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Effect of Light Conditions on the Contents of Glucosinolates in Germinating Seeds of White Mustard, Red Radish, White Radish, and Rapeseed

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The study was aimed at determining the effect of light conditions on contents of glucosinolates (GLS) in germinating seeds of white mustard, red radish, white radish, and rapeseed. The seeds were germinated in light and dark, at 25 °C, for up to 7 days. As compared to the nongerminated seeds, in seeds exposed to light and germinated for 4, 5, 6, and 7 days the content of total GLS was observed to decrease by 30 to 70% depending on the species. Germination in conducted the dark for the respective periods of time resulted in decreases of total GLS not exceeding 25%. The changes in the concentration of total GLS were attributed to aliphatic GLS predominating in seeds, yet in the case of white mustard to sinalbin belonging to aralkyl glucosinolates. Although seeds germinated in the dark, as compared to those exposed to light, were characterized by a higher total content of indole GLS, the percentage contribution of that group of compounds in white mustard, red radish, and white radish remained at a similar level, irrespective of germination time. Only in the case of rapeseed was the percentage of the sum of indole GLS observed to increase from 17 to up to 45% once the seeds were exposed to light and to 50% once they were germinated in the dark.

KEYWORDS: Glucosinolates; seeds; germination; light conditions

INTRODUCTION

The evidence for the importance of health-promoting bioactive compounds occurring in edible parts of vegetables belonging to cruciferous species has increased in the last few years. Epidemiological studies, supported by several in vitro and in vivo models, have suggested that the consumption of these vegetables appears to exert a protective effect on human health. Among several bioactive compounds investigated in cruciferous vegetables, such as vitamins, carotenoids, or phenols, glucosinolates (GLS) as an important class of secondary metabolites have received particular interest as potential cancer and chemopreventing agents. They are characterized by a wide variety of chemical structures of their side chains and on the basis of their nature can be divided into three groups constituted by aliphatic, aralkyl, and indole compounds (*1*). After destruction of plant cell tissues during chewing, cutting, crushing, and processing of a vegetable, intact GLS are hydrolyzed, among others, to their respective isothiocyanates and nitriles upon enzymatic and nonenzymatic transformations (*1*−*5*).

It was found that degradation products of indole GLS might act as anticarcinogens by decreasing carcinogen activation through the inhibition of phase I enzymes, increased detoxification by the induction of the phase II enzymes that affect xenobiotic transformations, as well as the inhibition of tumor cell growth and stimulation of apoptosis (*6*−*11*). Similar activity has also been observed in the case of isothiocyanates released from aralkyl and aliphatic GLS, such as glucotropaeolin, gluconasturtiin, glucoraphanin, glucoiberin, gluconapin, and sinigrin (*6*−*11*). Unfortunately, under some conditions, indole compounds have been reported to demonstrate mutagenic (*12*) and carcinogenic activity (*8, 13*).

The major source of GLS in the human diet is and undoubtedly will be cruciferous vegetables. Still, recently an immense interest is being observed in germinated seeds that may constitute an additional source of GLS in our food. Some works have also appeared on the health-promoting effects of extracts from germinated seeds (*14*−*18*).

Most of investigations carried out so far on GLS in developing plants have focused on the aspects of plant physiology and metabolic function of GLS, mainly in the later stages of ontogenesis (*19*−*27*).

Experiments aimed at investigating the obtained sprouts with a desired GLS profile as food products are sparse and encompass merely a few species of seeds from a vast family of cruciferous plants. Thus far, assays have been performed on sprouts of broccoli (*28*−*30*), cauliflower (*30*), cabbage (white, red, and Savoy) (*30*) as well as black mustard and Indian mustard (*29*). Even less data is available on the parameters of the germination process. While such parameters as temperature and humidity that provide optimal conditions for the growth and development

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Table 1. GLS Content (μmol/g D.M.) of White Mustard Seeds and Sprouts Germinated in the Dark (D) and in Light (L)

	aliphatic	aralkyl		indole				
	sinigrin	sinalbin	gluconasturtiin	4-hydroxyglucobrassicin	glucobrassicin	4-methoxyglucobrassicin	neoglucobrassicin	total
seeds	3.74	345.08	tr[b]	0.38	0.46	tr	0.07	349.72
days of germination (D)								
1	3.68[a] ± 0.08	350.71 ± 7.21	tr	0.36 ± 0.04	0.44 ± 0.09	tr	0.05 ± 0.01	355.24 ± 7.17
2	3.25 ± 0.20	322.58 ± 42.61	tr	0.30 ± 0.02	0.22 ± 0.03	0.16 ± 0.06	0.07 ± 0.02	326.58 ± 42.75
3	2.65 ± 0.05	281.79 ± 10.25	tr	0.23 ± 0.01	0.25 ± 0.03	0.35 ± 0.15	0.19 ± 0.13	285.46 ± 10.44
4	2.04 ± 0.27	258.50 ± 25.31	tr	0.23 ± 0.03	0.25 ± 0.04	0.85 ± 0.33	0.37 ± 0.24	262.24 ± 25.73
5	1.57 ± 0.24	272.17 ± 32.23	tr	0.26 ± 0.03	0.37 ± 0.06	1.98 ± 0.48	0.74 ± 0.09	277.09 ± 32.47
6	1.62 ± 0.29	262.67 ± 34.17	tr	0.25 ± 0.10	0.53 ± 0.08	2.12 ± 0.31	0.88 ± 0.11	268.07 ± 33.96
7	1.45 ± 0.17	265.28 ± 18.32	tr	0.25 ± 0.12	0.63 ± 0.08	2.19 ± 0.51	0.96 ± 0.17	270.76 ± 18.42
LSD[c]	0.35	47.53		0.13	0.11	0.56	0.23	47.71
days of germination (L)								
1	2.57 ± 0.16	263.39 ± 6.54	tr	0.43 ± 0.01	0.81 ± 0.04	tr	0.05 ± 0.01	267.25 ± 6.66
2	2.70 ± 0.19	274.83 ± 7.24	tr	0.38 ± 0.03	0.64 ± 0.10	0.09 ± 0.05	0.05 ± 0.01	278.69 ± 7.27
3	2.63 ± 0.18	282.51 ± 6.43	tr	0.13 ± 0.03	0.67 ± 0.03	0.25 ± 0.08	0.09 ± 0.01	286.28 ± 6.52
4	1.94 ± 0.45	221.56 ± 23.42	tr	0.11 ± 0.06	0.49 ± 0.23	0.44 ± 0.13	0.22 ± 0.14	224.76 ± 23.74
5	1.54 ± 0.15	211.25 ± 25.70	tr	0.18 ± 0.01	0.14 ± 0.02	1.05 ± 0.33	0.56 ± 0.20	214.72 ± 26.21
6	1.31 ± 0.58	185.95 ± 24.27	tr	0.20 ± 0.03	0.08 ± 0.01	1.69 ± 0.74	0.78 ± 0.28	190.01 ± 23.80
7	1.72 ± 0.10	243.35 ± 34.60	tr	0.22 ± 0.03	0.08 ± 0.02	2.76 ± 0.53	1.06 ± 0.28	249.19 ± 35.27
LSD	0.46	37.04		0.06	0.16	0.65	0.31	37.45

[a] Values are the means from three independent experiments ± SD ($n = 3$). [b] Trace, mean of three samples <0.05 μmol/g. [c] Least significant differences at $\alpha = 0.05$.

of plants should be, to some extent, adopted arbitrarily, time and light conditions could be modified to obtain a product with desired characteristics.

The reported study was aimed at determining the effect of germination time and light conditions on the contents of GLS in germinated seeds of white mustard, red radish, white radish, and rapeseed.

MATERIALS AND METHODS

Seeds. Single cruciferous seed samples were obtained from a local plant breeding station in Northeast Poland. The samples included white mustard (*Sinapis alba* L.), red radish (*Raphanus sativus* var. *sativus*), white radish (*Raphanus sativus* L.), and rapeseed (*Brassica napus* var. *oleifera*). The seeds were stored at room temperature in polyethylene bags until germination.

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 and dark, at 25 °C, for up to 7 days. Then, they were layered over a moist filter paper (qualitative medium-speed filter paper) to one-third of the depth of the paper. Seeds of each species were taken out off the incubator every 24 h, frozen in liquid nitrogen, and lyophilized. Germination of particular species of seeds was conducted in three independent replications.

Below are presented contents of dry matter (%) in nongerminated seeds as well as in seeds germinated for 1 up to 7 days in the dark (D) and under light (L), determined with the lyophilization method.

White mustard: 97.67 (seeds); D, 39.91 ± 1.54, 27.74 ± 1.87, 21.42 ± 1.19, 13.71 ± 1.76, 12.00 ± 1.52, 11.54 ± 1.25, 9.75 ± 0.76; L, 35.79 ± 3.30, 30.08 ± 2.36, 20.79 ± 2.21, 16.96 ± 1.42, 13.21 ± 1.52, 11.21 ± 1.31, 8.31 ± 1.32.

Red radish: 98.60 (seeds); D, 46.01 ± 2.32, 35.31 ± 1.59, 24.31 ± 3.38, 18.88 ± 2.15, 14.08 ± 1.58, 10.51 ± 0.58, 8.67 ± 1.03; L, 46.39 ± 2.69, 36.52 ± 0.27, 20.48 ± 1.85, 17.01 ± 0.96, 13.74 ± 0.15, 12.07 ± 0.46, 9.33 ± 0.62.

White radish: 98.58 (seeds); D, 44.42 ± 4.04, 29.92 ± 0.93, 20.98 ± 1.67, 17.49 ± 1.68, 13.89 ± 2.85, 11.53 ± 0.99, 9.28 ± 0.97; L, 46.69 ± 2.16, 36.36 ± 1.60, 25.64 ± 2.49, 17.94 ± 1.96, 15.35 ± 0.15, 13.03 ± 0.84, 9.76 ± 0.87,

Rapeseed: 96.89 (seeds); D, 52.84 ± 2.63, 32.36 ± 2.07, 23.04 ± 2.46, 14.67 ± 1.18, 13.67 ± 1.41, 12.93 ± 1.00, 9.83 ± 0.66, L, 47.95 ± 0.24, 41.91 ± 1.82, 26.07 ± 1.78, 21.41 ± 2.20, 14.75 ± 1.17, 11.30 ± 2.16, 10.01 ± 2.02.

Analysis of GLS. The content of GLS in plant material was estimated by means of high performance liquid chromatography (HPLC) according to the *Official Journal of European Communities* (*31*). Briefly, duplicate 200 mg samples of freeze-dried material were extracted thrice with boiling 70% methanol. Since there was a lack of glucotropaeolin in cabbage, its known amount was added to each sample just before the first extraction as an internal standard for the HPLC analysis. The isolation, desulphatation, and HPLC of GLS were carried out according to the modified method of Heaney et al. (*32*). Desulfo-GLS were separated in the HPLC system with an autoinjector (20 μL loop), Lichrospher 100 RP-18 (5 μm) column (250 × 4 mm,) and 1.2 mL/min flow rate at 32 °C by eluting with a gradient of water (A) and 20% acetonitrile (B) as follows: isocratically 1% B for 1 min, gradient to 99% B for 30 min (curve 3), isocratically 99% B for 6 min, linear gradient to 1% B for 5 min, and 1% B for 8 min. GLS were detected at $\lambda = 229$ nm. Individual GLS were identified by comparing their retention times with those of standards (sinigrin glucoraphanin, progoitrin, and gluconasturtiin) or on the basis of available literature data. The presence of aliphatic GLS not having a standard was also additionally confirmed with the GC-MS analysis of respective degradation products. To this end, samples of plant material were comminuted and incubated at a temperature of 30 °C for 24 h. The qualitative analysis of the volatile degradation products was conducted on a gas chromatograph Agilent 7890A equipped with a mass detector 5975C VL, as described earlier (*33*).

The sample content of GLS was quantified on the basis of the internal standard and relevant relative response factors (*31*).

Statistical Analysis. Data collated in tables are presented as the means ± SD ($n = 3$). Mean separation was determined by the least significant differences (Fisher's LSD) at $\alpha = 0.05$. The calculations were carried out with the Windows version of Statgraphics 5.1 (Statistical Graphics Corp. and Manugistics, Inc., Rockville, MD).

RESULTS AND DISCUSSION

Contents of individual GLS and total GLS in seeds and sprouts of white mustard, red radish, white radish, and rapeseed were collated in **Tables 1−4**. Types and contents of individual GLS determined in seeds are consistent with literature data (*1, 23, 25*). Seeds of white mustard red radish and white radish were characterized by the presence of one predominating compound. In white mustard seeds, it was sinalbin (belonging

Table 2. GLS Content (μmol/g D.M.) of Red Radish Seeds and Sprouts Germinated in the Dark (D) and in Light (L)

	aliphatic				indole			
	glucoraphanin	glucoraphenin	napoleiferin	4-MTB	4-hydroxy-glucobrassicin	glucobrassicin	4-methoxy-glucobrassicin	total
seeds	1.31	100.58	5.54	1.12	2.92	0.73	0.18	112.38
days of germination (D)								
1	1.24[a] ± 0.06	96.84 ± 4.04	5.59 ± 0.33	3.22 ± 0.70	3.85 ± 0.13	0.71 ± 0.02	0.23 ± 0.02	111.68 ± 3.83
2	0.84 ± 0.07	69.79 ± 4.73	4.29 ± 0.36	15.76 ± 5.72	3.26 ± 0.27	0.46 ± 0.01	0.43 ± 0.11	94.83 ± 10.49
3	0.84 ± 0.09	65.69 ± 5.20	2.49 ± 0.37	31.53 ± 9.53	3.11 ± 0.19	0.32 ± 0.02	1.13 ± 0.38	105.11 ± 14.91
4	0.79 ± 0.06	54.25 ± 4.70	3.30 ± 0.29	49.43 ± 8.29	2.98 ± 0.22	0.14 ± 0.03	1.13 ± 0.15	112.02 ± 13.19
5	0.61 ± 0.14	37.85 ± 5.37	2.73 ± 0.34	42.64 ± 14.00	2.38 ± 0.56	0.07 ± 0.02	1.32 ± 0.42	87.60 ± 19.09
6	0.78 ± 0.07	41.53 ± 5.22	2.95 ± 0.40	59.86 ± 26.01	2.41 ± 0.94	0.05 ± 0.02	2.19 ± 0.23	109.77 ± 32.44
7	0.76 ± 0.11	51.28 ± 1.82	2.50 ± 0.41	73.39 ± 11.34	2.45 ± 0.03	0.07 ± 0.01	3.19 ± 0.95	133.64 ± 11.73
LSD[b]	0.15	8.03	0.63	22.87	0.78	0.03	0.76	30.15
days of germination (L)								
1	1.11 ± 0.06	74.80 ± 3.35	7.70 ± 0.84	1.95 ± 0.43	3.46 ± 0.13	0.56 ± 0.01	0.07 ± 0.00	89.65 ± 4.63
2	1.00 ± 0.09	64.12 ± 7.23	7.40 ± 0.34	7.50 ± 1.31	3.05 ± 0.31	0.45 ± 0.09	0.08 ± 0.02	83.60 ± 8.67
3	1.04 ± 0.08	56.67 ± 5.36	5.34 ± 0.06	7.16 ± 2.32	1.71 ± 0.46	0.24 ± 0.06	0.62 ± 0.23	72.78 ± 8.40
4	1.00 ± 0.03	53.31 ± 12.92	4.51 ± 0.58	6.52 ± 0.83	1.18 ± 0.11	0.16 ± 0.05	0.62 ± 0.05	67.30 ± 13.94
5	1.12 ± 0.06	60.54 ± 2.66	4.14 ± 0.46	8.12 ± 2.51	0.71 ± 0.11	0.10 ± 0.02	1.05 ± 0.07	75.78 ± 5.53
6	0.95 ± 0.16	45.31 ± 9.16	3.63 ± 0.64	6.53 ± 5.70	0.52 ± 0.28	0.06 ± 0.00	1.59 ± 0.33	58.59 ± 16.23
7	1.26 ± 0.05	50.66 ± 5.48	3.70 ± 0.97	13.52 ± 6.62	0.75 ± 0.10	0.06 ± 0.01	3.90 ± 1.64	73.85 ± 11.08
LSD	0.15	12.91	1.09	6.30	0.44	0.08	1.12	18.47

[a] Values are the means from three independent experiments ± SD ($n = 3$). [b] Least significant differences at $\alpha = 0.05$.

Table 3. GLS Content (μmol/g D.M.) of White Radish Seeds and Sprouts Germinated in the Dark (D) and in Light (L)

	aliphatic				indole			
	glucoraphanin	glucoraphenin	napoleiferin	4-MTB	4-hydroxy-glucobrassicin	glucobrassicin	4-methoxy-glucobrassicin	total
seeds	2.75	165.14	12.57	1.57	6.20	0.40	0.06	188.69
days of germination (D)								
1	2.39[a] ± 0.11	130.42 ± 4.18	12.17 ± 3.54	2.44 ± 0.80	4.79 ± 0.25	0.33 ± 0.05	0.14 ± 0.00	152.68 ± 6.85
2	2.39 ± 0.14	117.12 ± 4.79	15.07 ± 0.34	18.00 ± 3.41	5.25 ± 0.20	0.21 ± 0.02	0.16 ± 0.01	158.20 ± 7.98
3	1.88 ± 0.13	84.91 ± 1.92	12.36 ± 0.39	38.57 ± 4.23	5.61 ± 0.42	0.11 ± 0.01	0.32 ± 0.09	143.76 ± 3.25
4	1.85 ± 0.08	74.37 ± 5.23	11.37 ± 0.44	46.62 ± 14.12	5.28 ± 0.51	0.07 ± 0.01	0.49 ± 0.21	140.05 ± 16.96
5	1.37 ± 0.30	88.35 ± 3.21	9.74 ± 0.82	64.04 ± 16.42	5.01 ± 0.33	0.09 ± 0.02	0.93 ± 0.36	169.53 ± 20.10
6	1.61 ± 0.27	80.15 ± 15.23	8.97 ± 1.83	50.59 ± 7.11	3.99 ± 0.60	0.06 ± 0.03	1.08 ± 0.17	146.45 ± 14.17
7	1.57 ± 0.14	82.13 ± 15.74	8.99 ± 0.52	86.74 ± 26.43	4.83 ± 0.86	0.02 ± 0.02	1.72 ± 0.19	186.00 ± 38.49
LSD[b]	0.33	15.68	2.75	23.38	0.87	0.05	0.33	33.06
days of germination (L)								
1	2.46 ± 0.12	143.53 ± 12.63	9.04 ± 0.39	1.56 ± 0.44	3.53 ± 0.28	0.39 ± 0.05	0.11 ± 0.01	160.62 ± 13.10
2	2.27 ± 0.15	115.33 ± 2.16	8.05 ± 0.40	10.86 ± 1.03	4.61 ± 0.82	0.28 ± 0.01	0.16 ± 0.02	141.56 ± 2.55
3	2.07 ± 0.31	108.01 ± 22.96	5.34 ± 0.72	6.11 ± 4.05	2.67 ± 0.57	0.26 ± 0.07	0.31 ± 0.07	124.77 ± 25.49
4	2.09 ± 0.38	105.65 ± 12.78	4.03 ± 0.45	5.77 ± 3.94	1.80 ± 0.51	0.22 ± 0.02	0.58 ± 0.19	120.14 ± 18.18
5	2.05 ± 0.09	82.37 ± 7.52	3.81 ± 0.51	7.09 ± 3.08	1.32 ± 0.26	0.13 ± 0.01	0.70 ± 0.11	97.47 ± 4.61
6	1.48 ± 0.28	50.37 ± 6.84	3.00 ± 0.62	2.41 ± 0.27	0.56 ± 0.10	0.05 ± 0.01	0.87 ± 0.29	58.74 ± 7.28
7	2.16 ± 0.52	64.65 ± 14.35	3.34 ± 0.19	16.15 ± 10.55	1.41 ± 0.65	0.03 ± 0.01	1.76 ± 0.42	89.50 ± 25.16
LSD	0.53	22.58	0.87	8.20	0.90	0.06	0.37	28.59

[a] Values are the means from three independent experiments ± SD ($n = 3$). [b] Least significant differences at $\alpha = 0.05$.

to aralkyl GLS) that constituted 99% of total GLS (**Table 1**); in red and white radish, it appeared to be glucoraphenin that constituted ~90% of total GLS (**Tables 2** and **3**); whereas rapeseed seeds were predominated by progoitrin and gluconapin constituting, respectively, ~55% and ~20% of total GLS (**Table 4**). In addition, rapeseed was characterized by a relatively high (17%) percentage contribution of indole GLS, which in both varieties of radish constituted only ~4% and in white mustard as little as ~0.3% to the total pool of GLS.

The process of germination evoked changes in contents of GLS in dry matter, while the dynamics and direction of those changes were determined by the species of seeds, light conditions, and time of germination. The effect of light exposure was especially tangible after four days of germination (**Figure 1**). As compared to the nongerminated seeds, in seeds germinated in light for 4, 5, 6, and 7 days the content of total GLS was observed to decrease by 30 to 70% depending on the species. Germination in the dark for the respective periods of time resulted in decreases of total GLS not exceeding 25%, whereas in the case of red radish germinated for 7 days, the content of GLS was higher by 20% than in the nongerminated seeds.

Results obtained for the effect of light exposure on contents of GLS are in agreement with data published by McGregor (*25*). As reported by that author, exposure of developing rapeseed seedlings to light caused a greater decline in GLS concentration than in the seeds germinated in the dark, and the effect of light exposure was visible after the sixth day of germination. Opposite results were obtained by Pérez-Balibrea et al. (*28*) during the germination of broccoli seeds. In seeds germinated for 3, 5, and 7 days, and exposed to light in the 16 h light/8 h dark cycle, the total content of GLS was higher by 33% as compared to seeds germinated in the dark. Since these authors provided only the content of total GLS expressed as mg of sinigrin per 100 g FW, their findings could not be confronted with results obtained in our experiment. Perhaps, the differences result from the use of various seed species and conditions of their light exposure,

Table 4. GLS Content (μmol/g D.M.) of Rapeseed and Sprouts Germinated in the Dark (D) and in Light (L)

	aliphatic					aralkyl	indole				
	progoitrin	napoleiferin	glucoalyssin	gluconapin	gluco-brassicanapin	gluconasturtiin	4-hydroxy-glucobrassicin	glucobrassicin	4-methoxy-glucobrassicin	neogluco-brassicin	total
seeds	3.44	tr[b]	0.14	1.29	0.48	0.08	1.08	0.06	tr	tr	6.68
days of germination (D)											
1	3.06[a] ± 0.25	tr	0.13 ± 0.01	1.14 ± 0.04	0.71 ± 0.12	0.11 ± 0.02	1.17 ± 0.27	0.08 ± 0.01	tr	tr	6.40 ± 0.50
2	3.23 ± 0.45	0.07 ± 0.01	0.11 ± 0.03	1.03 ± 0.06	0.54 ± 0.19	0.17 ± 0.01	0.95 ± 0.20	0.14 ± 0.04	0.14 ± 0.04	0.20 ± 0.12	6.58 ± 0.80
3	3.30 ± 0.33	0.09 ± 0.01	0.12 ± 0.02	0.84 ± 0.08	0.51 ± 0.06	0.11 ± 0.01	0.96 ± 0.05	0.23 ± 0.05	0.21 ± 0.02	0.56 ± 0.15	6.93 ± 0.33
4	2.99 ± 0.17	0.11 ± 0.01	0.12 ± 0.01	0.52 ± 0.07	0.46 ± 0.10	0.09 ± 0.01	0.67 ± 0.19	0.28 ± 0.03	0.36 ± 0.08	0.68 ± 0.18	6.28 ± 0.21
5	2.70 ± 0.25	0.18 ± 0.02	0.13 ± 0.03	0.37 ± 0.03	0.32 ± 0.07	0.08 ± 0.01	0.51 ± 0.04	0.43 ± 0.01	0.72 ± 0.20	1.11 ± 0.16	6.55 ± 0.07
6	2.66 ± 0.20	0.16 ± 0.04	0.17 ± 0.05	0.27 ± 0.01	0.27 ± 0.03	tr	0.45 ± 0.05	0.37 ± 0.00	0.86 ± 0.18	1.46 ± 0.29	6.67 ± 0.70
7	2.34 ± 0.22	0.20 ± 0.06	0.19 ± 0.06	0.22 ± 0.02	0.18 ± 0.03	tr	0.32 ± 0.04	0.38 ± 0.04	0.92 ± 0.17	1.61 ± 0.12	6.36 ± 0.26
LSD[c]	0.50	0.05	0.06	0.09	0.17	0.02	0.26	0.05	0.22	0.30	0.96
days of germination (L)											
1	3.15 ± 0.35	0.11 ± 0.02	0.15 ± 0.01	1.09 ± 0.16	0.42 ± 0.02	0.16 ± 0.00	1.60 ± 0.30	0.07 ± 0.01	tr	tr	6.75 ± 0.78
2	3.43 ± 0.15	0.14 ± 0.02	0.18 ± 0.05	1.11 ± 0.10	0.40 ± 0.02	0.14 ± 0.01	1.60 ± 0.08	0.14 ± 0.03	0.07 ± 0.03	0.12 ± 0.07	7.33 ± 0.15
3	3.09 ± 0.28	0.20 ± 0.06	0.21 ± 0.04	0.79 ± 0.06	0.32 ± 0.04	0.09 ± 0.01	0.81 ± 0.03	0.25 ± 0.04	0.14 ± 0.06	0.34 ± 0.13	6.24 ± 0.58
4	2.96 ± 0.44	0.22 ± 0.01	0.24 ± 0.09	0.61 ± 0.09	0.20 ± 0.03	tr	0.38 ± 0.10	0.37 ± 0.08	0.46 ± 0.05	0.47 ± 0.18	5.91 ± 0.89
5	2.09 ± 0.60	0.16 ± 0.00	0.21 ± 0.06	0.34 ± 0.02	0.14 ± 0.06	tr	0.13 ± 0.08	0.33 ± 0.06	0.50 ± 0.20	0.52 ± 0.11	4.42 ± 1.08
6	1.56 ± 0.57	0.18 ± 0.03	0.28 ± 0.06	0.27 ± 0.02	0.15 ± 0.03	tr	0.14 ± 0.06	0.27 ± 0.04	0.71 ± 0.24	0.47 ± 0.20	4.03 ± 1.14
7	1.68 ± 0.69	0.14 ± 0.02	0.20 ± 0.07	0.20 ± 0.04	0.10 ± 0.03	tr	0.09 ± 0.04	0.28 ± 0.09	0.70 ± 0.31	0.86 ± 0.09	4.26 ± 1.06
LSD	0.83	0.05	0.10	0.14	0.06	0.01	0.23	0.10	0.30	0.22	1.58

[a] Values are the means from three independent experiments ± SD ($n = 3$). [b] Trace, mean of three samples <0.05 μmol/g. [c] Least significant differences at $\alpha = 0.05$.

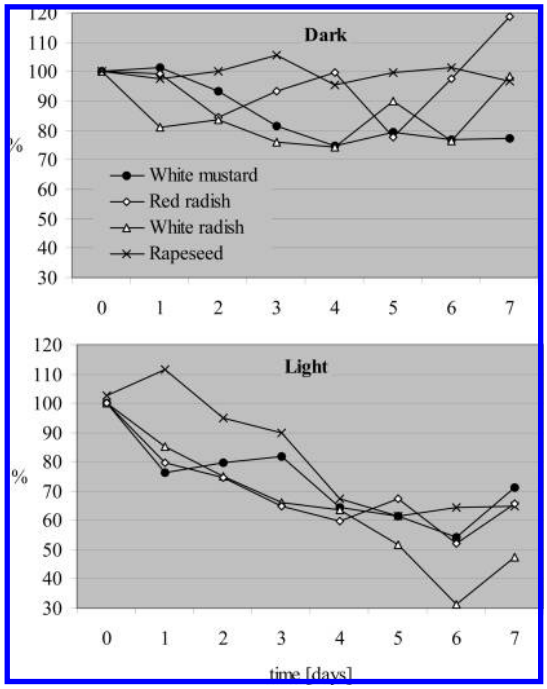

Figure 1. Changes in the total GLS concentration during germination.

i.e., the cited authors applied light cycles, whereas in our study, the seeds were exposed to light over the entire period of germination.

The influence of the germination process on contents of individual GLS during germination of seeds was intricate. Dynamics and direction of changes observed in individual compounds appeared to depend on seed species and time of germination. They were additionally modified by the light conditions.

Changes in contents of GLS in white mustard were determined by the predominating sinalbin belonging to aralkyl GLS (**Table 1**). As compared to the nongerminated seeds, the process of germination resulted in a decreasing concentration of that compound by ~5 to 55% depending on the period of germination and access of light. Nevertheless, irrespective of light conditions and germination time, the relative content of sinalbin was comparatively stable and reached ~98%. Germination also reduced the content of sinigrin, representing aliphatic GLS, in seeds of white mustard (**Table 1**). The effect of light exposure on the contents of sinalbin and sinigrin in germinating seeds of white mustard was especially tangible in the first two days and evoked greater decreases of both compounds.

The reduced concentration of sinalbin in seeds of white mustard was also observed by Bergmann (*22*). As postulated by that author, synthesis de novo of sinalbin is inhibited at early stages of plant development, while indole GLS are synthesized in the same plant tissues.

Contents of GLS in germinated seeds of red and white radish (**Tables 2** and **3**) were resultant of changes in concentrations of two aliphatic GLS, namely, glucoraphenin predominating in seeds and 4-methylothiobutenyl GLS (4-MTB GLS) predominating in mature plants of that species (*34*). The successive loss of glucoraphenin was accompanied by a respective increase in the content of 4-MTB GLS. Hence, irrespective of the time of germination, the percentage contribution of the sum of those compounds in the seeds of both varieties was relatively stable and accounted for ~90%. Light conditions were observed to significantly modify mutual ratios of both of these compounds. As compared to the light-exposed seeds, germination in the dark evoked greater losses of glucoraphenin and greater increases of 4-MTB GLS (**Tables 2** and **3**).

Extending the time of germination of red radish and white radish seeds resulted in diminishing concentrations of glucoraphanin and napoleiferin. The losses of glucoraphanin in both varieties were greater when the seeds were germinated in the dark. A similar effect was noted in the case of napoleiferin in red radish. In turn, an opposite tendency was reported during the germination of white radish, in which losses of napoleiferin were greater in seeds exposed to light.

The decreases of glucoraphenin and increases of 4-MTB GLS in both species of *Raphanus sativus* are convergent with data obtained by Cole (*23*) who, using the GC method, analyzed respective volatile degradation products of those GLS germinating in red radish seeds. The same dependency between both of those compounds was demonstrated by Barillari et al. (*35*) while germinating seeds of Kaiware Diakon also belonging to the species *Raphanus sativus*. Since both of the compounds differ

only in the oxidation degree of a sulfur atom in the side chain, the author suggests direct biological reduction of glucoraphenin to 4-MTB GLS during sprouting of *Raphanus sativus* seeds. In turn, the diminished concentrations of glucoraphanin observed in our experiment are consistent with results of studies published by Bellostas et al. (*30*) and Rangkadilok et al. (*29*).

Germination of rapeseed resulted in decreasing contents of progoitrin, gluconapin and glucobrassicanapin and increases in concentrations of napoleiferin and glucoalyssin. Losses of gluconapin appeared to be the greatest, irrespective of the light conditions applied. As compared to the nongerminated seeds, the content of gluconapin after 7 days of germination decreased by ~85%. Losses of progoitrin predominating in seeds were smaller and accounted for ~30 and 50% in seeds germinated without and with the access of light, respectively. Exposing the seeds to light also caused a gradual reduction in the content of glucobrassicanapin. In turn, germination in the dark evoked an interesting effect of an initial rise in its concentration to as much as ~50% followed by its progressive decrease.

Germination of rapeseed, irrespective of light conditions, evoked an increase in the content of napoleiferin occurring in those seeds in trace amounts (<0.05 μmol/g d.m.). The greatest increase of that compound (up to 0.22 μmol/g d.m.) was observed after four days of germination in light. Exposing rapeseed seeds to light also caused an increase in the content of glucoalyssin, whereas during germination in the dark, its content appeared to increase only in the last two days.

The diversified and complex effect of light conditions on contents of individual GLS, both of the group of aliphatic and indole ones, during the germination of rapeseed seeds was also reported by McGregor (*25*). As suggested by that author, changes in contents of individual GLS are linked with the development of organs and tissues in a developing young plant. Still, relatively lower decreases and in some cases increases in the contents of aliphatic GLS with 5 carbon atoms in the major side chain, such as glucobrassicanapin, napoleiferin, and glucoalyssin, as compared to gluconapin and progoitrin belonging to aliphatic GLS with four carbon atoms in the side chain, are consistent with findings reported by McGregor (*25*) and Kondo et al. (*36*). The lower contents of aliphatic GLS in germinated seeds of four out of the five examined varieties of *B. oleracea* were also observed by Bellostas et al. (*30*).

Despite a number of factors, including time and conditions of germination as well as chemical structure of GLS and finally species diversity of seeds, which significantly affected the direction and dynamics of changes in the contents of individual aliphatic GLS in germinating seeds, a general dependency may be observed between their total concentration and light conditions. Seeds exposed to light during germination were characterized by a lower content of aliphatic GLS than those germinated in the dark. An exception were rapeseed seeds germinated for 2 days (**Figure 2**). The effect of light exposure was especially tangible after 5 days of germination. It is likely that seed exposure to light enables photosynthesis of a seedling that provides its complete autotrophism and self-contained vegetation (*37*). Intensive development of a plant results in its increasing demand for basic compounds, e.g., amino acids and carbohydrates. Thus, the decrease in aliphatic GLS, synthesized from amino acids and glucose, may be linked with the inhibition of their synthesis or, even, with their metabolic transformations into precursor compounds (glucose and amino acids), which are essential compounds to the development of a young plant.

Changes in indole GLS appeared to proceed differently than those in aliphatic and aralkyl GLS. In all species examined,

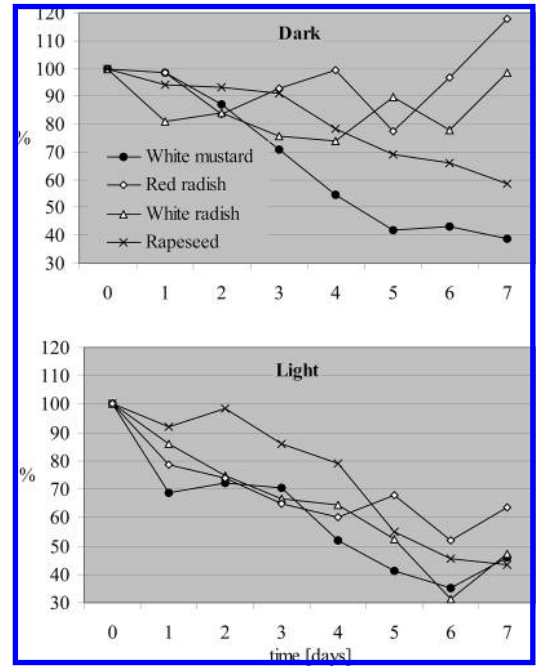

Figure 2. Changes in the aliphatic GLS concentration during germination.

irrespective of light conditions, a dynamic increase was observed in the content of 4-methoxyglucobrassicin. A rise was also noted in the content of neoglucobrassicin that occurred in seeds of white mustard and rapeseed. All species were characterized by a decreasing concentration of 4-hydroxyglucobrasicin. The dynamics of changes in the contents of those GLS differed depending on the species and light conditions. Changes in the content of glucobrassicin turned out to be the most complex. In rapeseed, its content was observed to increase, whereas in red radish and white radish, it decreased. In white mustard, the tendency of changes was determined by both light conditions and germination time.

When compared to the light-exposed seeds, the seeds germinated in the dark were usually characterized by a higher total content of indole GLS. An exception were rapeseeds and white mustard seeds germinated for 1 and 2 days (**Figure 3**).

During the germination of rapeseed, as a result of dynamic increases of, especially, neoglucobrassicin and 4-metoxyglucobrassicin, the percentage of total indole GLS increased from 17% to as much as 50% when the seeds were germinated in the dark (**Figure 4**). The dynamic increase in the contents of both those compounds also occurred during the germination of white mustard. Yet, the percentage contribution of indole GLS increased from merely 0.26 to ~1.6% after seven days of seeds germination in the dark, when the increase observed appeared to be the highest (**Figure 4**). The percentage of indole GLS in both varieties of radish remained at a similar level irrespective of germination time. An exception was red radish germinated for 7 days in light in which the content of indole compounds did not exceed 5%. However, it was not due to the increased content of indole compounds but to a change in the ratio between indole and aliphatic GLS evoked by the decrease of predominating aliphatic GLS.

The results obtained for contents of individual GLS are consistent with data published by other authors. As indicated by investigations of McGregor (*25*) carried out on rapeseed, development of a plant sprout, i.e., hypocotyl, and cotyledons

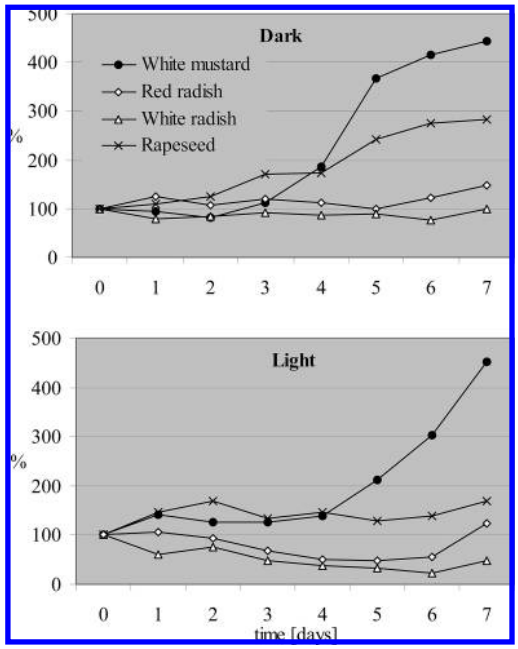

Figure 3. Changes in the indole GLS concentration during germination.

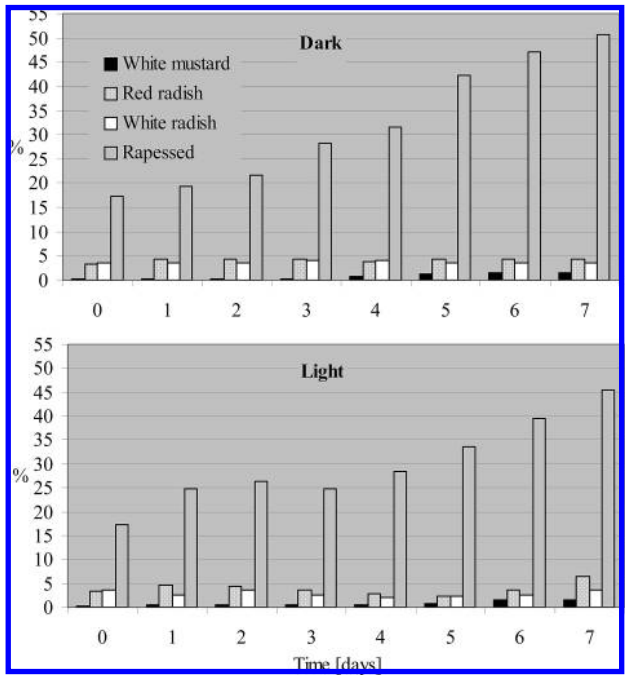

Figure 4. Relative contents of indole GLS in seeds and germinated seeds, expressed as the percentage of total GLS.

is mainly linked with the synthesis of glucobrassicin and, to a lesser extent, with the synthesis of 4-methoxyglucobrassicin. In turn, a study by McClellan et al. (*24*) proves 4-methoxyglucobrassicin to be the predominating GLS developing in the root of rapeseed. Thus, the greater increases of 4-methoxyglucobrassicin versus glucobrassicin observed in our experiment in all seeds examined might have resulted from both genetic differences as well as various conditions of seed germination, enhancing intensive development of the root system. In turn, the reduced content of 4-hydroxyglucobrassicin, observed irrespective of seed species, is consistent with data reported by both the above-mentioned authors and approximates those described by Bellostas et al. (*30*). In addition, Ballostas demonstrated dynamic increases in the concentration of neoglucobrassicin during the germination of seeds of three cultivars of cabbage, broccoli, and cauliflower. Results referring to contents of indole GLS in germinated seeds of red radish and radish are consistent with findings published by Chong and Bible (*38*). In examining changes in indole GLS evoked by SCN^- ions released during their degradation, these authors demonstrated that an intensive increase in the content of indole GLS during ontogenesis of red radish and radish occurs already after 30 days of young plant development.

As demonstrated by studies carried out so far, the effect of the germination process on the content of GLS is complex and determined by both species of seeds and germination conditions as well as the chemical structure of GLS. Because of the complexity of the biochemical transformations proceeding during the germination of seeds and the fact that GLS are secondary metabolites of plant metabolism, their content is determined by compounds essential to the development of a young plant: amino acids and glucose, which makes the dynamics and direction of changes in the contents of individual GLS highly diversified. Nevertheless, results obtained in our study enabled us to notice some general dependencies between time and light conditions of germination and contents of GLS in various species of germinated seeds. Therefore, they may prove useful in the selection of those species of seeds and the application of such parameters of the germination process that would ensure a food product with the optimum composition of GLS.

Irrespective of seed species, it was demonstrated that during germination in light, the total content of GLS was decreasing. The effect of light exposure was especially tangible after the fourth day of germination. In turn, during germination in the dark, both the dynamics and direction of changes were found to depend on the species of seeds. Changes in the content of total GLS were attributed to aliphatic GLS predominating in seeds and in the case of white mustard to sinalbin belonging to aralkyl GLS.

As compared to the light-exposed seeds, the ones germinated in the dark were characterized by a higher total content of indole GLS. The process of germination had no significant effect on the relative contents of indole GLS in white mustard and both species of *Raphanus sativus*, irrespective of light conditions. An exception were rapeseeds characterized by a relatively high contribution of indole GLS. During germination in the dark, the percentage of that group of compounds increased considerably from ~17 to 50%. Thus, germinated rapeseeds are worthy of notice as an additional source of indole GLS in our diet, while the other species enrich it mainly with aliphatic compounds (*30*).

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