Profiles of *S*-Alk(en)ylcysteine Sulfoxides in Various Garlic Genotypes

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Abstract

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The contents of major *S*-alk(en)ylcysteine sulfoxides (namely alliin, methiin and isoalliin) were determined in a set of 58 various garlic genotypes (22 flowering plant morphotypes, 14 semi bolting plants and 22 scape absent morphotype plants), representing the garlic collection of the *Allium* gene bank in the Czech Republic. The plants were cultivated in four successive years (2005–2008) and analysed immediately after harvest and subsequently after eight weeks of storage at 5°C. The total content of the three cysteine derivatives in fresh samples varied considerably between 3.35 mg/g fresh weigh and 12.77 mg/g fresh weight, with the mean of 7.50 mg/g fresh weight and the average relative proportions of alliin/methiin/isoalliin of 83/16/1. Upon 8-week storage at 5°C, the average total amount of *S*-alk(en)ylcysteine sulfoxides increased by 30% to 9.75 mg/g fresh weight, with the alliin/methiin/isoalliin ratio changing to 82/14/4. The data obtained were statistically evaluated using linear discrimination analysis to distinguish the differences between the years of harvest, between freshly harvested and stored samples, and between the individual morphotypes. While the year-to-year differences between the samples were statistically not very significant, the fresh and stored samples as well as the individual garlic morphotypes differed considerably in *S*-alk(en)ylcysteine sulfoxide content. Our results indicate that the content of *S*-alk(en)ylcysteine sulfoxides primarily depends on various genetic factors and post-harvest storage conditions, whereas the climatic conditions during the growth (e.g. temperature, irrigation) influence their level to a lesser extent. Various implications for the food and pharmaceutical industries are discussed.

Keywords: garlic; *Allium sativum*; genotypes; *S*-alk(en)ylcysteine sulfoxides; methiin; alliin; isoalliin; linear discrimination analysis

Garlic (*Allium sativum* L.), a member of the onion family Alliaceae, is a rich source of various *S*-alk(en)yl substituted cysteine sulfoxides, with *S*-allylcysteine sulfoxide (alliin) being the major derivative. Alliin is typically accompanied by significantly lower levels of *S*-methylcysteine sulfoxide (methiin) and (*E*)-*S*-(1-propenyl) cysteine sulfoxide (isoalliin) and, sometimes, trace quantities of *S*-ethylcysteine sulfoxide (ethiin) (Figure 1) (Kubec *et al.* 1999; Kubec & Dadáková 2008).

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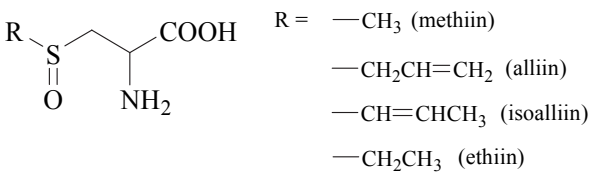


Figure 1. Structure of major *S*-alk(en)ylcysteine sulfoxides in garlic

These amino acids are enzymatically formed by hydrolysis of the corresponding γ-glutamyl-*S*-alk(en)ylcysteine storage dipeptides (Lancaster & Shaw 1989; Velíšek & Cejpek 2009). In the intact tissue, *S*-alk(en)ylcysteine sulfoxides are located in the cytoplasm. Following tissue disruption, a number of sulfur-containing compounds responsible for the characteristic alliaceous smell and taste of garlic, are generated from *S*-alk(en)ylcysteine sulfoxides by the action of the C-S lyase enzyme alliinase (EC 4.4.1.4), occurring in the vacuole, and by subsequent nonenzymatic transformations of the products thus formed (Lancaster & Collin 1981; Block 1992; Block *et al*. 2010).

Garlic is a relatively easily cultivated plant which can be grown year-round in regions with mild climate. Although sexual propagation of garlic is possible, nearly all garlic in cultivation is propagated asexually by planting individual cloves. Thus, garlic frequently displays a high degree of genotypic plasticity that is likely to be dependent on the soil type, moisture, latitude, altitude, and agricultural practices. As a result, numerous garlic genotypes have been obtained through selection of spontaneous mutations. Some genotypes have even lost the ability to form flower stalks and flowers (Volk *et al.* 2004). Most taxonomic systems divide garlic subspecies into bolting types (flowering plants) and nonbolting (scape absent)/incomplete bolting types (semi bolters). These two groups are taxonomically referred to as *A. sativum* ssp. *ophioscorodon* (syn. *A. sativum* ssp. *sagittatum*) and *A. sativum* ssp. *sativum*, respectively. Garlic growers often classify garlic varieties based on phenotypic character into hardneck garlic and softneck garlic (IPGRI 2001, Stavělíková & Faberová 2005).

Flowering plants belonging to the *A. sativum* ssp. *ophioscorodon* group include hardneck garlic morphotypes which form scapes or flower stalks. Many of these morphotypes produce a single layer of cloves around the stalk within the bulb and typically form 6–11 cloves. They are also known as bolting or hardneck morphotypes which are presumably closely related to wild garlic species. However, the hard flower stalk of these morphotypes makes their breeding quite difficult. Furthermore, flowering plant morphotypes are not suitable for long-term storage, as they form roots and start to shrivel within a few months after harvest. The group *Allium sativum* ssp. *sativum* includes the softneck morphotypes that are either nonbolting (scape absent) or produce only very weak stalks. Scape absent morphotypes do not produce flower stalks at all. Under stressful conditions, bulbils may be produced within the false stem. Semi bolter morphotypes produce flower stalks, but not flowers (Stavělíková & Faberová 2005). In general, most softneck types form 12–20 cloves arranged in three to six layers within the bulb. Scape absent morphotypes and semi bolters exhibit a high degree of geographical diversity and flower stalk formation can depend on the climatic conditions at the place of cultivation. They can be stored for 6–8 months without appreciable deterioration, thus much longer than the flowering plant morphotypes.

It has been well documented that the total content and relative proportions of the individual *S*-alk(en)ylcysteine sulfoxides in garlic are strongly affected by a number of genetic and environmental factors (e.g. climatic conditions, soil composition, irrigation, fertilisation, harvest date, etc.) (Randle & Lancaster 2002; Volk *et al*. 2004; Kamenetsky *et al*. 2005; Hughes *et al*. 2006; Ichikawa *et al*. 2006). However, the differences in *S*-alk(en)ylcysteine sulfoxide profiles of various garlic morphotypes have been so far studied only sporadically.

The main goal of this study was to determine the levels of the three main sulfur-containing amino acids (alliin, methiin, and isoalliin) in the cloves of different garlic genotypes and to investigate the changes in the contents of these sulfoxides during storage. Furthermore, the data obtained can serve for genotypisation of garlic accessions and, together with phenotypic descriptors, they can also help in the characterisation of garlic genetic resources available in the Czech Republic.

MATERIALS AND METHODS

Plant material. A set of 58 various garlic genotypes was obtained from the Plant Research Institute Prague, Department of Vegetables and Special Crops (Olomouc, Czech Republic). These

samples are included in the collection of vegetatively propagated *Alliums* for long day conditions. The genotypes could be divided into three main morphological types: (a) flowering plants (bolting garlic, 22 accessions, group F), (b) scape absent garlic (nonbolting garlic, 22 accessions, group N), and (c) semi bolting (producing scapes but never developing bulbils, 14 accessions, group S). More details about the individual accessions can be found in Table 1 and in Fáberová (2009) and Stavělíková and Faberová (2005).

The samples were planted in the autumn period (middle of October), grown under open field conditions and harvested at the end of June or beginning of July. The plants were stained with the combination of iprodian (255 g/l, Rowral Flo), chlorpyrifos-methyl (400 g/l, Reldan 40 EC), and carbendazin (500 g/l, Bavistin WG) for 20 minutes. The insecticides chlorpyrifos-methyl (400 g/l, Reldan 40 EC) and diazion (10%, Basudin 10G) were used to protect the plants against pests. The fertiliser used was Cererit (8% N, 13% P_2O_5, 11%

Table 1. Garlic samples analysed in this study

Sample No.	Evigez[a]	Morphotype	Donor Institution	Sample No.	Evigez[a]	Morphotype	Donor Institution
1	09H0100019	N	SUN	30	09H0100220	F	SUN
2	09H0100025	N	ROM	31	09H0100251	F	CZE
3	09H0100059	N	YUG	32	09H0100292	F	AUT
4	09H0100062	N	SUN	33	09H0100317	F	SUN
5	09H0100078	N	CZE	34	09H0100403	F	KOR
6	09H0100183	N	POL	35	09H0100498	F	CZE
7	09H0100186	N	POL	36	09H0100516	F	CZE
8	09H0100233	N	ROM	37	09H0100530	F	SUN
9	09H0100254	N	CZE	38	09H0100801	F	SVK
10	09H0100272	N	ESP	39	09H0100918	F	SUN
11	09H0100291	N	AUT	40	09H0100922	F	SUN
12	09H0100305	N	ITA	41	09H0100930	F	SUN
13	09H0100364	N	HUN	42	09H0101037	F	SUN
14	09H0100372	N	ROM	43	09H0101168	F	CZE
15	09H0100405	N	ROM	44	09H0101170	F	CZE
16	09H0100465	N	CZE	45	09H0100020	S	SUN
17	09H0100553	N	SUN	46	09H0100024	S	EGY
18	09H0100996	N	ITA	47	09H0100073	S	SVK
19	09H0101027	N	SUN	48	09H0100069	S	CZE
20	09H0101029	N	SUN	49	09H0100241	S	CZE
21	09H0100988	N	CZE	50	09H0100370	S	HUN
22	09H0101171	N	CZE	51	09H0100381	S	ROM
23	09H0100035	F	CHN	52	09H0100283	S	GBR
24	09H0100042	F	SUN	53	09H0100799	S	SVK
25	09H0100043	F	SUN	54	09H0100806	S	SVK
26	09H0100081	F	CZE	55	09H0100387	S	FRA
27	09H0100076	F	CZE	56	09H0100398	S	ESP
28	09H0100212	F	SUN	57	09H0101009	S	AUT
29	09H0100215	F	SUN	58	09H0100971	S	PRT

[a]Plant Genetic Resources Documentation in the Czech Republic (Fáberová 2009), Czech registered varieties were samples No. 22 (Lukan, morphotype N), No. 43 (Jovan, morphotype F), No. 44 (Blanin, morphotype F) (National List of Varieties 2009)

K_2O, 15% SO_4^2) in the amount of 20 g/m^2 (spring) and of 40 g/m^2 (autumn). Weeds had been removed manually from the field during the whole vegetative period.

Chemicals and reagents. *S*-Alk(en)ylcysteine sulfoxides (alliin, methiin and isoalliin) were prepared/isolated as described previously (Kubec & Dadáková 2008). Phosphate buffers were prepared by dissolving 7.8 g of dihydrogen phosphate in 1000 ml of distilled water and adjusting the pH to either 6.5 or 9.5 with 1M sodium hydroxide. The OPA derivatisation reagent was prepared by dissolving 140 mg of *o*-phthaldiadehyde in 5 ml of methanol. After the addition of 100 µl of *tert*-butylthiol (2-methylpropane-2-thiol), the solution was made up to 50 ml with 50mM KH_2PO_4 buffer (pH 9.5) (Velíšek *et al*. 1993).

Sample preparation. Garlic cloves (about 5 g) were carefully peeled, placed in 50 ml of methanol and gently boiled for 10 minutes to inactivate alliinase. Norleucine (10 mg) was added to the sample as an internal standard. The sample was then homogenised using a blender and filtered through a 0.45 µm cellulose acetate syringe-tip filter (Chromservis, Ltd., Prague, Czech Republic). An aliquot (100 µl) of the filtrate obtained was mixed with 900 µl of the OPA reagent and analysed by HPLC. All samples were analysed in duplicates using cloves of two plants. The limits of quantification (LOQ) were determined to be 0.03 mg/g fw for all three amino acids monitored. To determine dry matter content, garlic cloves were carefully peeled, subjected to brief microwave heating (650 W for 1 min) to inactivate alliinase and cut into approximately 1 mm thick slices. These slices were dried in an air flow oven at 70°C for 24 h and subsequently at 105°C until reaching constant weight.

High performance liquid chromatography. HPLC separations were performed on a Consta-Metric binary pump system (Watrex, San Francisco, USA), employing an AS 100 autosampler (20 µl injections), a SpectroMonitor UV detector (Thermo Scientific, Inc., Waltham, USA), and a C-18 reverse phase column (Synergi POLAR-RP 80A, 250 mm × 4.6 mm, 4 µm, Phenomenex, Torrance, USA). The chromatographic conditions were as follows: (A) 50mM KH_2PO_4 buffer (pH 6.5, solvent A) and methanol (solvent B), flow rate of 0.8 ml/min and the gradient A/B 59/41 (0 min), 25/75 (in 37 min), 25/75 (in 39 min) and 59/41 (in 50 min), detection wavelength of 337 nm. The column temperature was maintained at 37°C.

Statistical analysis. Descriptive statistics and linear discrimination analysis were performed using the software SPSS for Windows (Release 11.0.0, SPSS Inc., USA).

RESULTS AND DISCUSSION

In total, 58 different garlic accessions were cultivated for four consecutive years (2005–2008) and the contents and relative proportions of major *S*-substituted cysteine sulfoxides (alliin, methiin and isoalliin) in these samples were determined by HPLC. The cloves were analysed immediately after harvest and after storage at 5°C for 8 weeks. As the samples were grown each year at the same location, the effects of several variable factors (such as soil type, latitude and altitude) were eliminated. The total content of the three *S*-substituted cysteine sulfoxides in fresh samples varied considerably between 3.35 and 12.77 mg/g fw, with the mean of 7.50 mg/g fw (Table 2). The average relative proportions of alliin/methiin/isoalliin in freshly harvested samples were found to be 83/16/1. In general, the values found in the present study are consistent with the data reported previously (Table 3), except for the noticeably low relative abundance of isoalliin.

When the same batches of garlic bulbs were stored at 5°C for 8 weeks, the average total amount of *S*-alk(en)ylcysteine sulfoxides markedly increased from 7.50 mg/g to 9.75 mg/g fw (Table 2). For example, the average content of methiin increased from 1.18 to 1.37 mg/g fw (16% increase), whereas that of alliin changed from 6.25 to 8.04 mg/g fw (30% increase). However, the most striking difference was observed in the case of isoalliin whose average content increased nearly six times upon storage (from 0.06 to 0.35 mg/g fw). The ratio of alliin/methiin/isoalliin changed from 83/16/1 to 82/14/4 upon 8-week storage. It should be noted that the average content of dry matter changed only slightly during storage (from 38.7% to 39.2%). Thus, the observed increase in the content of *S*-alk(en)ylcysteine sulfoxides can be attributed to the conversion of the corresponding γ-glutamyl dipeptides to sulfoxides rather than to the loss of water. Similar observations of a several-fold increase of isoalliin content in garlic cloves were also reported by Lawson *et al*. (1991), Hughes *et al*. (2006), and Ichikawa *et al*. (2006). This phenomenon has very important implications for food

Table 2. The content of *S*-alk(en)ylcysteine sulfoxides in garlic cloves harvested in different years

Year/analyte	Fresh				Stored			
	Min.	Max.	Mean	S.D.	Min.	Max.	Mean	S.D.
2005								
Methiin (mg/g fw)	n.d.	2.50	1.11	0.69	0.18	3.00	1.23	0.67
Alliin (mg/g fw)	3.73	9.80	6.41	1.68	5.17	10.81	7.93	1.45
Isoalliin (mg/g fw)	n.d.	0.53	0.08	0.12	0.10	1.00	0.39	0.21
Sum of ACSO (mg/g fw)	4.13	11.32	7.61	2.11	5.89	14.15	9.56	1.98
Dry matter (%)	33.06	42.07	37.36	2.04	33.57	59.07	39.23	4.66
2006								
Methiin (mg/g fw)	0.26	2.77	1.08	0.61	0.31	3.52	1.21	0.72
Alliin (mg/g fw)	3.58	9.99	6.13	1.53	4.14	12.48	7.19	1.51
Isoalliin (mg/g fw)	n.d.	0.31	0.07	0.06	0.09	0.67	0.29	0.15
Sum of ACSO (mg/g fw)	3.90	12.77	7.28	2.01	4.78	16.36	8.69	2.12
Dry matter (%)	36.36	45.49	40.61	1.88	33.87	44.39	38.96	1.82
2007								
Methiin (mg/g fw)	0.33	2.93	1.21	0.57	0.40	2.81	1.33	0.62
Alliin (mg/g fw)	2.96	9.51	6.25	1.63	5.25	20.18	9.24	3.07
Isoalliin (mg/g fw)	n.d.	0.28	0.06	0.06	0.03	1.26	0.25	0.27
Sum of ACSO (mg/g fw)	3.35	11.53	7.52	2.06	6.08	23.27	10.82	3.61
Dry matter (%)	31.33	42.98	39.43	2.24	30.08	52.02	39.34	3.06
2008								
Methiin (mg/g fw)	0.28	2.71	1.33	0.62	0.35	4.07	1.70	0.78
Alliin (mg/g fw)	3.25	8.69	6.22	1.34	1.28	13.80	7.78	1.78
Isoalliin (mg/g fw)	n.d.	0.18	0.03	0.04	0.12	0.92	0.46	0.20
Sum of ACSO (mg/g fw)	3.61	10.38	7.58	1.75	1.75	18.36	9.94	2.44
Dry matter (%)	32.56	43.53	37.39	1.96	32.58	66.22	39.41	5.60
Average of 2005–2008								
Methiin (mg/g fw)	n.d.	2.93	1.18	0.63	0.18	4.07	1.37	0.72
Alliin (mg/g fw)	2.96	9.99	6.25	1.54	1.28	20.18	8.04	2.18
Isoalliin (mg/g fw)	n.d.	0.53	0.06	0.08	0.03	1.26	0.35	0.22
Sum of ACSO (mg/g fw)	3.35	12.77	7.50	1.98	1.75	23.27	9.75	2.71
Dry matter (%)	31.33	45.49	38.70	2.45	30.08	66.22	39.24	4.03

n.d. – not detected (< 0.03 mg/g fw); S.D. – standard deviation

industry. Although isoalliin is only a minor garlic constituent, its content is of particular importance from the technological point of view. It has been shown that isoalliin is the key precursor of the compounds causing undesirable blue or blue-green discoloration of various preparations made from garlic (Kubec *et al*. 2004; Kubec & Velíšek 2007). Therefore, long-term storage is not advisable for garlic intended to the production of garlic powder or paste. To avoid the formation of the undesirable discoloration, garlic should be processed as soon after harvest as possible.

The differences between the sulfoxide levels in the fresh and stored garlic cloves were also evaluated by linear discrimination analysis. To describe these two groups of samples statistically, one canonical discrimination function was extracted (with the group centroids having been located at –1.84 and 1.84) and the squared Mahalanobis distances to centroids were plotted against the discriminant scores in the canonical function (Figure 2). It can be seen that 96.6% of the originally grouped samples were correctly classified, indicating that the differences between the levels

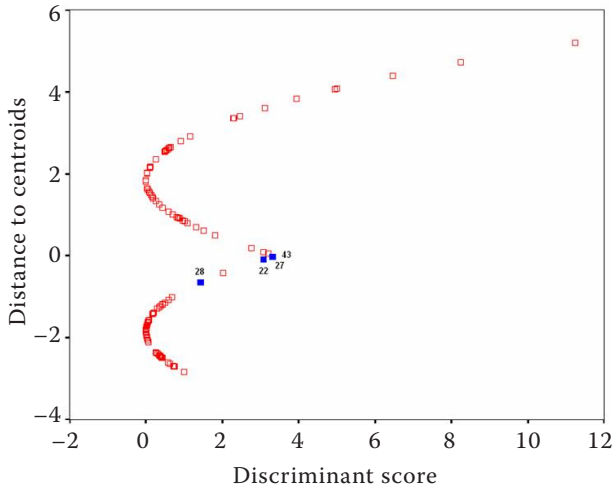


Figure 2. Discrimination between fresh and stored garlic bulbs

of *S*-alk(en)ylcysteine sulfoxides in the fresh and stored samples are statistically very significant. Only four out of the 58 samples of stored garlic were incorrectly classified, namely three samples of F type (No. 27, 28, and 43) and one sample of N type (No. 22).

As shown in Figure 3, the average temperature, sunshine duration, and precipitation differed significantly at the experimental location (Olomouc, Czech Republic) during the four years. Nevertheless, linear discrimination analysis of the data obtained did not reveal any significant year-to-year differences in the average total sulfoxide levels between the individual batches of samples. For example, only 49.1% of all samples were correctly classified using discriminant scores in the first two discriminant functions (Figure 4), indicating that the year-to-year differences in the content of *S*-substituted cysteine sulfoxides were statistically not very significant. The highest number of correctly classified samples harvested in 2006 was 62.1%, where eight samples were incorrectly classified as harvested in 2005 (samples No. 1, 2, 11, 13, 38, 39, 40, and 47), 10 samples were classified as harvested in 2007 (samples No. 6, 16, 18, 24, 33, 42, 43, 53, 56, and 58), and four samples were classified as grown in 2008 (samples No. 14, 35, 45, and 46).

As shown in Figure 2, the greatest difference in sulfoxide levels was found between the 2006 and 2008 sets of freshly harvested samples. In 2006, the cloves contained the smallest amounts of the two main sulfoxides, methiin (1.08 mg/g fw) and alliin (6.13 mg/g fw), and had the highest dry matter contents (40.61%). In 2008, the cloves had the highest methiin content (1.33 mg/g fw), a medium content of alliin (6.22 mg/g fw), and the lowest dry matter content (37.39%) (Table 2). Perhaps, unusually low temperatures between December 2005 and March 2006 could have influenced the chemical composition of the garlic harvested in 2006. Furthermore, at the end of 2005 and during almost the whole vegetation period of 2006 (till June 2006), the precipitation was above the average (Figure 3). On the contrary, the garlic samples harvested in 2008 were grown under average weather conditions (temperature, sunshine duration, and precipitation).

Significant variations in the sulfoxide content were observed between the three groups of different garlic morphotypes, i.e. 22 flowering plants (F), 14 semi bolters (S), and 22 scape absent (N) morphotypes (Tables 4 and 5). As can be seen, the lowest average content of the sulfoxides was observed in the group of semi bolting plants (5.90 mg/g fw). A considerably higher average level of the sulfoxides (8.10 mg/g fw) was determined in flowering plant morphotypes (F) (Table 4). Similar differences in the total content of the sulfoxides could be also

Table 3. The content of *S*-substituted cysteine sulfoxides in garlic cloves

Relative proportion (%)				Total content (mg/g fw)	References
Methiin	alliin	isoalliin	ethiin		
17	83	tr.	n.d.	3.65	Thomas and Parkin (1994)
5	84	11	n.d.	11.8	Yoo and Pike (1998)
6–11	89–94	tr.	1.0	5.3–12.2	Kubec *et al.* (1999)
14	76	10	n.d.	2.29	Krest *et al.* (2000)
10	81	9	n.d.	12.3	Kubec and Dadáková (2008)

tr. – traces; n.d. – not detected

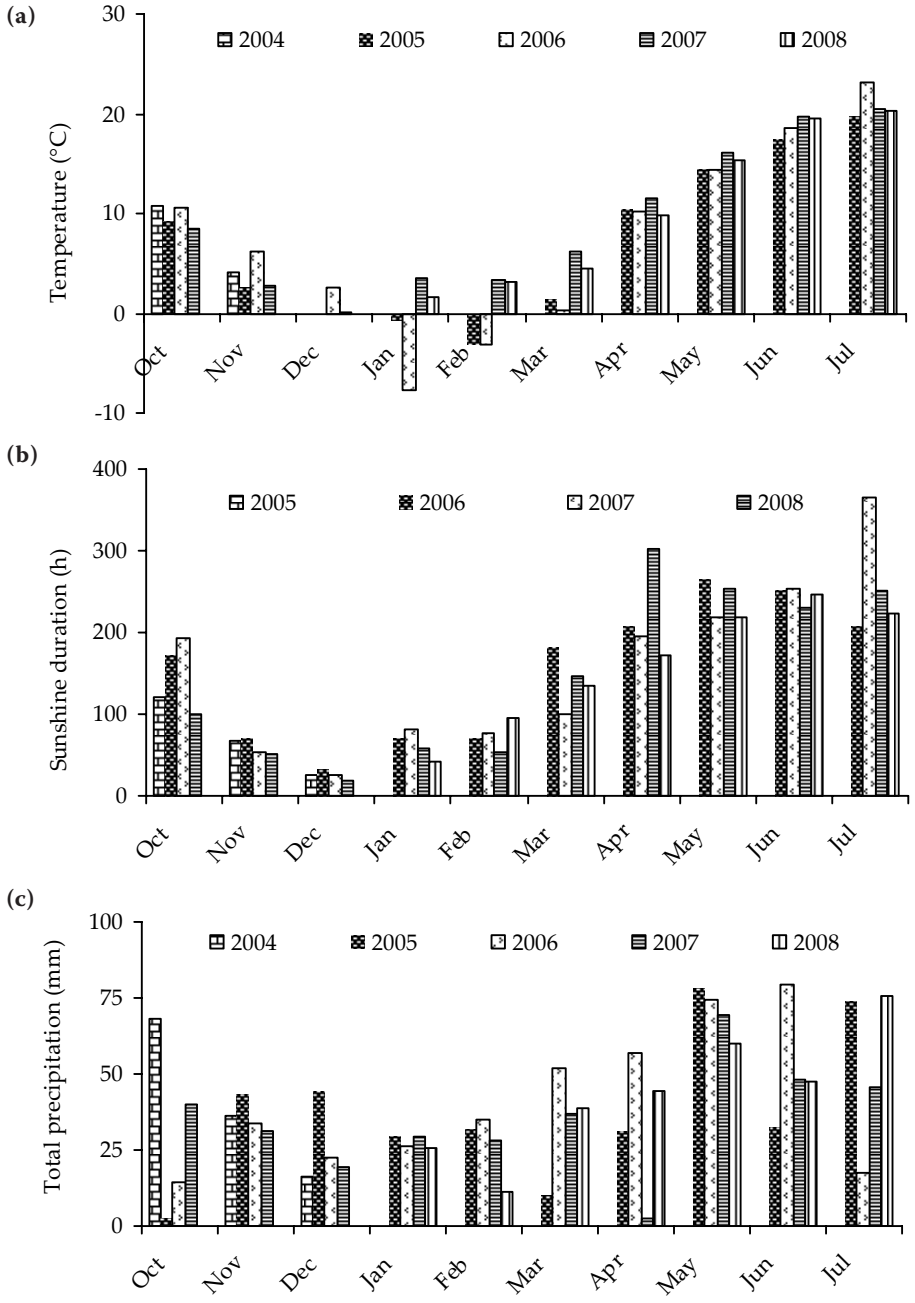


Figure 3. Climatic data recorded during years 2005–2008: (a) Mean air temperature, (b) Sunshine duration, (c) Total precipitation

observed between the samples stored at 5°C for 8 weeks (Table 5). The average levels of methiin, alliin, and isoalliin in freshly harvested/stored flowering plant morphotypes were 1.61/1.80, 6.41/8.30, 0.07/0.37 mg/g fw, respectively, in semi bolters 0.68/0.87, 5.12/7.32, 0.10/0.39 mg/g fw, respectively, and in scape absent plants 1.07/1.25, 6.81/8.22, 0.03/0.30 mg/g fw, respectively. The average dry matter content did not differ significantly between the three groups of morphotypes, varying in the range from 38.4~39.0% (fresh samples) to 38.8~40.0% (stored samples). The relative proportions of alliin/methiin/isoalliin were found to be 87/11/2, 86/13.5/0.5 and 79/20/1 in fresh cloves of semi bolters, scape absent samples, and flowering plant morphotypes, respectively. Upon 8-week storage, these proportions changed to 85/10/5, 84/13/3, and 79/17/4, respectively. As can be seen, the morphotypes belonging to *Allium sativum* ssp. *sativum* (nonbolting or semi bolting varieties)

Table 4. The content of *S*-alk(en)ylcysteine sulfoxides in various garlic morphotypes (before storage)

Year/analyte	Morphotype S				Morphotype N				Morphotype F			
	Min.	Max.	Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.	Mean	S.D.
2005												
Methiin (mg/g fw)	< LOQ	1.69	0.48	0.45	n.d. [d)]	1.83	1.09	0.57	0.34	2.50	1.54	0.61
Alliin (mg/g fw)	3.73	8.85	5.39	1.50	4.08	9.80	7.20	1.97	4.48	8.97	6.27	1.01
Isoalliin (mg/g fw)	0.08	0.38	0.23	0.08	n.d.	0.05	0.02	0.01	n.d.	0.53	0.06	0.11
Sum of ACSO (mg/g fw)	4.13	10.62	6.11	1.90	4.51	11.32	8.31	2.47	5.54	11.26	7.87	1.29
Dry matter (%)	33.94	39.2	36.68	1.85	33.48	41.90	37.71	1.98	33.06	42.07	37.45	2.20
2006												
Methiin (mg/g fw)	0.28	1.16	0.58	0.27	0.32	1.60	0.93	0.33	0.26	2.77	1.53	0.67
Alliin (mg/g fw)	3.58	7.76	4.81	1.25	4.46	8.85	6.52	1.39	4.78	9.99	6.57	1.37
Isoalliin (mg/g fw)	n.d.	0.18	0.07	0.05	n.d.	0.16	0.05	0.05	0.01	0.31	0.10	0.07
Sum of ACSO (mg/g fw)	3.90	8.76	5.46	1.47	4.86	9.95	7.50	1.67	5.52	12.77	8.20	1.92
Dry matter (%)	37.61	42.98	39.90	1.83	36.36	45.49	40.97	2.24	38.31	43.35	40.69	1.43
2007												
Methiin (mg/g fw)	0.39	1.85	0.79	0.39	0.33	1.77	1.10	0.38	0.63	2.93	1.60	0.59
Alliin (mg/g fw)	3.49	8.51	5.06	1.50	2.96	9.51	6.83	1.77	4.15	8.54	6.43	1.17
Isoalliin (mg/g fw)	n.d.	0.12	0.05	0.03	n.d.	0.24	0.04	0.05	n.d.	0.28	0.08	0.08
Sum of ACSO (mg/g fw)	3.90	9.77	5.90	1.85	3.35	10.90	7.96	2.11	5.03	11.53	8.12	1.62
Dry matter (%)	37.07	42.98	39.72	1.74	37.16	42.59	39.85	1.72	31.33	41.84	38.82	2.86
2008												
Methiin (mg/g fw)	0.36	1.55	0.88	0.35	0.28	1.79	1.16	0.41	0.65	2.71	1.78	0.65
Alliin (mg/g fw)	3.25	7.66	5.23	1.32	3.88	8.69	6.69	1.39	4.66	7.92	6.38	0.96
Isoalliin (mg/g fw)	n.d.	0.07	0.02	0.02	n.d.	0.06	0.01	0.02	0.01	0.18	0.06	0.05
Sum of ACSO (mg/g fw)	3.61	9.17	6.13	1.65	4.16	10.30	7.87	1.75	6.15	10.38	8.21	1.30
Dry matter (%)	34.88	42.14	37.16	1.94	33.37	43.53	37.45	2.26	32.56	40.08	37.46	1.72
Average of 2005–2008												
Methiin (mg/g fw)	< LOQ	1.85	0.68	0.39	n.d.	1.83	1.07	0.43	0.26	2.93	1.61	0.63
Alliin (mg/g fw)	3.25	8.85	5.12	1.38	2.96	9.80	6.81	1.64	4.15	9.99	6.41	1.13
Isoalliin (mg/g fw)	n.d.	0.38	0.10	0.10	n.d.	0.24	0.03	0.04	n.d.	0.53	0.07	0.08
Sum of ACSO (mg/g fw)	3.61	10.62	5.90	1.70	3.35	11.32	7.91	2.01	5.03	12.77	8.10	1.54
Dry matter (%)	33.94	42.98	38.37	2.32	33.37	45.49	39.00	2.51	31.33	43.35	38.60	2.48

Morphotype: S – semibolters, N – scape absent, F – flowering plants; S.D. – standard deviation; n.d. – not detected (< 0.03 mg/g fw)

contained considerably lower relative proportions of methiin than *A. sativum* ssp. *ophioscorodon* flowering plant varieties.

The data obtained by HPLC analysis of fresh cloves were subjected to linear discrimination analysis which divided these samples into the three aforementioned groups of morphotypes according to the similarity in their *S*-alk(en)ylcysteine sulfoxide and dry matter contents. Discriminant scores of the individual samples in functions 1 and 2 are plotted in Figure 5. The best classification was obtained for the set of 22 scape absent morphotypes (N) where all samples were correctly classified. Statistically very significant differences were also observed in the classification of the group of 14 semi bolters (S), as 13 samples (92.9%) were correctly classified and only one sample (No. 50) was classified incorrectly as a scape absent (N) morphotype. Of the 22 flowering plant morphotypes (F), 19 samples (86.4%) were correctly classified, one sample (no. 44) resembled a semi bolter morphotype (S), and two samples (No. 34

Table 5. The content of *S*-alk(en)ylcysteine sulfoxides of various garlic morphotypes (stored at 5 °C)

Year/analyte	Morphotype S				Morphotype N				Morphotype F			
	Min.	Max.	Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.	Mean	S.D.
2005												
Methiin (mg/g fw)	0.26	1.52	0.71	0.40	0.18	1.83	1.16	0.53	0.39	3.00	1.64	0.69
Alliin (mg/g fw)	5.48	9.53	7.66	1.17	5.44	10.70	7.82	1.58	5.17	10.81	8.22	1.49
Isoalliin (mg/g fw)	0.25	0.58	0.44	0.10	0.10	0.47	0.23	0.08	0.27	1.00	0.53	0.24
Sum of ACSO (mg/g fw)	6.57	11.54	8.81	1.53	5.89	12.63	9.21	2.01	6.62	14.15	10.39	1.99
Dry matter (%)	34.44	44.27	38.40	3.40	33.88	59.07	39.02	5.21	33.57	53.52	39.97	4.86
2006												
Methiin (mg/g fw)	0.31	1.22	0.67	0.30	0.40	1.67	1.01	0.36	0.60	3.52	1.76	0.81
Alliin (mg/g w)	4.14	8.26	5.90	1.17	5.11	9.40	7.27	1.10	5.89	12.48	7.92	1.58
Isoalliin (mg/g fw)	0.10	0.67	0.33	0.19	0.09	0.49	0.27	0.11	0.12	0.58	0.28	0.14
Sum of ACSO (mg/g fw)	4.78	9.46	6.91	1.33	5.84	11.16	8.54	1.43	7.32	16.36	9.96	2.28
Dry matter (%)	36.23	41.28	38.69	1.61	36.65	41.42	38.93	1.04	33.87	44.39	39.17	2.49
2007												
Methiin (mg/g fw)	0.49	1.79	0.85	0.34	0.40	2.81	1.30	0.60	0.80	2.77	1.67	0.59
Alliin (mg/g fw)	5.60	13.14	8.13	2.28	5.60	20.18	10.14	4.13	5.25	12.43	9.03	1.88
Isoalliin (mg/g fw)	0.07	1.26	0.30	0.35	0.04	1.06	0.26	0.26	0.03	0.67	0.22	0.21
Sum of ACSO (mg/g fw)	6.38	14.63	9.28	2.64	6.08	23.27	11.69	4.83	7.40	15.01	10.92	2.31
Dry matter (%)	36.80	41.91	39.19	1.54	35.82	52.02	39.42	3.40	30.08	48.86	39.36	3.51
2008												
Methiin (mg/g fw)	0.69	2.61	1.26	0.51	0.69	2.64	1.55	0.50	0.35	4.07	2.14	0.93
Alliin (mg/g fw)	4.71	11.24	7.56	1.49	6.01	10.21	7.65	0.97	1.28	13.80	8.04	2.48
Isoalliin (mg/g fw)	0.19	0.89	0.49	0.24	0.16	0.83	0.43	0.18	0.12	0.92	0.47	0.21
Sum of ACSO (mg/g fw)	5.94	14.45	9.31	2.01	7.30	12.90	9.63	1.38	1.75	18.36	10.65	3.31
Dry matter (%)	34.67	43.68	38.83	2.94	32.58	43.91	37.69	2.93	33.40	66.22	41.50	7.95
Average of 2005–2008												
Methiin (mg/g fw)	0.26	2.61	0.87	0.45	0.18	2.81	1.25	0.54	0.35	4.07	1.80	0.78
Alliin (mg/g fw)	4.14	13.14	7.32	1.77	5.11	20.18	8.22	2.55	1.28	13.80	8.30	1.92
Isoalliin (mg/g fw)	0.07	1.26	0.39	0.24	0.04	1.06	0.30	0.19	0.03	1.00	0.37	0.24
Sum of ACSO (mg/g fw)	4.78	14.63	8.58	2.14	5.84	23.27	9.77	2.99	1.75	18.36	10.48	2.50
Dry matter (%)	34.44	44.27	38.78	2.46	32.58	59.07	38.77	3.48	30.08	66.22	40.00	5.13

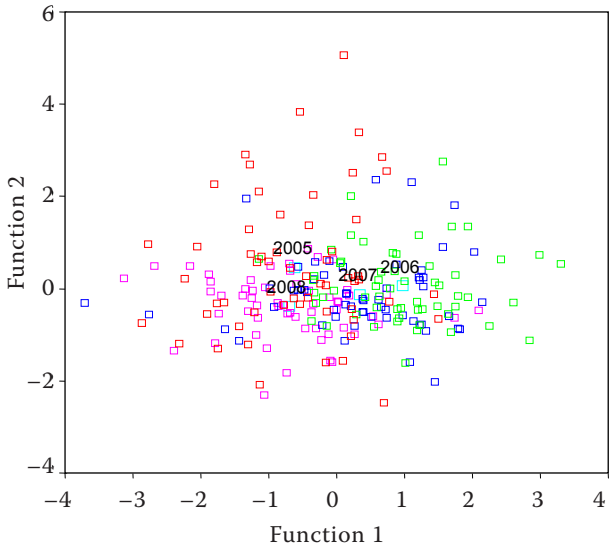
Morphotype: S – semibolters, N – scape absent, F – flowering plants; S.D. – standard deviation

and No. 37) were recognised as scape absent morphotypes (N).

Discrimination scores between the individual morphotypes stored for 8 weeks were statistically less significant than those between the fresh samples. Only 20 samples (90.9%) of scape absent morphotypes were correctly classified (samples No. 17 and No. 19 were classified as semi bolters). Correct classification was achieved with 12 semi bolters (85.7%), incorrectly classified were samples Nos. 50 and 57 which rather resembled the flowering plant and scape absent morphotypes, respectively. The flowering plant morphotypes were correctly classified in 95.5% (21 samples) and only sample No. 34 was classified as a semi bolter (Figure 6).

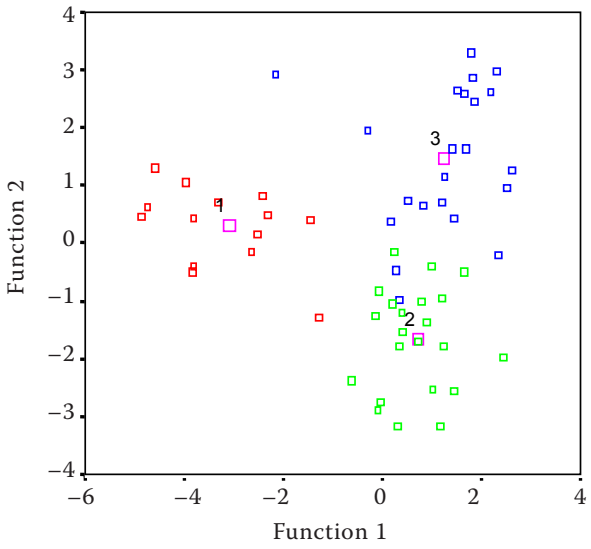
CONCLUSION

The profiles of major *S*-alk(en)ylcysteine sulfoxides (alliin, methiin and isoalliin) were determined in a large set of 58 garlic genotypes (22 flowering



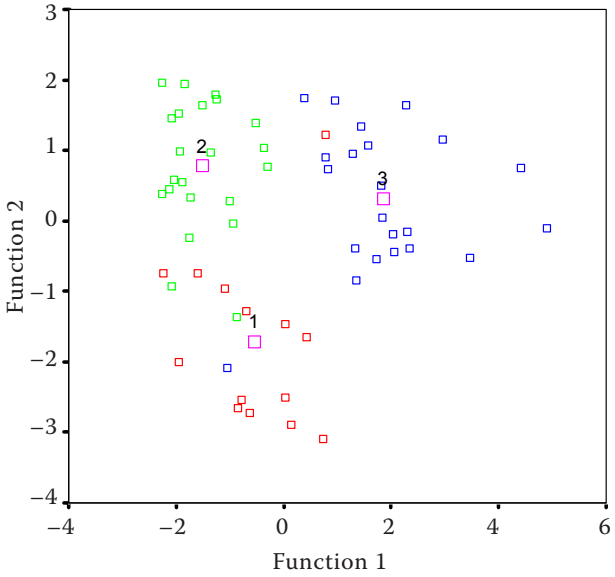
□ = group centroids, □ = 2005, □ = 2006, □ = 2007, □ = 2008

Figure 4. Discrimination between years 2005, 2006, 2007 and 2008



□ = group centroids, 1= semibolter morphotype, 2 = scape absent morphotype, 3 = flowering plant morphotype

Figure 5. Discrimination between fresh garlic morphotypes



□ = group centroids, 1= semibolters morphotype, 2 = scape absent morphotype, 3 = flowering plant morphotype

Figure 6. Discrimination between stored garlic morphotypes

plant morphotypes, 14 semi bolting plants, and 22 scape absent morphotypes). The sulfoxide levels were determined in the cloves immediately after harvest and subsequently after 8 weeks of storage at 5°C. To the best of our knowledge, this work represents one of the most extensive studies dealing with *S*-substituted cysteine sulfoxides in various garlic genotypes. Linear discrimination analysis of the data obtained revealed only minor year-to-year differences between the samples. On the other hand, statistical evaluation distinguished significant differences between the freshly harvested and stored samples as well as between the individual garlic morphotypes. Our results indicate that the content of *S*-alk(en)ylcysteine sulfoxides primarily depends on various genetic factors and post-harvest storage conditions, whereas the climatic conditions during the growth (e.g. temperature, irrigation) influence their levels to a lesser extent. It has been confirmed that the concentrations of all *S*-alk(en)ylcysteine sulfoxides markedly rise upon storage at 5°C, with isoalliin exhibiting the most striking, several-fold increase. These findings have important implications for food and pharmaceutical industries. To minimise the formation of the undesirable blue discoloration, garlic should be processed as soon after harvest as possible. On the contrary, pharmaceutical industry may prefer garlic with higher levels of free *S*-alk(en)ylcysteine sulfoxides, as these amino acids are precursors of numerous biologically-active compounds. Thus, for the preparation of garlic-based dietary supplements, garlic should be stored for several weeks before processing.

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