

## THE CONTENT OF TOCOPHEROLS IN *CRUCIFERAE* SPROUTS

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Germination (in light and dark, at 25°C, for up to 7 days) of cruciferous seeds was studied to determine the optimum conditions to maximize the synthesis of vitamin E in sprouts. In this study, the tocopherol content ( $\alpha$ -T,  $\beta$ -T,  $\gamma$ -T,  $\delta$ -T) was examined in four species of cruciferous seeds: rapeseed (*Brassica napus* var. *oleifera*), white mustard (*oleifera Sinapis alba* L.), radish (*Raphanus sativus* L.), and small radish (*Raphanus sativus* var. *sativus*). Using light conditions during germination of selected cruciferous seeds, the optimal time that would maximize the synthesis of vitamin E was found to be four and five days of germination. Under dark conditions, the optimal germination time was found to be 3 days for small radish and 4 days for radish and rapeseeds. After these times, up to the seventh day of germination, the vitamin E content reached a stable level. A negative correlation between  $\alpha$ -tocopherol and  $\gamma$ -tocopherol content was found for all investigated cruciferous seeds during the germination under light and dark conditions, with only one exception made for rapeseeds. In summary, it was found that a 100-gram portion of individual or mixed fresh cruciferous sprouts is able to cover the Recommended Dietary Allowance for vitamin E in the range of 15.3–21.6% for men and 19.1–29.5% for women.

### INTRODUCTION

While searching for new sources of functional food, special attention has been paid to sprouts from the *Cruciferae* family that are more and more often used in human diets. Sprouts are believed to have a greater nutritive value than seeds [Price, 1988]. The 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 a food ingredient. Apart from well-known nutrients such as tocopherols and ascorbic acid [Zieliński *et al.*, 2002 a], cruciferous seeds contain a wide range of non-nutrients, which exert a beneficial effect on human health. The major part of non-nutrients contains compounds with valuable antioxidative properties, including phenolic compounds and glutathione [Zieliński *et al.*, 2002 b].

Vitamin E is an essential nutrient for animals and humans since it is not synthesized in the body. A growing body of evidence indicates that a low intake of vitamin E is associated with a significantly increased risk of chronic diseases such as cardiovascular diseases and cancer. Epidemiological studies strongly indicate that a high dietary vitamin E intake protects against the development of these chronic diseases [Kelly, 1997]. Vitamin E appears to protect against atherosclerotic disease by inhibiting the oxidation of low-density lipoproteins. The influence of this antioxidant on cancer development is probably linked with its effects on the immune function. Vitamin E comprises all naturally occurring tocopherols (T) and tocotrienols (T3).

The biological activity of various tocopherols differs and declines in the order:  $\alpha$ -T >  $\beta$ -T >  $\gamma$ -T >  $\delta$ -T [Bourgeois, 1992], however, their antioxidant activity *in vitro* has been reported as follows:  $\alpha$ -T <  $\beta$ -T <  $\gamma$ -T <  $\delta$ -T [Belitz & Grosch, 1987]. Early *in vitro* studies showed a clear superiority of  $\alpha$ -T for trapping peroxy radicals followed by  $\beta$ -T,  $\gamma$ -T and  $\delta$ -T. Their relative reactivities with peroxy radicals were 100/60/25/27 [Burton & Ingold, 1981] and seemed to account in large part for their relative vitamin E activity. However, later studies showed that their relative reactivities in trapping singlet oxygen were 100/100/76/34 for  $\alpha$ -T/  $\beta$ -T/  $\gamma$ -T/  $\delta$ -T [Kaiser *et al.*, 1990], while  $\gamma$ -T was reported to be superior to  $\alpha$ -T in quenching nitrogen dioxide and peroxy nitrite radicals [Cooney *et al.*, 1993; Christen *et al.*, 1997]. The provided evidences show that the antioxidant activity of various tocopherols *in vitro* seems to be depend on the kind of reactive oxygen species and sources generating such species. The bio-availability of dietary vitamin E is affected by differences among ingested forms, processing methods, physiological factors (*e.g.* nutritional status), drugs and other dietary components [Erdman *et al.*, 1988]. This study was merited since the potential of dietary antioxidants to reduce the role of chronic diseases, such as coronary heart disease and other malignant diseases, is gaining a great deal of interest in the medical and scientific communities [Diplock, 1987; Burton & Traber, 1990; Gey, 1993; Cohn, 1997; Acuff *et al.*, 1994].

Previously, we showed that during germination, the ascorbic acid content was gradually increased as the germination time increased by an almost linear characteri-

stic curve and reached the peaks at day 4, depending upon the cruciferous seeds [Zieliński *et al.*, 2002 a]. Moreover, the amounts of ascorbic acid in the sprouts were a slightly higher when compared to the germination in the dark. In contrast, overall, germination of cruciferous seeds in the dark resulted in higher concentrations of GSH in the sprouts when compared with light conditions [Zieliński *et al.*, 2002 b]. It seems to be that ascorbic acid biosynthesis is partially required for preventing tocopherols against oxidation. Ascorbic acid, on the other hand, requires cellular thiols such as glutathione in order to be regenerated from its radical form [Basu, 1999].

Knowledge of the content of vitamin E in sprouts from cruciferous seeds is also important from the dietary point of view.  $\gamma$ -Tocopherol was found to be the main contributor of vitamin E content in cruciferous sprouts. This homologue possesses only 10–20% of the vitamin E activity of  $\alpha$ -tocopherol in bioassays, although it displays a similar efficiency as an antioxidant. Despite a higher dietary intake of  $\gamma$ -tocopherol than  $\alpha$ -tocopherol and similar rates of gastrointestinal absorption, the steady state concentrations of  $\gamma$ -tocopherol in plasma and tissue average 10–20% of these of  $\alpha$ -tocopherol [Cohn, 1997]. For this reason, the first objective of this study was to determine the content of tocopherols in selected germinated cruciferous seeds as a plant derived-diet. The second objective was to examine the germination time and light conditions to determine the optimum conditions to maximize the synthesis of vitamin E calculated within this paper as d- $\alpha$ -tocopherol equivalents ( $\alpha$ -TE) originating from the tocopherols being analysed.

## MATERIALS AND METHODS

**Material.** Single cruciferous seed samples were obtained from a local plant breeding station in the north-eastern Poland. The samples included rapeseed (*Brassica napus* var. *oleifera*), white mustard (*Sinapis alba* L.), radish (*Raphanus sativus* L.), and small radish (*Raphanus sativus* var. *sativus*).

**Seed germination.** The germination was carried out in an incubator (Cliambic cabinet, model Economic Delux EC00-065, Snijders Scientific b.v, Netherlands). The seeds were germinated in light and dark, at 25°C, for up to 7 days.

The seeds were layered over a moist filter paper (qualitative medium-speed filter paper) to one-third of the depth of the paper. Germinated seeds from each species were removed from the incubator every 24 h, frozen in liquid nitrogen and lyophilized. Germination was carried out in triplicate and tocopherol analysis in duplicate of each germination completed. All samples were also analysed in duplicate for dry matter, protein, fat and ash using the methods of the AOAC [1990] and, following this, the carbohydrate content was calculated.

**Analysis of tocopherols.** Tocopherols ( $\alpha$ -T,  $\beta$ -T,  $\gamma$ -T,  $\delta$ -T) were extracted by methanol (0.5 g of lyophilized sample/5 mL of solvent) for one minute using a Polytron homogeniser at full speed, at room temperature. The solvent was decanted after centrifuging (2000 g, 10 min), and the extraction was repeated on the residue using the same volume of solvent. The combined supernatants were evaporated in a rotary evaporator under vacuum, and then evaporated extracts were re-dissolved in 2 mL of n-hexane [Peterson, 1994]. The tocopherols were separated by high-performance liquid chromatography (HPLC) on Lichrospher Si 60 5- $\mu$ m particle size, 4  $\times$  250-mm column (Merck), according to the method described by Paterson and Qureshi [Paterson & Qureshi, 1993]. Twenty microlitres of each sample were injected into the column. The HPLC systems consisted of a Shimadzu model LC pump series 10 AD, and a Shimadzu RF-535 fluorescence detector. The mobile phase was 0.5% isopropanol in hexane. The flow rate was 1 mL/min, and the peaks were detected using an excitation wavelength of 295 nm and an emission wavelength of 330 nm. The tocopherol contents were calculated from the peak areas using the standard curves of tocopherols ( $\alpha$ -T,  $\beta$ -T,  $\gamma$ -T,  $\delta$ -T) obtained from Merck and Sigma. The vitamin E content, expressed in d- $\alpha$ -tocopherol equivalents ( $\alpha$ -TE) was calculated using the current biological activities of 1.0 for  $\alpha$ -T, 0.5 for  $\beta$ -T, 0.1 for  $\gamma$ -T, and 0.03 for  $\delta$ -T [Czajka-Marins, 1996; Eitenmiller *et al.*, 1998].

## RESULTS AND DISCUSSION

The contents of protein, ash, fat and carbohydrates means of lyophilized cruciferous seeds are presented in Table 1. Of the cruciferous seeds studied, rapeseeds had the

TABLE 1. The content of moisture, water, protein, ash, fat and carbohydrates means (%) in lyophilized cruciferous seeds and ready-to-eat lyophilized cruciferous sprouts collected after 4-day germination under dark (D) and light conditions (L).

Source	Moisture	Protein	Ash	Fat	Carbohydrates
Radish seeds	3.8 $\pm$ 0.1	28.6 $\pm$ 0.1	3.7 $\pm$ 0.1	37.5 $\pm$ 0.8	26.4 $\pm$ 0.8
Radish sprouts (D)	6.2 $\pm$ 0.3	28.3 $\pm$ 0.7	3.9 $\pm$ 0.5	28.7 $\pm$ 2.5	32.9 $\pm$ 3.0
Radish sprouts (L)	5.2 $\pm$ 0.2	28.5 $\pm$ 0.5	3.8 $\pm$ 0.5	28.2 $\pm$ 3.1	34.3 $\pm$ 2.2
Small radish seeds	3.6 $\pm$ 0.1	26.4 $\pm$ 0.1	4.7 $\pm$ 0.1	38.1 $\pm$ 0.3	27.3 $\pm$ 0.2
Small radish sprouts (D)	6.6 $\pm$ 0.6	27.6 $\pm$ 1.6	4.3 $\pm$ 0.4	23.5 $\pm$ 2.4	38.1 $\pm$ 1.9
Small radish sprouts (L)	5.5 $\pm$ 0.2	27.9 $\pm$ 1.7	5.0 $\pm$ 0.1	24.8 $\pm$ 0.5	37.9 $\pm$ 3.9
White mustard seeds	4.7 $\pm$ 0.1	28.5 $\pm$ 0.4	4.9 $\pm$ 0.1	27.3 $\pm$ 0.1	34.6 $\pm$ 0.6
White mustard sprouts (D)	4.9 $\pm$ 0.8	32.7 $\pm$ 0.4	5.2 $\pm$ 0.3	21.6 $\pm$ 6.1	35.6 $\pm$ 5.4
White mustard sprouts (L)	4.7 $\pm$ 0.9	32.6 $\pm$ 0.4	5.3 $\pm$ 0.2	21.0 $\pm$ 4.7	36.3 $\pm$ 3.4
Rapeseeds	3.8 $\pm$ 0.1	21.6 $\pm$ 0.5	3.7 $\pm$ 0.1	42.9 $\pm$ 0.6	28.1 $\pm$ 0.9
Rapeseed sprouts (D)	4.6 $\pm$ 0.4	20.8 $\pm$ 0.3	3.8 $\pm$ 0.5	32.6 $\pm$ 2.1	38.3 $\pm$ 2.7
Rapeseed sprouts (L)	4.3 $\pm$ 0.7	20.9 $\pm$ 0.5	3.9 $\pm$ 0.1	31.7 $\pm$ 0.7	39.2 $\pm$ 0.5

highest fat content, approximately 57%, 14% and 13% higher when compared to white mustard, radish and small radish seeds. Rapeseeds had also had the lowest content of protein, which was approximately 24% lower in respect to the remaining seeds (Table 1). The ash and carbohydrate contents ranged between 3.65–4.92% and 26.42–43.57%, respectively, showing the highest content for white mustard seeds. Because of the high fat content in these oil seeds and a the well-known antioxidant role of tocopherols in rancidity of edible oils from vegetable sources, higher levels of tocopherols can be expected when compared to the legume seeds and cereal grains [Grela *et al.*, 1993; Martinez De La Cuesta *et al.*, 1996]. Unfortunately, the cruciferous seeds are not eaten in a raw or cooked form. Since consumption of these seeds as sprouts has been popularized (germination is an inexpensive and simple method of improving nutritive value), the impact of the germination process (in light and dark, at 25°C, for up to 7 days) on their tocopherol content was addressed.

The content of tocopherols ( $\alpha$ -T,  $\beta$ -T,  $\gamma$ -T,  $\delta$ -T) during germination of cruciferous seeds under dark and light conditions is shown in Table 2 and Table 3. Of the cruciferous seeds studied, the main dominant tocopherol homologue was  $\gamma$ -T, reaching 82–86% of total tocopherols noted in radish, small radish and white mustard, and 64% of total tocopherols determined in rapeseeds. The rapeseeds contained the highest amount of  $\alpha$ -T, constituting up to 23% of total tocopherols whereas only 4% of the total tocopherols were present in small radish and white mustard seeds, and trace amounts (0.2% of the total) were detected in radish seeds.  $\beta$ -T and  $\delta$ -T were detected in all seeds, showing a rather low level. When vitamin E content was expressed as  $\alpha$ -TE, the highest content was found in rapeseeds (4.49 mg  $\alpha$ -TE/100 g d.m.) and small radish seeds (4.52 mg  $\alpha$ -TE/100 g d.m.), followed by white mustard (3.52 mg  $\alpha$ -TE/100 g d.m.)

and radish seeds (2.39 mg  $\alpha$ -TE/100 g d.m.). Moreover, the kind of tocopherols found in cruciferous seeds was quite different to that found in cereal grains [Zieliński *et al.*, 2001], but it was similar to that found in legume seeds grains [Grela *et al.*, 1993]. On the other hand, the cruciferous seeds were shown to be a richer source of tocopherols than cereal grains and legume seeds.

The  $\alpha$ -T content in cruciferous seeds studied increased gradually as the germination time increased by an almost linear characteristic curve, reaching a nearly stable level between the fifth and the seventh day of the process under light or dark conditions. A similar observation between germination time and  $\alpha$ -tocopherol content was found by Yang *et al.* [2001] during germination of wheat grains under dark conditions. Since  $\alpha$ -T was found in cruciferous seeds at a low level, its synthesis during germination may be taken as an important parameter in the examination of germination process to improve the nutritional value of cruciferous sprouts.

The  $\beta$ -T content also increased during germination of all cruciferous seeds studied up to the last day of the process with only one exception made for white mustard seeds where a decrease in the content of this isomer was noted over the germination period under dark conditions. A similar effect on the  $\beta$ -T content was found in rapeseed seeds germinated under light conditions.

Since  $\gamma$ -T was found to be the main tocopherol homologue in the seeds, its changes may reflect germination as the time of intense metabolic activity. It was found that the  $\gamma$ -T contents in radish, small radish and rapeseeds germinated under dark conditions increased up to the third day of the process, and after that time linearly decreased up to the end of germination, reaching the levels lower than those recorded for the ungerminated seeds (Table 2). This tendency was also observed for white mustard sprouts

TABLE 2. The content of tocopherols ( $\alpha$ -T,  $\beta$ -T,  $\gamma$ -T,  $\delta$ -T) during germination of cruciferous seeds under dark conditions [mg/100 g d.m.].

Cruciferae seeds	Day of germination							
	0	1	2	3	4	5	6	7
Radish								
$\alpha$ -T	0.05	0.08±0.02	0.61±0.08	5.63±0.55	7.67±0.38	8.89±0.17	9.47±0.78	10.61±0.70
$\beta$ -T	0.67	1.31±0.11	1.27±0.05	2.39±0.15	2.49±0.16	2.76±0.08	3.02±0.06	3.98±0.29
$\gamma$ -T	19.06	19.19±0.29	27.35±0.43	44.81±1.00	33.40±0.55	25.69±0.73	15.71±0.52	11.45±0.55
$\delta$ -T	3.39	3.00±0.12	5.85±0.09	7.82±0.36	7.09±0.32	5.17±0.26	3.39±0.19	2.90±0.09
Small radish								
$\alpha$ -T	1.25	0.63±0.09	2.25±0.16	8.61±0.31	9.86±0.44	10.34±0.75	10.38±0.72	11.27±0.44
$\beta$ -T	1.13	0.57±0.04	1.28±0.16	1.75±0.08	1.99±0.15	2.11±0.16	2.12±0.13	2.59±0.08
$\gamma$ -T	26.19	17.33±0.69	23.23±1.66	27.75±1.31	15.88±0.39	10.46±1.18	4.74±0.49	4.13±0.18
$\delta$ -T	2.78	1.39±0.11	2.19±0.26	2.58±0.19	1.65±0.04	1.29±0.24	0.63±0.07	0.71±0.10
White mustard								
$\alpha$ -T	0.80	0.90±0.12	0.80±0.15	0.66±0.06	0.79±0.19	1.03±0.08	1.62±0.18	3.20±0.12
$\beta$ -T	1.87	1.83±0.24	0.90±0.27	0.44±0.06	1.63±0.15	1.56±0.17	1.23±0.28	1.29±0.30
$\gamma$ -T	17.73	16.78±0.86	12.70±0.76	3.05±0.21	1.41±0.19	3.07±0.18	0.92±0.02	1.26±0.18
$\delta$ -T	0.11	0.11±0.01	0.21±0.01	0.23±0.01	0.20±0.01	0.14±0.01	0.15±0.01	0.17±0.01
Rapeseed								
$\alpha$ -T	3.05	5.13±0.57	2.49±0.31	5.70±0.55	10.44±0.50	11.05±0.27	11.51±0.33	11.92±0.49
$\beta$ -T	1.15	1.76±0.16	1.03±0.09	4.02±0.34	4.32±0.51	4.85±0.47	5.64±0.63	5.97±0.42
$\gamma$ -T	8.47	11.73±1.46	5.19±0.79	5.36±0.25	14.89±1.05	8.25±0.84	5.57±0.42	9.08±0.73
$\delta$ -T	0.55	0.68±0.12	0.34±0.06	1.31±0.10	1.50±0.21	1.27±0.03	1.24±0.08	1.18±0.08

TABLE 3. The content of tocopherols ( $\alpha$ -T,  $\beta$ -T,  $\gamma$ -T,  $\delta$ -T) during germination of cruciferous seeds under light conditions [mg/ 100 g d.m.].

Cruciferae seeds	Day of germination							
	0	1	2	3	4	5	6	7
<b>Radish</b>								
$\alpha$ -T	0.05	0.11 $\pm$ 0.02	0.21 $\pm$ 0.02	2.95 $\pm$ 0.24	5.68 $\pm$ 0.81	8.54 $\pm$ 0.06	8.84 $\pm$ 0.20	9.84 $\pm$ 0.14
$\beta$ -T	0.67	0.85 $\pm$ 0.06	0.70 $\pm$ 0.09	1.25 $\pm$ 0.06	1.05 $\pm$ 0.08	1.76 $\pm$ 0.04	1.85 $\pm$ 0.18	2.47 $\pm$ 0.22
$\gamma$ -T	19.06	17.53 $\pm$ 0.47	15.77 $\pm$ 0.59	22.51 $\pm$ 0.76	15.60 $\pm$ 0.58	17.58 $\pm$ 0.83	17.36 $\pm$ 0.59	10.97 $\pm$ 0.58
$\delta$ -T	3.39	3.06 $\pm$ 0.35	2.32 $\pm$ 0.33	4.07 $\pm$ 0.11	2.75 $\pm$ 0.12	3.45 $\pm$ 0.25	3.23 $\pm$ 0.25	2.15 $\pm$ 0.03
<b>Small radish</b>								
$\alpha$ -T	1.25	0.52 $\pm$ 0.06	1.12 $\pm$ 0.09	1.40 $\pm$ 0.14	1.52 $\pm$ 0.18	9.14 $\pm$ 0.54	8.47 $\pm$ 0.04	9.66 $\pm$ 0.42
$\beta$ -T	1.13	0.65 $\pm$ 0.07	0.60 $\pm$ 0.07	0.26 $\pm$ 0.02	0.15 $\pm$ 0.01	0.18 $\pm$ 0.01	0.17 $\pm$ 0.04	0.76 $\pm$ 0.07
$\gamma$ -T	26.19	12.95 $\pm$ 1.06	8.93 $\pm$ 1.41	4.93 $\pm$ 0.53	5.35 $\pm$ 0.15	6.09 $\pm$ 0.43	4.91 $\pm$ 0.52	2.71 $\pm$ 0.21
$\delta$ -T	2.78	1.23 $\pm$ 0.14	1.01 $\pm$ 0.17	0.35 $\pm$ 0.04	0.16 $\pm$ 0.01	0.83 $\pm$ 0.05	0.69 $\pm$ 0.01	0.47 $\pm$ 0.04
<b>White mustard</b>								
$\alpha$ -T	0.81	1.62 $\pm$ 0.15	1.53 $\pm$ 0.19	3.01 $\pm$ 0.27	2.93 $\pm$ 0.09	8.79 $\pm$ 0.45	6.93 $\pm$ 0.58	7.27 $\pm$ 0.48
$\beta$ -T	1.87	2.98 $\pm$ 0.31	2.19 $\pm$ 0.44	2.06 $\pm$ 0.34	2.86 $\pm$ 0.15	3.87 $\pm$ 0.31	2.52 $\pm$ 0.32	2.11 $\pm$ 0.13
$\gamma$ -T	17.73	20.95 $\pm$ 0.86	19.24 $\pm$ 0.96	16.52 $\pm$ 0.81	11.58 $\pm$ 0.48	8.34 $\pm$ 0.58	3.33 $\pm$ 0.26	3.49 $\pm$ 0.25
$\delta$ -T	0.11	0.17 $\pm$ 0.04	0.13 $\pm$ 0.02	0.13 $\pm$ 0.01	0.12 $\pm$ 0.01	0.11 $\pm$ 0.01	0.04 $\pm$ 0.01	0.05 $\pm$ 0.01
<b>Rapeseed</b>								
$\alpha$ -T	3.05	4.67 $\pm$ 0.54	8.20 $\pm$ 0.41	8.93 $\pm$ 0.09	9.57 $\pm$ 0.09	10.09 $\pm$ 0.24	10.49 $\pm$ 0.13	10.64 $\pm$ 0.15
$\beta$ -T	1.15	1.61 $\pm$ 0.21	3.41 $\pm$ 0.14	1.31 $\pm$ 0.12	1.56 $\pm$ 0.10	1.38 $\pm$ 0.11	1.14 $\pm$ 0.16	0.92 $\pm$ 0.11
$\gamma$ -T	8.47	11.05 $\pm$ 1.09	20.08 $\pm$ 0.15	18.86 $\pm$ 0.55	11.50 $\pm$ 0.24	5.93 $\pm$ 0.65	4.29 $\pm$ 0.58	3.05 $\pm$ 0.34
$\delta$ -T	0.55	0.74 $\pm$ 0.15	1.43 $\pm$ 0.12	1.19 $\pm$ 0.10	0.45 $\pm$ 0.10	1.35 $\pm$ 0.21	2.04 $\pm$ 0.15	1.13 $\pm$ 0.11

where a linear decrease throughout the germination period was found. A similar observation was made when the dark conditions were changed into the light conditions (Table 3). However, under these conditions, a linear decrease in the  $\gamma$ -T content was noted in germinated small radish seeds, but not in white mustard seeds. These findings indicate that light conditions may affect the tocopherol contents in germinated cruciferous seeds. It was also confirmed by an analysis of the  $\delta$ -T contents in germinated seeds where no clear relationship between the contents of this isomer and germination time was provided under both light conditions.

When the contents of all analysed tocopherols were expressed in terms of the biological activity of vitamin E, the final result reflected the tocopherol changes due to the germination course. The vitamin E content of germinated small radish, radish, rapeseed and white mustard seeds

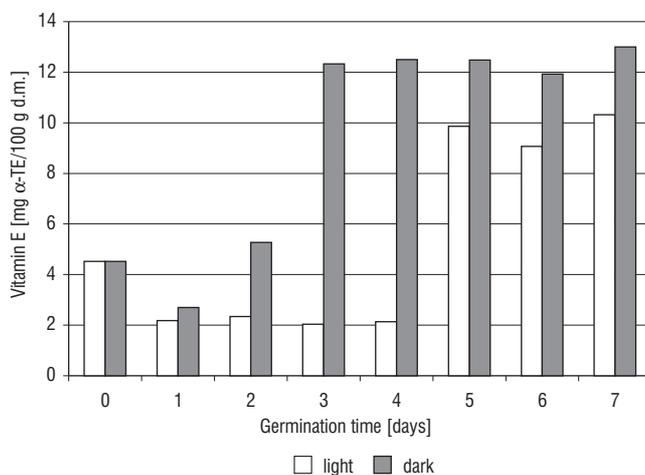


FIGURE 1. Time course of vitamin E activity during germination of small radish seeds under light and dark conditions.

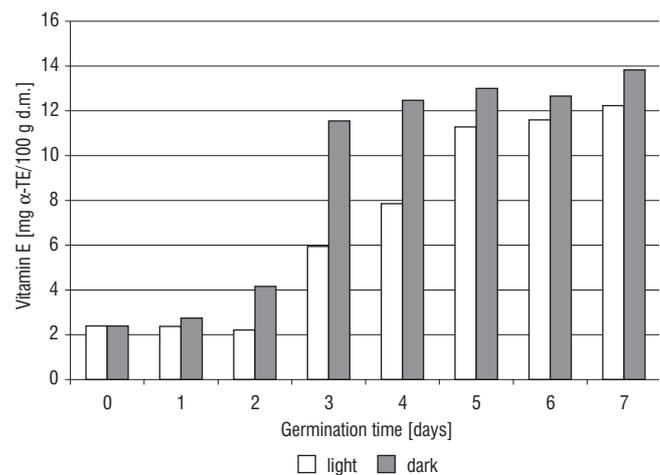


FIGURE 2. Time course of vitamin E activity during germination of radish seeds under light and dark conditions.

under dark and light conditions are shown in Figures 1, 2, 3 and 4, respectively. These figures clearly indicate that the dark conditions can maximize the synthesis of vitamin E in respect to small radish (Figure 1), radish (Figure 2) and rapeseeds (Figure 3). In this case, the optimal germination time was found to be 3 days for small radish and 4 days for radish and rapeseeds. After these times, up to the seventh day of germination, the vitamin E content reached a stable level. Previously, it was shown that the investigated sprouts revealed the best sensory profile qualities after the fourth day of germination [Troszyńska *et al.*, 2002]. This finding indicates that ready-to-eat sprouts of small radish, radish and rapeseeds provide the optimum vitamin E content for consumers, being three times higher than that in seeds. This beneficial finding was also accompanied by a lower level of fat and carbohydrates in the sprouts when compared to the initial seeds (Table 1). In contrary, the ger-

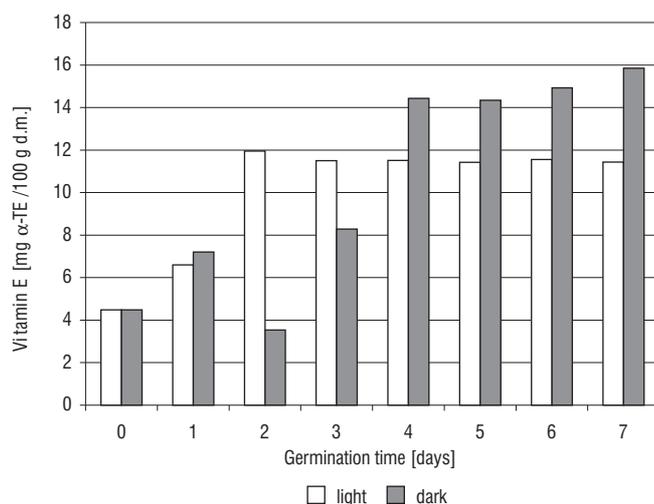


FIGURE 3. Time course of vitamin E activity during germination of rapeseeds under light and dark conditions.

mination of white mustard seeds under light conditions provided more vitamin E than in the dark (Figure 4).

The effect of light was also shown in other sprouts. Seibold [1990] kept the sprouts in light for several more days after germination and found that light was a very important factor in the biosynthesis of  $\beta$ -carotene in germinated wheat. Other studies also reported that exposure of etiolated bean sprouts to artificial light for 24 h increased the provitamin A content [Farhangi & Valadon, 1982]. It seems to be that biosynthesis of some vitamin E isomers requires degradation of others. It has been observed that increases in  $\alpha$ -T have been accompanied by a decrease in  $\gamma$ -T in germinating seeds (Table 3).

We performed also a correlation studies between  $\alpha$ -T and  $\gamma$ -T contents during germination of cruciferous seeds. It was found that the correlation coefficient between  $\alpha$ -T vs  $\gamma$ -T content in the course of germination under dark condition reached -0.641, -0.092, -0.423 and 0.247 for small radish, radish, white mustard seeds and rapeseeds, respectively. The difference between correlation coefficients was statistically significant for small radish and radish, small radish and rapeseeds and white mustard and rapeseeds ( $P \leq 0.05$ ). A similar finding was noted for

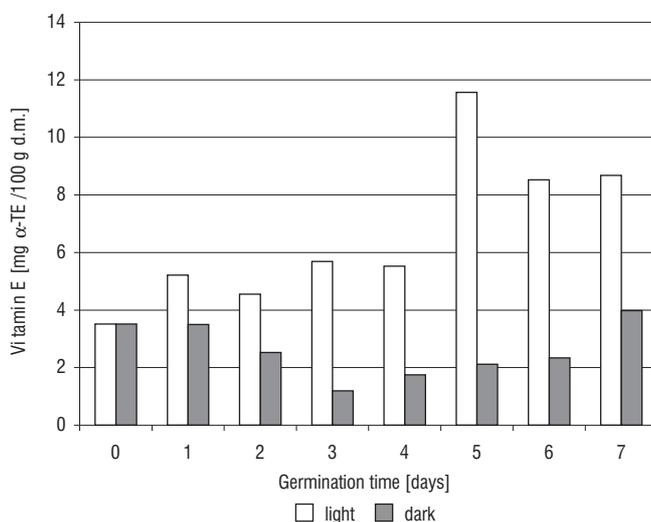


FIGURE 4. Time course of vitamin E activity during germination of white mustard seeds under light and dark conditions.

germination under light conditions where the correlation coefficient was -0.500, -0.466, -0.881 and -0.199 for small radish, radish, white mustard seeds and rapeseeds, respectively. In this case, the difference between correlation coefficients was statistically significant for small radish and white mustard, followed by white mustard and rapeseeds ( $P \leq 0.05$ ). These data, despite the unclear data for rapeseeds, indicate some negative correlations between the content of  $\alpha$ -tocopherol and  $\gamma$ -tocopherol during germination since the  $\alpha$ -tocopherol is required by the human body and the  $\gamma$ -tocopherol is the most abundant in plants [Burton & Traber, 1990; Cohn, 1997].

Fontaine *et al.* [1995] suggested that antioxidants, including tocopherols, might play a crucial role in seed dormancy breakage and germination. Antioxidants synthesized during germination are essential to the protection of the new seedling by reducing the rate of initiation or preventing the propagation of free radicals created during germination [Osawa *et al.*, 1985].

In this study, the vitamin E content was computed from all tocopherols being present in seeds and sprouts, showing that sprouts obtained under dark conditions represent a better source of the vitamin E as well as  $\alpha$ -T and  $\gamma$ -T than germinated cereals or legume seeds [Yang *et al.*, 2001; Zieliński *et al.*, 2001].

The vitamin E content in the edible portion of fresh sprouts was calculated after taking into account the above points and observing that sprouts collected after four days of germination under dark conditions represent the maximized synthesis of vitamin E. It was found that 100 grams of fresh sprouts of small radish, radish and rapeseed supply about 2.36, 2.18 and 2.12 mg of vitamin E ( $\alpha$ -TE), respectively. One exception was made for white mustard sprouts, for which the maximized synthesis of vitamin E was noted after 5 days of germination under light conditions. After this, about 1.53 mg  $\alpha$ -TE may be supplied by 100-gram portion of these fresh sprouts. Currently, the Recommended Dietary Allowance for vitamin E, expressed as  $\alpha$ -tocopherol equivalents, is 10 mg for men and 8 mg for women [RDA, 1989]. A healthy man is rarely deficient in vitamin E because of its wide distribution in food. However, vitamin E deficiency may still arise in some rare cases, such as in patients with lipid malabsorption syndromes [Muller, 1994] or in patients with familial isolated vitamin E deficiency (FIVE deficiency) [Traber *et al.*, 1992]. A prolonged and severe deficiency of vitamin E gives rise to a neurological syndrome characterised by ataxia, tendon areflexia and muscle weakness [Harding, 1987]. In the case of a significantly increased risk of chronic diseases such as cardio-vascular disease and cancer, the level of 23–100 mg is required [Diplock, 1987; Andlauer & Furst, 1999]. The findings, outlined above, highlight two important areas of current vitamin E research. First, the public health benefit that arises from a high intake of vitamin E from a diet (20–50 mg) over a long period of time (lifestyle habit) and second, the potential of vitamin E, albeit in very high doses (250–500 mg) to help treat patients following the onset of clinical symptoms (antioxidant therapy).

The results of this study indicate that a 100-gram portion of individual or mixed fresh cruciferous sprouts is able to cover the Recommended Dietary Allowance for vitamin E

in the range of 15.3–21.6% for men and 19.1–29.5% for women. The data obtained indicate that a four-day germination of cruciferous seeds under dark conditions results in ready-to-eat sprouts which represent a rich source of vitamin E.

## CONCLUSIONS

1. Using different light conditions during germination of selected cruciferous seeds, the optimal time to maximize the synthesis of vitamin E was found after the fourth and the fifth day of germination.

2. During germination in the dark, the activity of vitamin E in sprouts of small radish, radish and rapeseeds was higher when compared to that of the respective sprouts germinated under light conditions.

3. The maximized synthesis of vitamin E in white mustard seeds was noted after 5 days of germination under light conditions.

4. A negative correlation between the contents of  $\alpha$ -tocopherol and  $\gamma$ -tocopherol was found for all of the investigated cruciferous seeds during germination under light conditions as well as during germination in the dark (with only one exception made for rapeseeds).

5. A 100-gram portion of individual or mixed fresh cruciferous sprouts is able to cover the Recommended Dietary Allowance for vitamin E in the range of 15.3–1.6% for men and 19.1–29.5% for women.

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## TOKOFEROLE W KIEŁKACH NASION *CRUCIFERAE*

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W pracy badano zawartość witaminy E w kiełkach wybranych nasion roślin krzyżowych (rzepak, gorczyca, rzodkiew, rzodkiewka) wyprodukowanych z udziałem i bez udziału światła w temperaturze 25°C. Zawartość poszczególnych tokoferoli ( $\alpha$ -T,  $\beta$ -T,  $\gamma$ -T,  $\delta$ -T) monitorowano co 24 godz. przez 7 dni.

Ustalono, że największe nagromadzenie tokoferoli w badanych nasionach, niezależnie od gatunku, miało miejsce po 4-tym i 5-tym dniu kiełkowania w świetle (tab. 3), natomiast kiełkowanie nasion bez udziału światła prowadziło do optymalnej syntezy witaminy E w kiełkach rzodkiewki już po trzech dniach (rys. 1), a w kiełkach rzodkwi i rzepaku – po 4 dniach (rys. 2, 3). Po tym czasie, aż do końca kiełkowania (7 dni), jej zawartość utrzymywała się na stałym poziomie. Ponadto, w kiełkach otrzymanych z większości nasion, poza rzepakiem, stwierdzono ujemną korelację pomiędzy zawartością  $\alpha$ - i  $\gamma$ -tokoferolu i to niezależnie od tego czy proces prowadzono w świetle czy bez. Obliczono, że 100-g porcja 4- lub 5-dniowych kiełków, wyprodukowanych z badanych nasion, bądź ich mieszanina, może pokryć dzienne zapotrzebowanie na witaminę E w zakresie 15.3–21.6% dla mężczyzn oraz 19.1–29.5% dla kobiet.