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ANTIOXIDANT CAPACITY AND ITS COMPONENTS OF CRUCIFEROUS SPROUTS

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Key words: antioxidant capacity, cruciferous seeds, germination, sprouts, antioxidants

This paper describes the antioxidant capacity of cruciferous sprouts and its components in the course of germination under light conditions. The content of soluble proteins (SP), reduced glutathione (GSH), L-ascorbic acid (AH_2), tocopherols (α-T, β-T, γ-T, δ-T) and total phenolic compounds (TPC), and finally Trolox equivalent antioxidant capacity (TEAC) of the seeds and sprouts were determined in this respect. The results obtained were used for calculating the contribution of these compounds to total antioxidant capacity (TAC) of seeds and sprouts. The TAC of the samples was calculated as the sum of TEAC obtained by ABTS test (formed by TPC and AH_2 presence) plus sum of the antioxidant capacities provided by tocopherols, soluble proteins and GSH. The percentage contribution of TPC was corrected by the content of AH_2 due to the overestimated TPC values determined by Folin-Ciocalteu (FC) reagent, and a simple correction method is shown. Both corrected TPC and AH_2 contribution was above 99%, 83% and 59% in radish, small radish and rapeseeds and about 97%, 73% and 71% in 5-day sprouts, respectively. The contribution of SP, GSH and T (α-T, β-T, γ-T, δ-T) in forming the antioxidant screen of the seeds and sprouts was of a minor importance since it did not exceed 6% in the seeds and approximately 2% in the sprouts collected after the fourth day of germination. This study indicates TPC and AH_2 to be the most important antioxidants in cruciferous sprouts.

INTRODUCTION

One of the new functional foods are sprouts which are a result of one of the leading ways to increase the use of different seeds in human nutrition by the popularisation of their consumption in a germinated form [Kuo & Van Middlesworth, 1988]. Germination is an inexpensive and simple method of improving nutritive value, and several studies have reported higher levels of nutrients and lower values of antinutrients in germinated food seeds and grains compared to the ungerminated originals [Raman, 1984; King & Perwastien, 1987; Honke *et al.,* 1998]. Germination incorporates events that commence with the uptake of water by the quiescent dry seed and terminates with the emergence of the embryonic axis, usually the radicle. It is a time of intense metabolic activity, involving subcellular structural changes, respiration, macromolecular syntheses and, finally, cell elongation. While searching for new sources of functional food, special attention has been paid to sprouts from the cruciferous family that are more and more often used in human diets. Recently, cruciferous sprouts were evaluated for their sensory quality in terms of consumers' acceptance [Troszyńska *et al.,* 2002]. Sensory characteristics of cruciferous sprouts showed that sprouts were acceptable after 4 days of germination. Sprouts are believed to have a greater nutritive value than seeds [Price, 1988] and addition of sprouted seeds to food can further modify its taste and texture [Finley, 1978]. The sprouts may thus become a potential source of nutritious food or food ingredient. However, data compiled on the effect of germination on the antioxidant content and property of cruciferous sprouts as well as their contribution to the total antioxidant capacity are sparse. Little is only known in respect of the germinated lentils and soybean [Zieliński, 2003; Fernandez-Orozco *et al*., 2003]. Therefore, the soluble proteins, reduced glutathione, ascorbic acid, tocopherols and phenolic compounds contents and antioxidant capacity of small radish, radish and rapeseeds during germination under light condition were addressed in this study. Then, the contribution of antioxidants to the total antioxidant capacity of cruciferous seeds and sprouts harvested up to the seventh day of germination was calculated as well.

MATERIALS AND METHODS

Bovine serum albumin (fraction V; BSA), reduced glutathione (γ-glutamyl-cysteinyl-glycine; GSH), oxidized glutathione (GSSG), L-ascorbic acid, metaphosphoric acid, myoglobin from horse heart, (±) catechin, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, U.S.A.). Tocopherol standards (α-T, β-T, γ-T, δ-T) were obtained from Merck and Sigma. All other reagents of reagent-grade quality were from POCh, Gliwice, Poland.

Cruciferous seeds samples including small radish (*Raphanus sativus var. sativus*), radish (*Raphanus sativus* L.) and rapeseed (*Brassica napus var. oleifera*), were obtained from a

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plant breeding station in the North-East Poland. The seeds were stored at room temperature in polyethylene bags until germination.

Cruciferous seeds (25 g) were soaked in 125 mL of distilled water at room temperature and shaken every 30 min. After 4 h the water was drained off and the seeds were transferred to an incubator (Cliambic Cabinet, model Economic Deluxe EC00-065, Snijders Scientific b.v, Netherlands). The seeds were germinated in light at 25°C for up to 7 days. A part of germinated seeds was removed from the incubator every 24 h, frozen in liquid nitrogen and lyophilized. The germination was carried out in triplicate.

The concentration of soluble proteins (SP) in the phosphate buffer extracts was measured using the protein microassay with bovine serum albumin (BSA) as a standard [Bradford, 1976].

Reduced glutathione (GSH) was extracted from the lyophilized samples with 1% methaphosphoric acid to obtain a final concentration of exactly 20 mg/mL. Following mixing by 4 min and centrifugation at 13,000 × g at 4°C for 5 min, the supernatant was diluted 1:10 (v/v) with 100 mmol/L sodium phosphate buffer containing 1 mmol/L EDTA (pH 7.5), giving final pH 7.2. Then, total glutathione (GSx) was determined by the enzyme recycling method modified for use in a microplate reader EF 340 (Biotek Instruments Inc, USA) according to Tietze [1969]. The detailed protocol of the assay was described previously [Zielinski *et al*., 2002]. Data were calculated as μmoles of GSH per gram of dry matter of sprouts.

Ascorbic acid (AH_2) was determined using the HPLC method as described by Oruna-Concha *et al.* [1998]. Fifty milligrams of lyophilized samples were extracted with 5 mL of 5% methaphosphoric acid for two minutes using vortex. Following centrifugation at 13,000 g for 10 min at 4°C in a Beckman GS-15 R centrifuge, 1 mL of the supernatant was extracted with 200 μL HPLC-grade heptane. Finally, the supernatant was centrifuged at 8,000 × g for 10 min at 4°C and then the heptane layer was carefully removed. If the supernatant remained opaque further 200 μL of heptane was added and the procedure was repeated. This lipid extraction step was repeated until the sample was clear. Finally, an aliquot of the acid extract was injected into a chromatographic column. The HPLC apparatus used consisted of a Shimadzu liquid chromatograph equipped with an LC – 10ADvp pump, SPD - 10A detector, Rheodyne 10 μL injection loop and an SCL-10Avp system controller. The data were collected using a computer software Class-8000 v.1.20 (all from Shimadzu Corp. Kyoto, Japan). The analysis was done with a LiChrospher 100 RP-18 column (4 × 250 mm) of particle size 5 μm and a HPLC quard column LiChrospher RP-18 (5 μm). The mobile phase was HPLC grade water brought to pH 2.2 with metaphosphoric acid; the flow rate was 1.0 mL/min, the detection wavelength was 254 nm. Quantitation used the external standard method and concentration of ascorbic acid was determined with reference to a standard curve of 0.1–6.3 μmol/L of AH_2.

Tocopherols (α-T, β-T, γ-T, δ-T) were determined by HPLC after extraction with methanol (0.5 g of lyophilized sample/5 mL of solvent) as previously described [Zieliński, 2003].

Total phenolic compounds (TPC) from the samples were determined according to Shahidi & Naczk [1995] after extraction by 80% methanol (1/10; m/v) for 2 h at room temperature, followed by centrifugation at 12,000 × g at 4°C, and evaporation at 37°C under vacuum. Dry extracts were dissolved in methanol to obtain a concentration of 2.5 mg/mL. Exactly 0.25 mL of aliquot of the methanol extract solution were mixed with 0.25 mL Folin-Ciocalteu reagent (previously diluted with water 1:1 v/v), 0.5 mL of saturated sodium carbonate (Na_2CO_3) solution and 4 mL of water. The mixture was left at room temperature for 25 min and then centrifuged at 2500 × g for 10 min. Supernatant absorbance was measured at 725 nm using a spectrophotometer (UV-160 1PC, Shimadzu, Japan). The results were expressed as (±) catechin equivalents.

The relative abilities of antioxidants to scavenge radical cation $ABTS^{\bullet+}$ were measured by a spectrophotometric technique in comparison with the antioxidant potency of Trolox according to Miller & Rice-Evans [1996]. The radical cation $ABTS^{\bullet+}$, produced by the ferrylmyoglobin radical generated from metmyoglobin (MetMb) and H_2O_2 in the presence of the peroxidase, is a blue/green chromogen with characteristic absorption at 734 nm. The determination of TEAC was carried out as follows: 0.5 mL of ABTS solution (375 μmol/L) was mixed with 0.5 mL of MetMb solution (6.25 μmol/L) and 0.125 mL of 80% methanol extracts. The mixture was pre-incubated for 3 min to 37°C and transferred to a cuvette placed in a temperature-controlled recording spectrophotometer (UV-160 1PC with CPS-Controller, Shimadzu, Japan) adjusted to 37°C. Then, 0.125 mL of H_2O_2 solution (3750 μmol/L) was added at time zero and absorbance was recorded at 734 nm The length of the lag phase before the reaction starts was used for calculating TEAC values. The length of the lag phase was defined as the time noted for the initiation of true color development. The standard curve was constructed with different Trolox solution up to a concentration of 2 mmol/L.

The contribution of SP, GSH, AH_2, T (α-T, β-T, γ-T, δ-T) and TPC to the total antioxidant capacity (TAC) of the germinated seeds was calculated. To this end, the following TEAC values of individual compounds were taken: 0.63 for albumin, 0.90 for reduced glutathione, 0.99 for ascorbic acid, 0.97 for tocopherols and 2.40 for catechin [Rice-Evans & Miller, 1994; Rice-Evans *et al.,* 1996] and confirmed experimentally. The soluble protein contents were recalculated as μmoles of albumin per gram of dry matter of sprouts. Calculation of fraction of antioxidant capacity derived from tocopherols was based upon the presumption that each tocopherol and Trolox possesses a single aromatic -OH, and quenches reactive oxygen species (ROS) on an equimolar basis *in vitro* [Handelman *et al.,* 1999]. The following point of view was taken into account when the contribution of the individual compounds to the total antioxidant capacity(TAC) of the samples was calculated. At first, the mean content of investigated compounds, expressed as μmoles of individual compound per gram of dry matter, was multiplied by their relative potential with respect to Trolox. After that, the result was divided by the TAC values of the seeds or sprouts, and finally expressed as percentage of contribution. The TAC of the samples was calculated as the sum of TEAC obtained by ABTS test (formed by TPC and AH_2 presence) plus sum of the antioxidant capacities provided by tocopherols, soluble proteins and GSH.

RESULTS AND DISCUSSION

Contents of soluble proteins (SP) decreased gradually in a linear fashion during sprouting (Figure 1). All seeds contained a similar level of SP, ranging from 166.3 mg/g d.m. (rapeseed) to 197.5 mg/g d.m. (small radish). A large reduction in SP by approximately 68%-80% was noted in ready-to--eat cruciferous sprouts after 4 days of germination. The term "ready-to-eat" was based on the sensory quality of sprouts evaluated in the previous work [Troszyńska *et al.,* 2002]. Longer germination of up to 7 days caused little changes in SP content, reflecting that the germination of up to 4 days was a time of intense metabolic activity in which seedling was initially supported by metabolites produced by the hydrolysis and conversion of the major stored reserves, proteins, carbohydrates and oils.

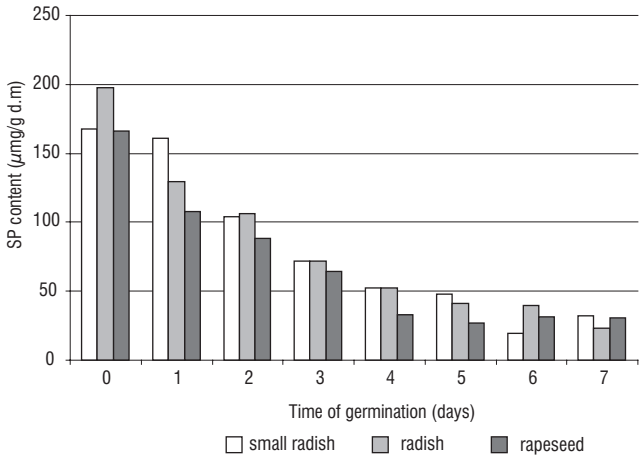

FIGURE 1. Time course of soluble protein changes during germination of cruciferous seeds.

Of the cruciferous seeds studied, rape had the highest GSH content (1.62 μmol/g d.m.), twice higher than that of radish seeds and approximately six times higher than that of small radish (Figure 2). During germination of small radish seeds for up to seven days no changes were found in GSH. In contrast, a progressive fall was found in GSH of the studied radish seeds over the entire germination period (5-fold fall). A similar trend was observed in reduction of GSH contents throughout germination of rapeseeds, as noted with radish, (10-fold fall). Average GSH content in sprouts collected on the fourth, fifth, sixth and seventh day of germination under light conditions was 0.23, 0.18, 0.20 and 0.19 μmol/g d.m. for small radish, rapeseed and radish, respectively. The results obtained indicate that sprouts originated from cruciferous seeds, especially those collected after the fourth day of germination, still represent a good source of GSH with concentrations comparable to these of fresh and cooked vegetables and fresh fruits [Valencia *et al.,* 2001]. They were also two times richer in GSH than hydrothermally processed cereal grains, like wheat, barley, rye and oat [Zieliński & Rzedzicki, 2001]. Reduced glutathione is a primary component of physiological systems responsible for the protection against oxidant and free-radical-mediated injury [Shan *et al.,* 1990]. While the role of glutathione as an important cellular antioxidant is generally known, several aspects of the functions of this compound remain debatable [Bartosz, 1996]. In plants, glutathione and ascorbic acid form the antioxidant system which together with defence enzymes, such as superoxide dismutases, peroxidases, and catalases protect them against damages caused by reactive oxygen species generated during photosynthesis, photorespiration, respiration and other reactions of cellular metabolism [Asada, 1992].

Ascorbic acid is an important component in the fresh and dried food, therefore small radish, radish and rapeseeds and sprouts were analysed for AH_2 content. Of the cruciferous seeds studied, unlike other food grains, radish and small radish had only trace amounts of AH_2 (0.13 and 0.23 μmol/g d.m., respectively) while no AH_2 was found in rapeseed. However, an appreciable amounts of ascorbic acid (1.14–1.41 μmol/g d.m.) was found by other authors in different rapeseed varieties [Sattar *et al.,* 1995]. During sprouting AH_2 content gradually increased with the progress of germination by almost linear fashion and reached the peak on day 5 or 6, depending on the kind of sprout (Figure 3). In this respect, the content of ascorbic acid, estimated on dry weight basis revealed the highest levels in small radish and rapeseed sprouts (31.8 and 28.9 μmol/g d.m., respectively) and then in radish (23.2 μmol/g d.m.).

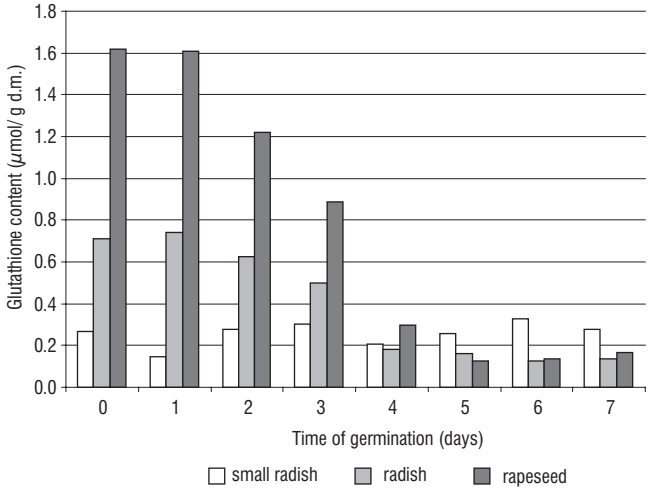

FIGURE 2. Time course of reduced glutathione changes during germination of cruciferous seeds.

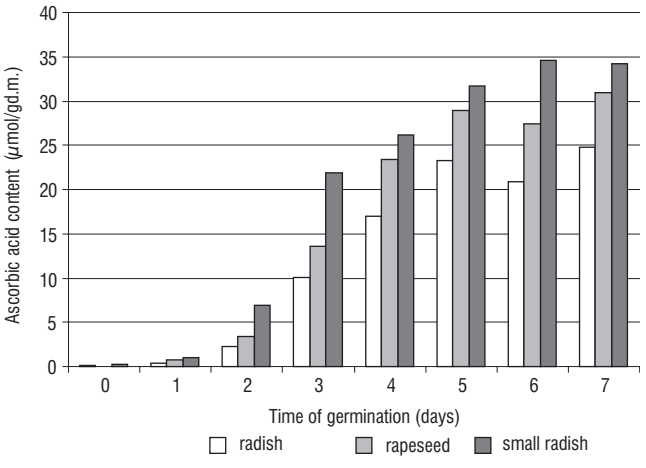

FIGURE 3. Time course of ascorbic acid changes during germination of cruciferous seeds.

TABLE 1. The content of tocopherols (α-T, β-T, γ-T, δ-T) during germination of cruciferous seeds (μmol/g d.m.).

Cruciferae seeds	Day of germination							
	0	1	2	3	4	5	6	7
Radish								
α-T	0.001	0.003 ± 0.001	0.005 ± 0.001	0.069 ± 0.006	0.132 ± 0.019	0.198 ± 0.001	0.205 ± 0.005	0.229 ± 0.003
β-T	0.016	0.020 ± 0.001	0.017 ± 0.002	0.030 ± 0.001	0.025 ± 0.002	0.042 ± 0.001	0.044 ± 0.004	0.059 ± 0.005
γ-T	0.457	0.421 ± 0.011	0.378 ± 0.014	0.540 ± 0.018	0.374 ± 0.014	0.422 ± 0.019	0.417 ± 0.012	0.263 ± 0.014
δ-T	0.084	0.076 ± 0.009	0.058 ± 0.008	0.101 ± 0.003	0.068 ± 0.003	0.086 ± 0.006	0.080 ± 0.006	0.053 ± 0.001
Small radish								
α-T	0.029	0.012 ± 0.001	0.026 ± 0.002	0.033 ± 0.003	0.035 ± 0.004	0.212 ± 0.013	0.197 ± 0.001	0.224 ± 0.009
β-T	0.027	0.016 ± 0.002	0.014 ± 0.002	0.006 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.018 ± 0.002
γ-T	0.629	0.311 ± 0.025	0.214 ± 0.034	0.118 ± 0.013	0.128 ± 0.004	0.146 ± 0.010	0.118 ± 0.012	0.065 ± 0.005
δ-T	0.069	0.031 ± 0.003	0.025 ± 0.004	0.009 ± 0.001	0.004 ± 0.001	0.021 ± 0.001	0.017 ± 0.001	0.012 ± 0.001
Rapeseed								
α-T	0.071	0.108 ± 0.013	0.190 ± 0.009	0.207 ± 0.002	0.222 ± 0.002	0.234 ± 0.006	0.244 ± 0.003	0.247 ± 0.003
β-T	0.028	0.039 ± 0.005	0.082 ± 0.003	0.031 ± 0.003	0.037 ± 0.002	0.033 ± 0.003	0.027 ± 0.004	0.022 ± 0.003
γ-T	0.203	0.265 ± 0.026	0.482 ± 0.004	0.453 ± 0.013	0.276 ± 0.006	0.142 ± 0.016	0.103 ± 0.014	0.073 ± 0.008
δ-T	0.014	0.018 ± 0.004	0.036 ± 0.003	0.029 ± 0.002	0.011 ± 0.002	0.034 ± 0.005	0.051 ± 0.004	0.028 ± 0.003

The data represent the mean values for repeated germination process in triplicate.

These levels of AH_2 were almost stable up to the seventh day of germination. It was shown that germinated cruciferous seeds collected after 4 or 5 days of germination, when the sprouts were ready to eat, had been an excellent source of vitamin C with concentrations comparable to fresh vegetables and fruits [Chauhan *et al.,* 1998] and could be used as components in certain food formulations as well as could represent a new kind of ready-to-eat vegetables designed for direct consumption in a fresh form. Alternatively, the sprouts when dried, could be converted to respective flours by standard procedures and the obtained flour could be employed for the fortification of cereal-based human diets for better nutrition.

The content of tocopherols (α-T, β-T, γ-T, δ-T) during germination of cruciferous seeds is shown in Table 1. In the cruciferous seeds studied, the main vitamin E isomer was γ-T, reaching 82-86% of total tocopherols noted in radish and small radish, and 64% of total tocopherols determined in rapeseeds. The rapeseeds contained the highest amount of α-T, constituting up to 23% of total tocopherols, whereas this isomer made only 4% of total tocopherols in small radish, and trace amount (0.2% of total) in radish seeds. β-T and δ-T were detected in all seeds rather at low levels. The cruciferous seeds showed to be a richer source of tocopherols than cereal grains and legume seeds [Zieliński *et al.,* 2001]. The α-T content in cruciferous sprouts studied increased gradually as the germination time increased by almost linear manner, reaching the nearly stable level between the fifth and seventh day of the process. A similar observation between germination time and α-tocopherol content was found by other authors during germination of wheat grains [Yang *et al.,* 2001]. Since α-T was found in cruciferous seeds at a low level, its production during germination may be taken as an important parameter in the examination of germination process to improve the nutritional value of cruciferous sprouts. The β-T content showed fluctuation in narrow range in the entire germination period. Since γ-T was found to be the main vitamin E isomer in the seeds, its changes may reflect germination as the time of intense metabolic activity. The linear decrease in the γ-T content was noted in germinated small radish. Moreover, the γ-T contents in rapeseeds increased up to the third day of the process, and after that time linearly decreased till the end of germination, reaching a lower level than that noted in the ungerminated seeds (Table 1). It seems that biosynthesis of some vitamin E isomers requires degradation of the others. It was noted that an increase in α-T was accompanied by a decrease in γ-T in germinating seeds. In order to show it, a correlation was studied between α-T and γ-T contents during germination of cruciferous seeds. It was found that the correlation coefficient between α-T *vs*. γ-T content in the course of germination was -0.50, -0.28 and -0.20 for small radish, radish and rapeseeds, respectively.

A slight increase in TPC contents was noted due to the germination for 7 days (Figure 4). In this respect, ready-to-eat rapeseed, radish and small radish sprouts, collected after

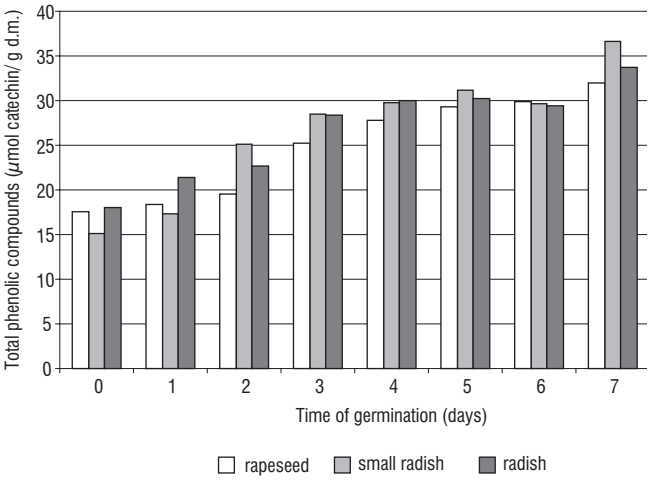

FIGURE 4. Time course of total phenolic compounds changes during germination of cruciferous seeds.

TABLE 2. The Trolox equivalent antioxidant capacity (TEAC) of cruciferous seeds during germination (X ± SD). TEAC determined in 80% methanol extracts.

Day of germination	TEAC (μmol Trolox/g d.m.)		
	Small radish	Radish	Rapeseed
0	40.8	48.6	67.8
1	62.3 ± 6.1	56.3 ± 4.4	74.3 ± 4.3
2	86.2 ± 2.3	52.8 ± 8.6	69.6 ± 3.0
3	79.5 ± 4.8	69.5 ± 7.0	77.9 ± 5.6
4	81.0 ± 8.6	68.8 ± 3.7	76.8 ± 13.7
5	81.2 ± 4.1	70.0 ± 3.3	73.2 ± 3.9
6	77.4 ± 2.6	69.6 ± 11.2	69.4 ± 1.8
7	84.5 ± 8.8	72.0 ± 1.2	67.0 ± 7.0

the fourth day of the process, contained approximately 58%, 66% and 96% more phenolics compounds when compared to the contents noted in the respective seeds. It was previously reported that phenolic acid esters and free phenolic acids constituted as much as 80% and 16% of total phenolic compounds of rapeseeds meals, respectively [Krygier *et al.,* 1982]. Moreover, the presence of flavonoids was reported in cruciferous plant extracts of *Brasica napus* (leaves) and *Sinapis alba* (seeds) [Bjergegaard *et al.,* 1994]. It is common knowledge that synapic acid is the predominant one among other phenolic compounds present in the cruciferous seeds and sprouts. However, in our opinion, total phenolic compounds (TPC) of the seeds and sprouts should be rather expressed as catechin equivalents. It enables a comparison of TPC content of cruciferous sprouts with that of other food of plant origin as well as sprouts obtained from the legume seeds and cereal grains in this respect. Secondly, it makes evaluation of this paper more appropriate when compared to those previously published by our group.

On the basis of the evaluation carried out in 80% methanol extracts, the results of Trolox equivalent antioxidant capacity (TEAC) of germinated cruciferous seeds up to the seventh day of the process are compiled in Table 2. The rapeseeds had higher TEAC (67.8 μmol Trolox/g d.m.) than radish and small radish seeds by approximately 30% and 40%, respectively. The germination up to 4 day influenced mainly TEAC of small radish and radish sprouts. For example, ready-to-eat small radish and radish sprouts showed higher TEAC by 99% and 42% when compared to the seeds, whereas the increase noted in rapeseed sprouts did not exceed 13%. No changes were noted in TEAC due to the germination after the fourth day of sprouting.

The contribution of SP, GSH, AH_2, T (α-T, β-T, γ-T, δ-T) and TPC to the total antioxidant capacity (TAC) of the germinated seeds was calculated, and the total contribution of antioxidants investigated in this study was estimated. The initially calculated sum of contributions of particular antioxidants exceeded 100% for radish, small radish and rapeseed sprouts collected after the third and fourth day of the germination untill the end of the process, respectively. It was suggested that these unexpected results originated from the overestimated TPC values determined by Folin-Ciocalteu reagent (FC). Obviously, the FC reagent is non-specific to phenolic compounds as it can be reduced by many non-phenolic compounds (*e.g.* AH_2, Cu(I), *etc*.) [Huang *et al.,* 2005]. Since a rapid biosynthesis of ascorbic acid was observed during sprouting, additional experiment was performed to show how FC reagent could be reduced by AH_2 under conditions applied for determination of TPC. In this case, separate and mixed catechin and ascorbic acid solutions in 80% methanol were prepared with a concentration ranging from 1 mmol/L to 4 mmol/L. The above concentrations reflected the amounts of AH_2 and TPC found in sprouts. However, for the analytical purposes, these solutions were 5-fold diluted using 80% methanol. There was found a dose-dependent linear relationship between concentrations of individual ascorbic acid solutions, catechin solutions and their mixes *vs.* absorbance of the samples in Folin-Ciocalteu assay (Figure 5). Then, the corrected TPC contribution to TEAC of extract was calculated individually for each sample listed in Table 3 using the following formula:

$$\text{corrected TPC contribution} = \{[TPC-(AH_2{*}K)]{*}AA_C\}/\ TAC$$

where: TPC = not corrected total phenolic compounds in the extract (μmol (±) catechin equivalents/ g d.m.); AH_2 = ascorbic acid in the extract (μmol ascorbic acid /g d.m.); K = ratio of the slope of AH_2 linear regression to the slope of (±) catechin linear regression; AA_C = Trolox equivalent antioxidant activity of (±) catechin (AA_C=2.4); TAC = total antioxidant capacity of the extract calculated as the sum of TEAC obtained by ABTS test (formed by TPC and AH_2 presence) plus sum of the antioxidant capacities provided by tocopherols, soluble proteins and GSH (μmol Trolox/ g d.m.).

The calculated contribution of SP, GSH, AH_2, T (α-T, β-T, γ-T, δ-T) and both corrected and not corrected TPC to the TAC of the germinated seeds is presented in Table 3. It was found that the main contributor in TEAC of the seeds were TPC while in sprouts TPC and AH_2. The average con-

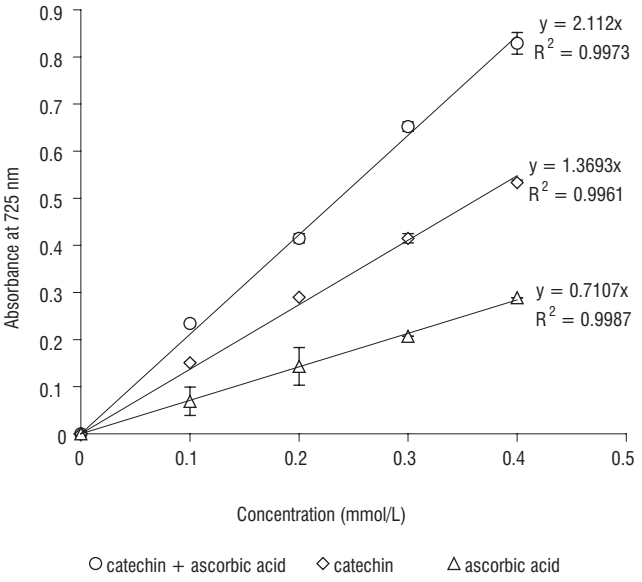

FIGURE 5. Concentration-response curve for the absorbance at 725 nm for ascorbic acid, catechin and a mixture of ascorbic acid and catechin solutions in 80% methanol. Values represent means ± standard deviation (n=3). Linear regression analysis is based on regression through averaged data set at each antioxidant concentration. The formula of linear regression are illustrated in the insert.

TABLE 3. The contribution of tocopherols, phenolic compounds, ascorbic acid, glutathione and soluble proteins to the total antioxidant capacity (TAC) of cruciferous seeds due to the germination (%).

Cruciferae seeds	Day of germination							
	0	1	2	3	4	5	6	7
Radish								
T	1.1	0.9	0.8	1.0	0.8	1.0	1.0	0.8
TPC*	99.3 (99.7)	86.7 (87.6)	94.2 (99.3)	80.3 (95.3)	72.6 (103.0)	61.1 (101.8)	63.0 (99.9)	68.5 (110.9)
AH2	0.2	0.6	4.1	13.9	24.0	32.3	29.2	33.6
GSH	1.2	1.1	1.0	0.6	0.2	0.2	0.2	0.2
SP	3.5	2.0	1.8	0.9	0.7	0.5	0.5	0.3
Total contribution	**105.3**	**91.3**	**101.9**	**96.7**	**98.3**	**95.1**	**93.9**	**103.4**
Small radish								
T	1.7	0.6	0.3	0.2	0.2	0.4	0.4	0.4
TPC*	82.9 (84.1)	62.7 (64.6)	56.3 (68.8)	51.0 (84.9)	41.6 (87.4)	39.8 (87.9)	46.4 (91.2)	50.1 (103.0)
AH2	0.5	1.5	7.7	26.9	31.7	38.2	43.8	39.7
GSH	0.6	0.2	0.3	0.3	0.2	0.3	0.4	0.3
SP	3.5	2.3	1.1	0.8	0.6	0.5	0.2	0.3
Total contribution	**89.2**	**67.3**	**65.7**	**79.2**	**74.3**	**79.2**	**91.2**	**90.8**
Rapeseed								
T	0.4	0.5	1.1	0.9	0.7	0.7	0.6	0.5
TPC*	59.4 (59.4)	55.9 (57.2)	59.2 (64.9)	54.9 (75.9)	41.3 (85.5)	46.6 (94.8)	57.9 (102.3)	56.9 (113.3)
AH2	0	1.0	4.6	16.9	29.7	38.6	38.6	45.1
GSH	2.1	1.9	1.5	1.0	0.3	0.2	0.2	0.2
SP	2.1	1.3	1.1	0.7	0.4	0.3	0.4	0.4
Total contribution	**64.0**	**60.6**	**67.5**	**74.4**	**72.4**	**86.4**	**97.7**	**103.1**

The data represent the means for repeated germination process in triplicate. * The uncorrected TPC contribution is shown in the brackets.

tribution of TPC to the TEAC of sprouts collected between the fourth and the seventh day of germination was higher than the contribution of AH_2 by 2.3 times for radish sprouts, by 1.3 times for rape sprouts, and by only 1.1 times for small radish sprouts. Ascorbic acid contribution in the seeds was negligible while AH_2 contribution in ready-to-eat radish, small radish and rapeseed sprouts was 24%, 32% and 30%, respectively. An excellent agreement was also found in contribution of AH_2 to the TEAC (Table 3) and quantity changes in AH_2 during germination showed on Figure 3. Both AH_2 and TPC formed above 98%, 80% and 78% of overall antioxidant capacity of radish, small radish and rape seeds and sprouts, respectively. The contribution of SP, GSH and T (α-T, β-T, γ-T, δ-T) in forming the antioxidant screen of the seeds and sprouts was of a minor importance since it did not exceed 6% in the seeds. Moreover, the contribution of these compounds decreased during the germination and did not exceed 2% in ready-to-eat sprouts. Our findings are consistent with previously described contribution of low molecular weight antioxidants to the total antioxidant capacity of legume sprouts [Fernandez-Orozco *et al.,* 2003; Zieliński, 2003].

CONCLUSIONS

The data provided in this study indicate that bioactive compounds originating from cruciferous seeds undergo considerable changes during germination as a result of complex physiological and biochemical processes occurring in the sprout formed. It was shown that phenolic compounds and ascorbic acid were the most important antioxidants in cruciferous sprouts obtained during germination. Evidence to date suggests that antioxidants can work synergistically and after consumption contribute to the total antioxidant protection of human body. For this reason, the germinated cruciferous seeds, as avaluable source of antioxidants, should be wider recommended in human nutrition.

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POJEMNOŚĆ PRZECIWUTLENIAJĄCA KIEŁKÓW NASION ROŚLIN KRZYŻOWYCH ORAZ JEJ SKŁADNIKI

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W pracy badano pojemność przeciwutleniającą (TEAC) kiełkowanych do 7 dni w świetle nasion rzodkwi, rzodkiewki i rzepaku, oraz przeanalizowano w uzyskanych kiełkach zawartość białka rozpuszczalnego (SP), zredukowanego glutationu (GSH), kwasu L-askorbinowego (AH_2), tokoferoli (T) oraz związków fenolowych ogółem (TPC). Uzyskane dane wykorzystano do obliczenia udziału tych związków w kształtowaniu całkowitej pojemności przeciwutleniającej (TAC) kiełków. Przedstawiono prostą metodę korekcji udziału związków fenolowych w TAC uwzględniającą zawartość AH_2 w kiełkach. Badania pokazały, że skorygowany udział związków fenolowych oraz kwasu askorbinowego w kształtowaniu pojemności przeciwutleniającej gotowych do spożycia kiełków rzodkwi, rzodkiewki i rzepaku stanowił ponad 97%, 73% i 71% (tab. 3). Udział białek rozpuszczalnych, zredukowanego glutationu oraz tokoferoli w kształtowaniu TEAC nasion nie przekraczał 6%, natomiast w 4 dniowych kiełkach wynosił około 2% (tab. 3). Przeprowadzone badania wskazują, że zarówno związki fenolowe jak i kwas L-askorbinowy stanowią najważniejsze przeciwutleniacze obecne w kiełkach rzodkwi, rzodkiewki i rzepaku.