Content of Glucosinolates in Cruciferous Vegetables Grown at the Same Site for Two Years under Different Climatic Conditions

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Individual glucosinolates (GLS) were determined in vegetables of three Cruciferae species: *Brassica oleracea* L. (white cabbage, red cabbage, Savoy cabbage, Brussels sprouts, cauliflower, kale, kohlrabi), *Brassica rapa* L. (turnip), and *Raphanus sativus* L. (red radish, black radish, and white radish) produced in two years. The cultivars were compared for the contents of total-, indole-, and aliphatic GLS. In both years, the total content of GLS was highest in black radish, and all examined *R. sativus* vegetables contained the greatest amount of aliphatic GLS. Neither the level nor the identity of GLS differentiated among the vegetables of the other cultivars grown in the same year. Comparison of the GLS contents of the same cultivar in two production years, which differed in temperature and rainfall rate, showed that low average 10-day rainfall and high average temperature during the vegetation period significantly increased the GLS content of vegetables. This suggests that the year × cultivar interaction modified the GLS content of vegetables.

Keywords: *Glucosinolates; cruciferous vegetables; climatic conditions*

INTRODUCTION

Glucosinolates (GLS) comprise a group of thioglucosides naturally occurring in cruciferous vegetables. The products of hydrolysis of these compounds, formed by enzymatic or nonenzymatic action, are biologically active compounds with diverse effects on human health. The products of GLS breakdown, particularly the aralkyl and indole products, have been shown to act as anticarcinogens by inhibition of the phase I enzymes and induction of the phase II enzymes that affect the xenobiotic transformations (Bailey and Williams, 1993; Verhoeven et al., 1997). Similar effects are also ascribed to some degradation products of the aliphatic GLS (Verhoeven et al., 1997). Negative effects of degradation products, especially of the aliphatic GLS, are related to their harmful influence on the thyroid gland, particularly at iodine deficiency in the organism (Michajlovski, 1986).

Cruciferous vegetables are the main source of GLS in human diet. It is known that particular species, varieties, and cultivars differ with regard to the type and amount of the GLS present. Irrespective of the genetic factors determining the identity of GLS and their content of particular vegetables, the latter is also modified by environmental factors, for example, soil and agronomic treatments (Fenwick et al., 1983). Among agronomic factors, the intensity of sulfur fertilization is of primary importance. For sulfur-deficient soil, sulfur fertilizers were reported to increase the GLS content of rapeseeds two or three times (Withers and O'Donnell, 1994; Zhao et al., 1993), whereas nitrogen fertilizers were reported to reduce the GLS content (Zhao et al., 1993). In addition, the soil type modifies the fertilization effect. At low sulfur fertilization of light soils, the GLS content of plants can be reduced by 40% as compared to those grown on heavy soils. This phenomenon is ascribed to increased sulfur deficit in light soil because of its easier leaching (Josefsson, 1970). The GLS content can therefore be modified by climatic conditions. Increased space between growing vegetables was also reported to decrease the GLS content of different cabbage cultivars and Brussels sprouts (MacLeod and Nussbaum, 1997; MacLeod and Pikk, 1978). The GLS concentration of plant was found to vary during the growing season (McGregor, 1988; Rosa et al., 1996) and day-and-night cycle (Rosa et al., 1994).

A variety of factors influencing the ultimate GLS content of vegetables causes the broad range of values reported by different authors for vegetables of the same variety. For example, the average GLS content ranges from ∼160 to >250 mg/100 g for Brussels sprouts (Fenwick et al., 1983; Carlson et al., 1987), from 40 to ∼80 mg/100 g for cauliflower (Carlson et al., 1985; Sones et al., 1984a), from 40 to 90 mg/100 g for white cabbage, from 30 to ∼95 mg/100 g for red cabbage, from 60 to >200 mg/100 g for Savoy cabbage (Sones et al., 1984b; VanEtten et al., 1976; Ciska et al., 1994), and from 10 to 70 mg/100 g for radish [after Fenwick et al. (1983) and Carlson et al. (1985)].

High consumption of cruciferous vegetables and biological effects of the products of GLS hydrolysis have motivated the studies on the factors determining the GLS content of vegetables. Compared to rapeseeds, not much research work has been done in this field for vegetables.

The objective of this work was to evaluate genetic and environmental influences on GLS by comparing the contents of total, indole, and aliphatic GLS of vegetables

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from two crops of different cruciferous cultivars grown under different conditions of rainfall and temperature.

MATERIALS AND METHODS

Plant Material. The vegetables of three species of Cruciferae were studied: *Brassica oleracea* L. [white cabbage (cv. Kamienna Glowa), red cabbage (cv. Haco), Savoy cabbage (cv. Langedijker Dauer), Brussels sprouts (cv. Maczuga), cauliflower (cv. Rapid), kale (cv. Srednio Wysoki Zielony), kohlrabi (cv. Delikates); *Brassica rapa* L. [turnip (cv. Goldball)]; and *Raphanus sativus* L. [red radish (cv. Tetra Iłowiecka), black radish (cv. Murzynka), and white radish (cv. Agata)]. The vegetables were obtained from the experimental garden of the Chair of Horticulture, University of Agriculture and Technology, Olsztyn, Poland. The commercial cultivars commonly grown in Poland were used. The growing conditions were typical for cruciferes. The studies were carried out in 1993 and 1994. Differences in the seed and harvest times did not exceed 7 days in either years. Immediately after harvest, the vegetables were sampled as follows: vertical angle segments about $^1/_{16}$ of a head were cut from the heads of white, red, and Savoy cabbages; 20−50 buttons, depending on the plant size, and 5 leaves were collected at random from the stems of Brussels sprouts and kale, respectively; a quarter of cauliflower was vertically cut from the edible part; whole red radish knots devoid of leaves were randomly selected; turnip, black radish, white radish, and kohlrabi were divided into halves after washing, drying with a blotting paper, and peeling. Seventeen samples of each cultivar ($n = 17$) were collected and analyzed each year of the study except for turnip and white radish, which were sampled only in 1993 and 1994, respectively. After weighing, the samples were frozen in liquid nitrogen and lyophilized.

Analytical Methods. GLS were extracted from the material according to the method reported in the *Off. J. Eur. Communities* (1990). Briefly, duplicate 200 mg samples of freeze-dried material were extracted three times with boiling 70% methanol. Because all of the cultivars examined lacked glucotropaeolin, its known amount was added to each sample just before the first extraction as an internal standard for the HPLC analysis. The isolation, desulfatation, and HPLC of GLS were carried out according to a modified version of the method of Heaney et al. (1986). Desulfo-GLS were separated in the HPLC system with an autoinjector (20 μL loop), a Spherisorb ODS-2 3 Micron column (150 × 4.6 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, linear gradient to 99% B for 30 min (curve 3), isocratically 99% B for 6 min, linear gradient to 1% B for 5 min, 1% B for 8 min. GLS were detected at $\lambda = 229$ nm. Individual GLS were identified by comparing the retention times with those for standards or on the basis of available literature data for 4-methylthiobut-3-enyl-GLS (Carlson et al., 1985) and glucoraphenin (Carlson et al., 1985, 1987). The sample content of GLS was quantified on the basis of the internal standard and relevant relative response factors (*Off. J. Eur. Communities*, 1990).

Statistical Analysis. One-factor variance analysis and multiple-comparison Duncan's test were used to analyze the results for the significance of differences between the mean GLS contents of the vegetables grown in the same year and of the same cultivar grown in two years. The effect of the year × cultivar interaction on the GLS content was analyzed using two-factor variance analysis in an orthogonal system, assuming the cultivar as the first factor and the experimental year as the second factor. The calculations were carried out with an ANOVA statistical packet (Statistica for Windows ver. 6.0, StatSoft, Tulusa, OK). All significance of differences was determined at $P \leq 0.05$.

RESULTS AND DISCUSSION

In the vegetables analyzed, 17 GLS were found: 4 indole, 1 aralkyl, and 12 aliphatic (Table 1). The results

Table 1. GLS Content (Milligrams per 100 g) of Vegetables Grown in 1993 (A) and 1994 (B)[a]

		aliphatic													indole				aralkyl	
		glucoiberin	progoitrin	sinigrin	gluco-raphanin	gluco-raphenin	glucoapo-leiferin	gluco-alyssin	gluco-napin	gluco-iberverin	gluco-brassi-canapin	glucerucin	4-methylthio-but-3-enyl GLS	glucono-nastrurtiin	4-hydroxy-gluco-brassicin	gluco-brassicin	4-methoxy-gluco-brassicin	neogluco-brassicin
white cabbage	A	4.46 ± 2.37	1.44 ± 0.46	19.98 ± 6.84	tr	–	–	–	0.56 ± 0.37	1.24 ± 0.74	–	na	–	0.29 ± 0.28	0.36 ± 0.35	7.46 ± 2.23	3.59 ± 1.00	0.55 ± 0.26
	B	12.38 ± 6.14	3.73 ± 1.60	22.73 ± 11.32	0.26 ± 0.13	–	–	–	0.63 ± 0.22	2.52 ± 0.59	–	–	–	0.42 ± 0.18	1.07 ± 0.37	35.84 ± 9.82	9.74 ± 5.98	0.53 ± 0.25
red cabbage	A	4.01 ± 3.36	3.29 ± 2.53	3.02 ± 2.45	3.99 ± 4.06	–	–	–	2.17 ± 1.79	0.72 ± 0.44	–	1.18 ± 0.87	–	tr	1.44 ± 0.42	8.50 ± 2.21	1.75 ± 0.55	tr
	B	13.61 ± 5.30	8.45 ± 4.34	16.68 ± 12.07	18.19 ± 9.07	–	–	–	4.52 ± 4.16	0.28 ± 0.11	–	0.52 ± 0.30	–	0.58 ± 0.25	1.44 ± 0.78	30.72 ± 17.05	2.72 ± 1.68	0.63 ± 0.19
Savoy cabbage	A	21.24 ± 8.61	1.50 ± 0.84	18.59 ± 3.13	tr	–	–	–	0.94 ± 0.44	1.11 ± 0.61	–	tr	–	tr	0.74 ± 0.32	15.13 ± 5.12	1.56 ± 0.40	0.55 ± 0.33
	B	10.35 ± 3.88	0.96 ± 0.74	15.52 ± 6.98	0.45 ± 0.32	–	–	–	1.17 ± 0.82	tr	–	tr	–	0.45 ± 0.38	0.67 ± 0.43	36.56 ± 12.95	6.09 ± 2.51	tr
Brussels sprouts	A	6.41 ± 4.12	7.13 ± 3.54	25.26 ± 8.96	0.56 ± 0.39	–	–	–	2.26 ± 0.99	0.20 ± 0.14	–	tr	–	0.51 ± 0.47	1.56 ± 0.85	25.25 ± 9.14	3.20 ± 0.83	0.64 ± 0.47
	B	13.90 ± 5.86	7.81 ± 3.42	21.97 ± 9.87	0.87 ± 0.69	–	–	–	2.64 ± 1.81	tr	–	–	–	0.69 ± 0.39	5.10 ± 2.54	29.02 ± 9.32	7.50 ± 3.24	1.91 ± 1.34
kale	A	13.38 ± 7.92	0.21 ± 0.24	2.21 ± 1.30	0.69 ± 0.60	–	–	–	–	tr	–	tr	–	tr	2.10 ± 0.79	43.44 ± 14.13	1.50 ± 0.81	1.84 ± 1.71
	B	15.97 ± 6.22	0.85 ± 0.41	22.72 ± 6.88	0.79 ± 0.33	–	–	–	tr	0.57 ± 0.27	–	–	–	0.68 ± 0.29	4.45 ± 2.41	92.13 ± 26.33	12.13 ± 5.62	0.76 ± 0.37
cauliflower	A	0.44 ± 0.78	0.90 ± 0.31	1.33 ± 1.17	tr	1.09 ± 0.37	–	–	tr	0.58 ± 1.01	–	tr	–	tr	0.47 ± 0.39	12.34 ± 8.26	0.59 ± 0.28	1.78 ± 0.98
	B	6.61 ± 2.97	1.21 ± 0.28	5.94 ± 2.11	0.35 ± 0.21	0.54 ± 0.68	–	–	tr	2.87 ± 1.58	–	0.19 ± 0.9	–	tr	0.65 ± 0.33	18.29 ± 4.51	2.12 ± 0.46	3.81 ± 4.01
kohlrabi	A	2.95 ± 1.31	2.05 ± 1.04	0.43 ± 0.57	8.73 ± 3.64	1.33 ± 0.34	–	–	tr	1.18 ± 0.81	–	4.48 ± 2.39	–	0.12 ± 0.16	1.11 ± 0.76	4.81 ± 1.91	0.18 ± 0.15	1.09 ± 0.68
	B	3.84 ± 2.02	1.42 ± 1.48	0.77 ± 1.32	7.83 ± 5.50	1.94 ± 5.36	–	–	0.60 ± 0.86	2.42 ± 1.51	–	8.44 ± 3.79	–	0.13 ± 0.22	2.04 ± 0.65	5.38 ± 2.72	0.32 ± 0.13	1.89 ± 1.42
rape	A	–	20.49 ± 6.90	–	–	–	0.18 ± 0.12	0.46 ± 0.34	2.95 ± 1.74	–	3.72 ± 2.06	0.19 ± 0.14	–	10.21 ± 4.94	7.05 ± 2.11	3.54 ± 1.22	2.23 ± 1.73	2.23 ± 0.94
red radish	A	–	0.31 ± 0.18	–	0.24 ± 0.14	14.99 ± 7.62	tr	–	–	–	–	0.36 ± 0.17	64.89 ± 18.25	tr	2.04 ± 1.03	2.66 ± 1.34	1.74 ± 0.87	0.41 ± 0.22
	B	–	0.37 ± 0.33	–	0.08 ± 0.16	1.56 ± 0.68	tr	–	–	–	–	0.39 ± 0.17	56.13 ± 30.18	tr	1.03 ± 0.76	1.31 ± 0.96	2.65 ± 0.96	tr
black radish	A	–	0.30 ± 0.08	–	tr	3.90 ± 2.17	0.68 ± 0.11	–	–	–	–	2.25 ± 0.65	292.29 ± 51.80	tr	1.77 ± 0.99	2.78 ± 1.44	1.97 ± 0.89	2.15 ± 0.86
	B	–	0.10 ± 0.13	–	0.29 ± 0.27	12.87 ± 10.09	–	–	–	–	–	3.12 ± 0.79	310.02 ± 45.43	tr	3.69 ± 1.44	1.77 ± 1.16	0.90 ± 0.48	tr
white radish	B	–	0.16 ± 0.05	–	tr	3.01 ± 1.82	tr	–	–	–	–	1.11 ± 0.52	119.13 ± 25.08	tr	1.76 ± 0.91	0.64 ± 0.36	0.61 ± 0.24	tr

[a] Values are means ± SD ($n = 17$). tr, trace, <0.05 pM/g dm. –, not detected. na, not analyzed.

for the identity and content of GLS in the vegetables confirmed the literature data (Fenwick et al., 1983; Nugon-Baudon and Rabot, 1994) except those for white and black radishes. For the former, the content of major 4-methylthiobut-3-enyl-GLS was 9- or 2-fold higher than the contents reported by, respectively, Mullin and Sahasrabudhea (1977) and Carlson et al. (1985). For black radish, the content of 4-methylthiobut-3-enyl-GLS was over twice as high as that reported by Carlson et al. (1985).

Glucobrassicin, glucoiberin, and sinigrin were the dominating GLS in all vegetables of *B. oleracea* species, except sinigrin in kohlrabi. Depending on cultivar, these three GLS accounted for ~10% to >65% of the total. In addition, apart from the compounds mentioned above, progoitrin also dominated in red cabbage and Brussels sprouts, and glucoraphanin dominated in kohlrabi and red cabbage. Kohlrabi was distinguishable by a high relative content of glucoerucin. The presence of 4-methylthiobut-3-enyl-GLS was characteristic of the cultivars of *R. sativus* species. Depending on cultivar, this compound accounted for 75−95% of the total. Turnip, belonging to *B. rapa* species, was dominated by progoitrin (38%), gluconasturtiin (20%), and 4-hydroxyglucobrassicin (13%), the last two being characteristic of turnip. The contents of gluconasturtiin and 4-hydroxyglucobrassicin of other cultivars did not exceed 1 and 5% of the total, respectively. The same cultivars produced in two years were generally characterized by the same dominating GLS except cauliflower, in which the relative glucoiberin contents were about 2 and 15% in 1993 and 1994, respectively. Similar percentage differences were found for sinigrin in kale and for glucoraphenin in radish.

Among the vegetables studied in both experimental years, black radish had the highest mean content of GLS (Table 2). In the two years, the Duncan test selected black radish as one set of means (a), so the GLS content of black radish was significantly ($P \leq 0.05$) higher than that of other vegetables. Among the vegetables grown in 1993, red radish was also distinguishable (one set of means b), the GLS content of which, despite being 3-fold lower than in black radish, was significantly higher than that of other vegetables produced in that year. In 1994, the GLS content of red radish did not, however, differ significantly from the GLS content of Savoy cabbage, and both vegetables were in the set of means f. The lower GLS contents had only cauliflower and kohlrabi (set of means g). Similarly, kale was only distinguishable among the vegetables grown in 1994, although with the GLS content being twice as low as that of black radish. The results for the Duncan test showed that, irrespective of year, the differences in the GLS content of other examined vegetables were small. Each year, the vegetables could be assigned to different sets of means within which the mean total GLS contents did not differ significantly ($P \leq 0.05$). For example, the GLS contents of white cabbage, red cabbage, Savoy cabbage, and Brussels sprouts grown in 1994 differed insignificantly because all of them were in the set of means e, despite also being in other set of means. Slight differentiation among vegetables was found in 1993 in which each of the cultivars, except red radish and black radish, was in a different set of means and these overlapped (Table 2). For example, in 1993, the total GLS content of white cabbage was not significantly different from the GLS contents of Savoy cabbage, kale, and turnip as all of

Table 2. GLS Content and Relative Contents of Aliphatic and Indole GLS of Vegetables Grown in Two Years[a]

	total		aliphatic				indole			
	mg/100 g		mg/100 g		% total		mg/100 g		% total	
	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994
white cabbage	39.93 ± 10.55 efg	89.85 ± 20.85* de	27.68 ± 9.44 de	42.25 ± 13.79* def	69.3 ± 7.4 d	47.0 ± 8.0* e	11.96 ± 2.41 def	47.18 ± 11.86* bc	30.0 ± 7.5 cd	52.5 ± 8.0* c
red cabbage	30.07 ± 9.09 fgh	98.34 ± 24.39* de	18.38 ± 8.15def	62.25 ± 15.80* cd	61.1 ± 10.1 e	63.3 ± 7.4 d	11.69 ± 2.36 def	35.51 ± 13.22* cd	38.9 ± 10.3 b	36.1 ± 7.5 d
Savoy cabbage	61.36 ± 15.74 cde	72.22 ± 18.76 ef	43.38 ± 12.14c	28.45 ± 10.93* efg	70.7 ± 5.4cd	39.4 ± 8.5* f	17.98 ± 5.38 cd	43.32 ± 11.53* bcd	29.3 ± 5.6cde	60.0 ± 8.6* b
Brussels sprouts	72.98 ± 21.43 cd	91.41 ± 24.51* de	41.82 ± 11.35 c	47.19 ± 16.28 cde	57.3 ± 4.3 e	51.6 ± 6.3* e	30.65 ± 72.98 b	43.53 ± 24.51 * bc	42.0 ± 4.3 b	47.6 ± 6.5* c
kale	65.37 ± 18.74 cde	151.05 ± 39.25* b	16.49 ± 9.92def	40.90 ± 11.59* def	25.2 ± 9.7 g	27.1 ± 2.8 g	48.88 ± 14.39 a	109.47 ± 39.25* a	74.8 ± 9.7 a	72.5 ± 2.8 a
cauliflower	19.52 ± 12.62 gh	42.58 ± 10.08* g	4.34 ± 3.13ef	17.71 ± 5.91* fg	22.2 ± 7.6 g	41.6 ± 9.2* f	15.18 ± 9.71 cde	24.87 ± 7.10* e	78.4 ± 7.6a	58.4 ± 9.2* b
kohlrabi	28.46 ± 6.76 fgh	37.02 ± 10.85* g	21.15 ± 5.77 de	27.26 ± 10.66 efg	74.3 ± 6.5cd	73.6 ± 11.1 c	7.19 ± 2.13 ef	9.63 ± 2.85* f	25.3 ± 6.6 de	26.0 ± 11.3 e
turnip	53.25 ± 14.51 def		27.99 ± 8.89 de		52.6 ± 7.7 f		15.05 ± 8.89 cde		28.3 ± 4.1 cde	
red radish	87.64 ± 22.09 b	63.52 ± 32.66* f	80.79 ± 20.61 b	58.53 ± 30.97* cd	92.2 ± 1.9 b	92.1 ± 3.3 b	6.85 ± 2.14 ef	4.99 ± 2.19* f	7.8 ± 1.9 f	7.9 ± 3.3 f
black radish	308.09 ± 54.47 a	332.76 ± 49.22 a	299.42 ± 53.19 a	326.40 ± 48.69 a	97.2 ± 0.7 a	98.1 ± 0.6* a	8.67 ± 2.37 ef	6.36 ± 1.99* f	2.8 ± 0.7 g	1.9 ± 0.6* g
white radish		126.42 ± 26.20 c		123.41 ± 25.44 b		97.6 ± 0.6 a		3.01 ± 1.17 f		2.4 ± 0.7 g

[a] Values are means ± SD ($n = 17$). An asterisk denotes statistically significant difference between-year ($P \leq 0.05$); same letters in the column denote Duncan's sets of means, i.e., between-cultivar means are not significantly ($P \leq 0.05$) different.

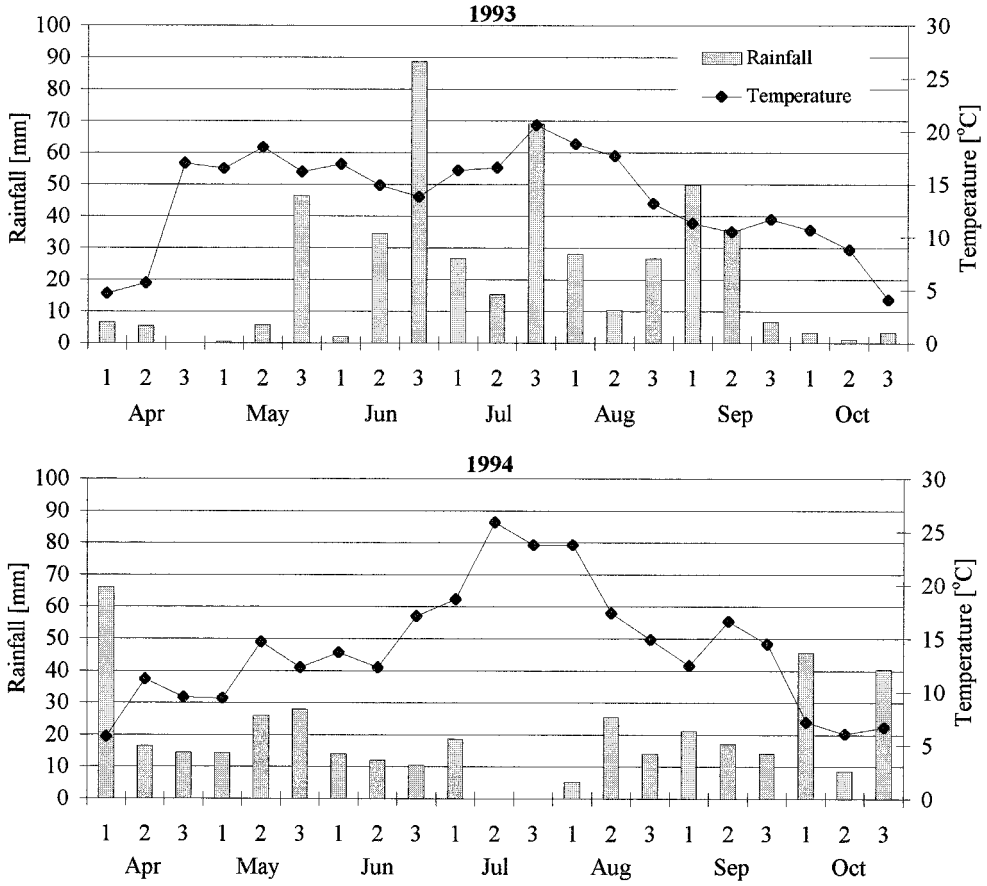

Figure 1. Total 10-day rainfall and average 10-day temperature from April to October for Olsztyn area.

these vegetables were in three sets of means sharing the common set of means e. Also, the contents of aliphatic and indole GLS differed between the analyzed cultivars to a small degree. The greatest amount of aliphatic GLS was found in black and white radishes from *R. sativus* species (sets of means a and b) (Table 2). The aliphatic GLS content of red radish grown in 1994 did not differ significantly from the contents of white and red cabbages, Brussels sprouts, and kale (sets of means c and d). A high relative content of aliphatic GLS (sets of means a and b) was characteristic of all cultivars of *R. sativus* species. Irrespective of year, the proportion of aliphatic GLS in these cultivars exceeded 90%, which was significantly higher than in other vegetables.

In the two production years, the highest content of indole GLS (set of means a) was found for kale. A high relative content of indole GLS, that is, >70% of the total, was characteristic of kale and cauliflower grown in 1993. In 1994, the percentage of indole GLS in cauliflower was significantly smaller (set of means e) than in other cultivars of *B. oleracea* species.

The results for the Duncan test on the homogeneity of means showed then slight differentiation among cultivars with regard to the contents of total GLS, aliphatic GLS, and indole GLS. Despite the fact that the cultivars from different sets of means differed significantly in the GLS content, the differentiation among cultivars was slight because they formed different sets of means that were different in two experimental years and, in addition, overlapped. Variation in the content of individual GLS, as evidenced by high standard deviations, often >50% of the mean (Table 1), was the possible reason for slight differentiation among particular cultivars.

The GLS contents of the same cultivar grown in two consecutive years were compared statistically. The results are presented in Table 2. The vegetables grown in 1994 generally had significantly ($P \leq 0.05$) higher content of GLS than those grown in 1993. Among the vegetables produced in 1993 significantly higher contents of total, indole, and aliphatic GLS were found in red radish and only the contents of aliphatic and indole GLS were higher in, respectively, Savoy cabbage and black radish (Table 2). Because the vegetables were grown in two consecutive years on the same kind of soil and under the same agronomic treatments, it should be assumed that the differences in climatic conditions during the vegetation period were the main factor that differentiated the GLS contents of the vegetables.

The climatic conditions during the growing season in 1993 and 1994 were different, being especially unfavorable in 1994 due to an exceptionally dry and hot summer. In 1993, the average total 10-day rainfall during the vegetation period was 30 mm (Figure 1). In the last 10 days of June and August, the total rainfall amounts were about 80 and 70 mm, respectively. In 1994, the conditions were completely reverse and extremely unfavorable. The total rainfall in particular 10-day periods of the spring and summer did not exceed 20 mm. July was characterized by a complete lack of rain after the first 10 days at the average temperature of 25 °C.

The higher GLS content of the vegetables grown in 1994 compared with those grown in 1993 is comparable to results reported by Freeman and Mossadeghi (1973),

who showed that vegetables grown in water deficiency had higher GLS content than those grown under more favorable conditions. As these authors suggest, the observation of a higher GLS content of vegetables during dry conditions may be related to the increased synthesis of amino acids and sugars, which are precursors in the biosynthesis of GLS. However, the content of these metabolites is not directly dependent on water conditions because intense and long-lasting water deficiency was found to lower the metabolite contents of vegetables [after Freeman and Mossadeghi (1973)].

An additional factor that may have contributed to the reduction of GLS content of the vegetables grown in 1993, in which the total rainfall was high, might be sulfur defciency in the soil due to sulfur leaching. Because the sulfur contents of soil and vegetables were not determined, it is uncertain whether the sulfur deficiency actually took place. As the studies by Zhao et al. (1993, 1994) and Withers and O'Donell (1994) showed, the sulfur deficit in soil influenced much more the content of aliphatic GLS than indole GLS. This effect was most probably associated with the inhibition of synthesis of methionine, the sulfur amino acid being the basic substrate in the aliphatic GLS biosynthesis (Zhao et al., 1994), as opposed to tryptophan, a non-sulfur amino acid and precursor in the indole GLS biosynthesis.

Therefore, if sulfur deficit was the reason for the inhibition of GLS synthesis in vegetables grown in 1993, much greater decreases in aliphatic GLS content than in indole GLS content should be expected. In contrast to this, white cabbage, Savoy cabbage, and Brussels sprouts grown in 1993 contained significantly ($P \leq 0.05$) more aliphatic GLS than the cultivars grown in 1994 (Table 2). The differences were as large as 30 and 20% for Savoy cabbage and white cabbage, respectively. The lower content of aliphatic GLS was found only for cauliflower and black radish, at respective differences of 20% and hardly 1%.

Although the lack of information about the contents of sulfur in soil and vegetables does not allow exclusion of a certain effect of heavy rainfall in 1993 on a decrease in the GLS content of vegetables, the results obtained suggest that unfavorable water conditions in 1994 were the main reason for different GLS contents. It can then be assumed that the water deficit enhanced the GLS synthesis in vegetables grown in 1994. It is therefore difficult to explain why the GLS content of radish was decreased. It can only be supposed that because of its small size this cultivar is more sensitive to water deficiency, and long-lasting water deficiency inhibited the GLS synthesis in radish.

The results obtained for the GLS content of the vegetables grown in two experimental years were statistically analyzed using a two-factor variance analysis. The results are presented in Table 3. The variance analysis showed that the GLS content of the vegetables studied was dependent on both the cultivar and production year (climatic conditions during the vegetation period), although the differentiation among vegetables grown in the same year was slight (Table 2). A significant effect of the factors mentioned above on the GLS content of vegetables is expressed in p level values lower than 0.05 (p level $\ll 0.05$). In addition, the GLS content of vegetables was modified by the cultivar × year interaction (Table 3). The significance of the interaction means that the effect of climatic conditions on the GLS contents of cultivars was significantly different. A different response of particular cultivars to different climatic conditions is clearly visible from the ratio of aliphatic and indole GLS. As compared with 1993, the percentages of aliphatic GLS in the vegetables grown in 1994 were lower in three cultivars and higher in two cultivars, and their differences were insignificant for four other cultivars (Table 2).

Table 3. Effects of Cultivar (1), Year (2), and Interactions (1 × 2) on GLS Content of Vegetables ($P \leq 0.05$)

GLS		df	MS	F emp	p level
total	1	8	263369.13	388.82	0
	2	1	67939.86	100.30	1.88E−20
	1 × 2	8	9706.93	14.33	1.39E−17
	error	228	677.35		
aliphatic	1	8	298323.78	650.21	0
	2	1	9103.58	19.84	1.21E−05
	1 × 2	8	3607.19	7.86	1.46E−09
	error	228	458.81		
% total	1	8	19654.38	411.43	0
	2	1	1228.00	25.71	7.13E−07
	1 × 2	8	1868.16	39.11	0
	error	228	47.77		
indole	1	8	17341.13	161.97	0
	2	1	25928.17	242.18	0
	1 × 2	8	3588.89	33.52	0
	error	228	107.06		
% total	1	8	19541.65	400.78	0
	2	1	1175.73	24.11	1.52E−06
	1 × 2	8	1890.12	38.76	0
	error	228	48.76		

The results obtained in this work showed that the GLS content separated to a small degree the cultivars grown in the same year. It was also shown that climatic conditions can greatly affect the GLS content of vegetables thus, modify their amount of dietary consumption. It seems then reasonable that nutritional assessment of the GLS intake with respect to the type of GLS in the daily food ration should consider the range of GLS content of different species and cultivars instead of the mean GLS content of particular vegetables. Such a procedure seems to be reasonable the more that many factors, including unpredictable climatic conditions, associated with growing vegetables could modify in different ways the total, indole, and aliphatic GLS contents of particular cultivars.

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