it is possible to enhance original antioxidant capacity of food sprouts, when the sprouts are grown on selenium solution.
Furthermore, we think it is important to make microbiological analysis, because germination conditions, for example temperature, pH, all this will create an ideal environment for the growth of microorganisms (Cobo Molinos et al., 2009; NACMCF, 1999). So we had the goal to determine, how the concentration of selenium affect the total plate count, coliform bacteria count and Staphylococcus aureus count of sprouts.

MATERIALS AND METHODS

Wheat sprout (*Triticum aestivum*) and green pea sprout (*Pisum sativum*) were used during our examination, which were germinated in selenium solution of increasing concentration**.** The selenium was used in the form of sodium selenite (Na_2SeO_3•$5H_2O$) (Fluka, Buchs, Switzerland) and sodium selenate (Na_2SeO_4) (Sigma-Aldrich, Steinheim, German) dissolved in deionized water. In case of solution, which contains 2 sort of selenium species, we calculated the proper trace element concentration referred to selenium. In the experiment, with regards to selenite and selenate 0.1; 1; 10 mg dm^{-3}, selenium concentrations was applied along with control treatment and distilled water. It took 5 days for wheat sprout to germinate while pea sprout took 4 days.

Determination of antioxidant activity of sprouts.
The sprouts were frozen, lyophilized, pulverized and then 25 mg cm^{-3} concentration solutions were prepared from the sample in Eppendorf tubes to order the investigation of antioxidant activity.
Distilled water was used to prepare the solution and the antioxidant capacity of water soluble (ACW) and methanol was used for the determination of antioxidant capacity of lipid soluble (ACL). In this way, the prepared sample was centrifuged 10 minutes, 2000 U min^{-1} rpm revs, in „2-16 Sartorius" (Sigma) laboratory centrifuge. By this method filtrate was used to obtain supernatant to take the measurement.
The antioxidant capacity was determined using a recently developed based research by Popov and Lewin (1999) using PHOTOCHEM equipment. The PHOTOCHEM applies the method of photochemiluminescencia, where the basic feature includes molecules, what are excited with UV light, which causes free radical reactions played out by a thousand times faster than in normal conditions. Superoxide anions are released from the test mixture, externally added photochemical sensitizers components as a result of excitation, which are eliminated in proportion of the antioxidant activity compounds of sample.
Then the rest of superoxide anions reacts with the specific superoxide anion of a

photochemical detector compound, which was added to the sample, as a result of this reaction the photons were emitted. The instrument measures this specific chemiluminescence issued by photochemical reactions, which in other words determines the antioxidant capacity of the sample indirect way.

Analysis of vitamin C and tocopherol content of sprouts.

The sample preparation to determine the vitamin C content of sprouts was made on the basis of Gyémánt and Kandra (2006). Vitamin C content was determined photometrically at 496 nm from the solution. The reducing property of vitamin C was used for the determination, where the equivalent amount of Fe (II) ions was generated from Fe (III) ions, which form a colored complex with the a,a-dipyridyl reagent.

The tocopherol content of sprouts was determined by high performance liquid chromatography (HPLC). Hexane was used for the extraction of the tocopherol, then one hour of stirring, and filtration, moreover evaporation was performed.

Microbiological analysis

Tryptone-glucose-yeast (TGY) agar medium was used to determine the total plate count of sprouts by MSZ EN ISO 4833:2003 standard. Plates were incubated for 72±3 hours, between aerobic conditions, at 30ºC. Colinstant medium was used to determine coliform count of sprouts by ISO 4832:2006 standard. Time of incubation was 24±2 hours, between aerobic conditions, at 30ºC.

The determination of *Staphylococcus aureus* count was made on the basis MSZ EN ISO 6888-1 (2000) international standard on Baird-Parker agar, supplemented with egg yolk and tellurite emulsion. Plates were incubated for 48±2 hours, between aerobic conditions, on 37ºC. Colonies grown on plates were counted after incubation period. Petri dishes were taken into consideration, were the number of colonies was between 15 and 300.

Statistical method

For the statistical analysis we used One-Way analysis of variance (ANOVA) and Tukey-test. The significance was evaluated at the $P< 0.05$ level. All statistical analyses were performed using SPSS v.13.0.

RESULTS AND DISCUSSIONS

Water-soluble and fat-soluble antioxidant activity of sprouts and their vitamin C, and tocopherol content

The water-soluble antioxidant activity was illustrated in Tables 1-2. This values show that selenite and selenate treatment resulted in similar concentration of antioxidant properties compounds of sprouts.

However, while the selenite treatment increased significantly the antioxidant capacity of both sprouts, then the selenate treatment increased the amount of antioxidants only to 1 mg dm^{-3} treatment and decreased in the case of largest applied treatment, but it was higher than of the control.

On the basis of Tables 1-2, the water-soluble antioxidant capacity of wheat sprouts was much greater than pea sprouts as a conclusion.

Table 1. The water-soluble antioxidant capacity of 4 days old pea sprouts grown on solution containing selenite or selenite, in the case of control; 0.1; 1; 10 mg dm-3 Se treatments (µg mg-1 dry mass), (n=3)

Treatments	Antioxidant capacity of pea sprouts (ACW)	
	Selenite treatment	Selenate treatment
control	0.101^{a}±0.001	0.101^{a}±0.001
0.1	0.238^{a}±0.014	0.201^{a}±0.119
1	0.235^{a}±0.022	0.287^{a}±0.032
10	0.327^{b}±0.131	0.200^{a}±0.006

Table 2. The water-soluble antioxidant capacity of 5 days old wheat sprouts grown on solution containing selenite or selenite, in the case of control; 0.1; 1; 10 mg dm-3 Se treatments (µg mg-1 dry mass), (n=3)

Treatments	Antioxidant capacity of wheat sprouts (ACW)	
	Selenite treatment	Selenate treatment
control	6.42^{a}±0.08	6.42^{a}±0.08
0.1	6.24^{a}±0.60	7.32^{a}±0.61
1	7.05^{a}±0.28	9.83^{b}±1.99
10	7.23^{a}±0.63	9.30^{a}±0.61

We assumed, that this difference in antioxidant capacity can be explained by examining the date of Tables 3-4, which shows that ascorbic acid content of wheat sprouts were lot more higher originally. In our opinion, probably due to the high water-soluble (antioxidant) ascorbic acid content, the antioxidant capacity of wheat sprouts was considerably greater than of pea sprouts.

Measuring fat-soluble antioxidant activity, neither selenite nor selenate treatments did not affect significantly the amount of the fat-

soluble compounds with antioxidant properties in sprouts (Tables 5-6).

It can also be concluded from Tables 5-6, that the value of antioxidant activity of pea sprouts exceeded with few tenths the antioxidant activity of wheat sprouts. We supposed that this result is associated with different tocopherol isomers of sprouts.

Table 3. The ascorbic acid content of 4 days old pea sprouts grown on a solution containing selenite or selenite in the case of control; 0.1; 1; 10 mg dm-3 Se treatments, (mg/100 g), (n=3)

Treatments	Ascorbic acid content of pea sprouts	
	Selenite treatment	Selenate treatment
control	94.0^{a}±6.3	94.0^{a}±6.3
0.1	67.9^{a}±26.7	106^{a}±50
1	89.1^{a}±37.8	141^{a}±137
10	93.6^{a}±19.4	93.6^{a}±6.7

Table 4. The ascorbic acid content of 5 days old wheat sprouts grown on a solution containing selenite or selenite, in the case of control; 0.1; 1; 10 mg dm-3 Se treatments (mg/100 g), (n=3)

Treatments	Ascorbic acid content of wheat sprouts	
	Selenite treatment	Selenate treatment
control	251^{a}±27	251^{a}±27
0.1	451^{b}±87	534^{b}±36
1	572^{c}±50	312^{a}±116
10	259^{a}±64	530^{b}±2

It can also be established based on the tocopherol chromatograms of sprouts (Figures 1-2), that α-isomer was accumulated mainly in the wheat sprouts, beside this their structural analogue, the tocotrienol appeared also on the chromatogram, till γ-isomer dominated in the pea sprouts. Since γ-isomer had higher antioxidant activity because of their chemical structure (Dietrich et al., 2006), this is an explanation, why the measured antioxidant values of pea sprout exceeded with a few tenths the antioxidant activity of wheat sprout.

Table 5. The lipid-soluble antioxidant capacity of 4 days old pea sprouts grown on a solution containing selenite or selenite, in the case of control; 0.1; 1; 10 mg dm-3 Se treatments (µg mg-1 dry mass), (n=3)

Treatments	Antioxidant capacity of wheat sprouts (ACL)	
	Selenite treatment	Selenate treatment
control	1.75^{a}±0.14	1.75^{a}±0.14
0.1	1.53^{a}±0.13	1.43^{b}±0.13
1	1.45^{a}±0.07	1.68^{a}±0.23
10	1.47^{a}±0.28	1.15^{b}±0.17

Table 6. The lipid-soluble antioxidant capacity of 5 days old wheat sprouts grown on a solution containing selenite or selenite, in the case of control; 0.1; 1; 10 mg dm-3 Se treatments (µg mg-1 dry mass), (n=3)

Treatments	Antioxidant capacity of wheat sprouts (ACL)	
	Selenite treatment	Selenate treatment
control	1.03^{a}±0.08	1.03^{a}±0.08
0.1	1.22^{a}±0.09	1.01^{a}±0.18
1	1.16^{a}±0.04	1.23^{a}±0.03
10	1.03^{a}±0.11	1.18^{a}±0.09

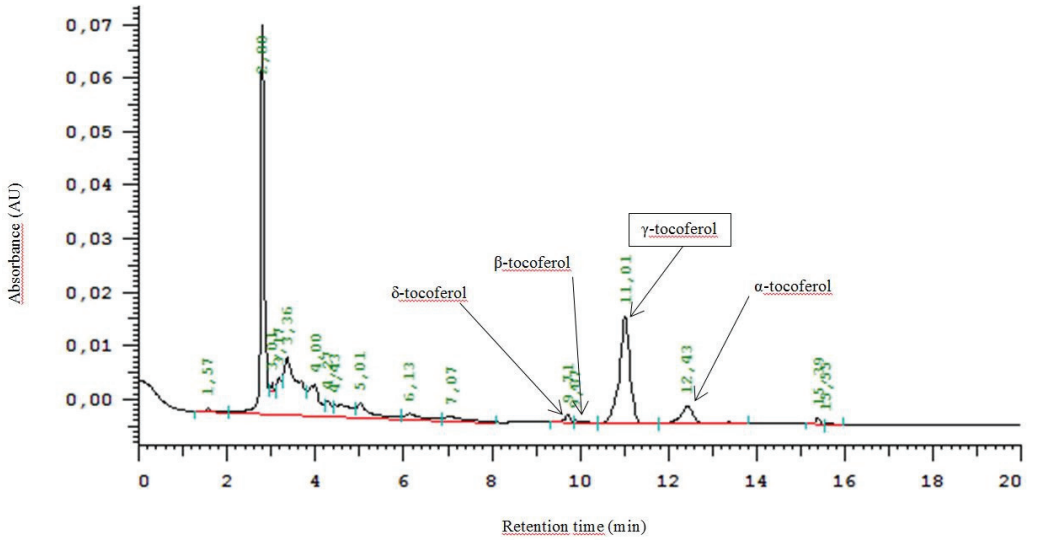

Figure 1. HPLC chromatogram of pea sprouts

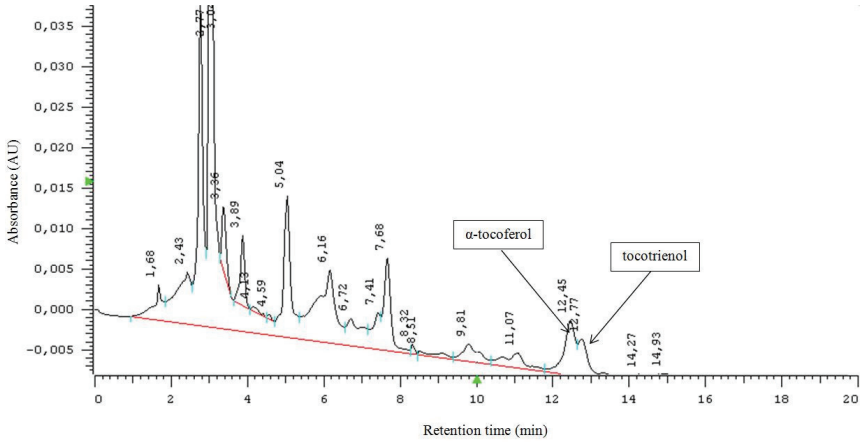

Figure 2. HPLC chromatogram of wheat sprouts

Results of our microbiological analysis.

On the basis of our microbiological analysis it is established that *Staphylococcus aureus* was not present either in control or treated samples.

The results of our microbiological analysis of wheat sprouts are summarized in Tables 9-10. Based on these results, although there was some fluctuation in the coliform and total plate count because of effect of treatments, however there was some significant decrease only between the control and 10 mg dm^{-3} selenite treatments in the case of coliform count, and between the control and 0.1 mg dm^{-3} selenate treatments in the case of total plate count.

The results of our microbiological analyses in the case of wheat sprout are presented in Tables 9-10. The treatments were effective in the case of wheat sprout, namely the coliform count decreased owing to the treatments because the highest concentration of selenite treatment resulted nearly 50 % decreasing of coliform count, while the selenate treatment caused approximately 70 % decreasing of coliform count, compared to the control wheat sprout.

Table 7. Coliform count (103 CFU g-1) of 4 days old pea sprouts grown on a solution containing selenite or selenate, in the case of control; 0.1; 1; 10 mg dm-3 Se treatments (n=3)

Treatments	Coliform count	
	Selenite treatment	Selenate treatment
control	9400^{a}±566	9400^{a}±566
0.1	4600^{a}±849	2900^{a}±1556
1	4700^{a}±1838	6850^{a}±3040
10	1450^{b}±636	4700^{a}±3253

Table 8. Coliform count (103 CFU g-1) of 4 days old pea sprouts grown on a solution containing selenite or selenite, in the case of control; 0.1; 1; 10 mg dm-3 Se treatments (n=3)

Treatments	Ttotal plate count	
	Selenite treatment	Selenate treatment
control	302^{a}±172	302^{a}±172
0.1	133^{a}±83	36^{b}±20
1	124^{a}±74	100^{a}±28
10	120^{a}±69	55^{a}±7

Table 9. Coliform count (103 CFU g-1) of 5 days old wheat sprouts grown on a solution containing selenite or selenate, in the case of control; 0.1; 1; 10 mg dm-3 Se treatments, (n=3)

Treatments	Coliform count	
	Selenite treatment	Selenate treatment
control	42.3^{a}±28.8	42.3^{a}±28.8
0.1	37.7^{a}±15.4	98^{a}±41.6
1	38^{a}±14.6	58.7^{a}±26.9
10	23.2^{a}±14.6	13.9^{b}±5.9

Table 10. Coliform count (103 CFU g-1) of 5 days old wheat sprouts grown on a solution containing selenite or selenate, in the case of control; 0.1; 1; 10 mg dm-3 Se treatments (n=3)

Treatments	Total plate count	
	Selenite treatment	Selenate treatment
control	1653^{a}±145	1653^{a}±145
0.1	1913^{a}±1294	828^{a}±698
1	2443^{a}±929	1193^{a}±634
10	157^{b}±99	521^{a}±82

CONCLUSIONS

Evaluating our experimental results, we concluded that the increasing concentrations of selenite or selenate treatments affected

primarily the water-soluble antioxidant capacity of sprouts. The water-soluble antioxidant capacity of wheat sprout was much higher than the values measured in pea sprout, which can be associated with the fact, that we measured a lot more higher ascorbic acid content in the case of wheat sprout and it is well known that the ascorbic acid is one of the most important antioxidant compound in the wheat sprout. We concluded on the basis of our microbiological results, that the highest concentrations of selenite or selenate treatments had significantly anti-microbial effect in the case of wheat sprouts. However the coliform and total plate count showed no clear decreasing effect, although the values of treatments in both cases obtained were below the actual or control values.

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