Variability in S-alk(en)yl-L-cysteine sulfoxides in garlic within a seven-month period determined by a liquid chromatography – tandem mass spectrometry method– Supplementary material

Plant Foods for Human Nutrition

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Agricultural practices used for garlic at all localities

Only healthy cloves, treated with 5% Sulka (Agrobio Oprava, Czech Republic) against *Aceria tulipae* and funghi, have been planted in three-row plots, in fields after NPK fertilization. Herbicide pendimethalin (Stomp SC, BASF, Germany, 1600 g/ha) was applied after planting and repeatedly during vegetation period; in addition to hand weeding. In the spring, plants were sprayed with a mixture of insecticides cypermethrin (30 mg/ha) and chlorpyrifos (300 mg/ha) (Nurelle D, Agrobio Oprava, Czech Republic) and lambda-cyhalothrin (50 g/ha, Markate 50, Sumi Agro Czech, Czech Republic) twice. As funghicide, azoxystrobin was used (250 g/ha, Ortiva, Agrobio Opava, Czech Republic, and then twice, in 14-day interval, Amistar, 150 g/ha, Syngenta Supply AG, Switzerland). In addition, root boosters were applied. During vegetation period, garlic scapes had been pinched when appeared. Nitrogen fertilization was applied according to standard practices. After machine harvest, soil was brushed away and bulbs with roots and shoots still attached were allowed to dry under ambient conditions (20 °C, suitable air circulation) for two weeks, and then stored at 4 °C.

Table S1 Target compounds analyzed in positive electrospray mode

Compound	Elemental formula	Ion type	MRM quantitative transition m/z	MRM qualitative transition m/z	Retention time (min)
Methiin	$C_4H_9NO_3S$	$[M+H]^+$	152 > 88	152 > 88	4.58
Isoalliin	$C_6H_{11}NO_3S$	$[M+H]^+$	178 > 88	178 > 74	4.23
Ethiin	$C_5H_{11}NO_3S$	$[M+H]^+$	166 > 88	166 > 88	4.12
Alliin	$C_6H_{11}NO_3S$	$[M+H]+$	178 > 88	178 > 74	3.54
Propiin	$C_6H_{13}NO_3S$	$[M+H]^+$	180 > 88	180 > 70	3.42
Butiin	$C_7H_{15}NO_3S$	$[M+H]^+$	194 > 88	194 > 88	2.88
Norleucine (ISTD)	$C_6H_{13}NO_2$	$[M+H]^+$	132 > 86	132 > 69	1.40

ISTD - internal standard; MRM - multiple reaction monitoring

Table S2 Linearity response ranges and limits of detection (LOD) of target S-alk(en)yl-L-cysteine sulfoxides (ACSOs).

	LOD (µg/ml)	LOD (g/kg garlic)	Linear range (µg/ml)	R^2
Methiin	0.015	2.3	0.1-20	0.9925
Isoalliin	0.100	15.5	0.1-10	0.9972
Ethiin	0.050	7.8	0.1-10	0.9992
Alliin	0.100	15.5	0.1-20	0.9968
Propiin	0.010	1.6	0.02-20	0.9976
Butiin	0.020	3.1	0.1-20	0.9980

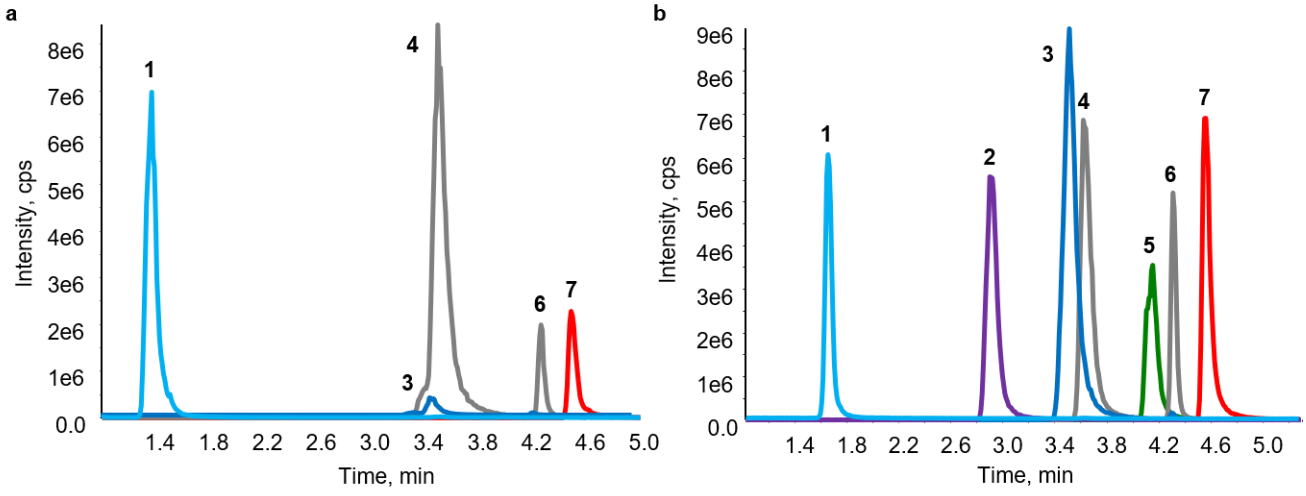

Fig. S1 LC-MS/MS chromatograms of: a - garlic extract; b – standard mixture 20 µg/ml.
1 – norleucin (ISTD);2 – butiin; 3 - propiin; 4 – alliin, 5 – ethiin, 6 – isoalliin, 7 - methiin

Table S3 List of analyzed garlic samples with determined dry weight and S-alk(en)yl-L-cysteine sulfoxide (ACSO) content

					ACSOs			
Sampling	Sampling date	Cultivar	Locality	Dry weight (%)	Aliin (g/kg dw)	Isoaliin (g/kg dw)	Methiin (g/kg dw)	Propiin (g/kg dw)
Sampling I	29.06.2016	Havran	Boritov	30.94	44.21	1.53	11.59	1.32
Sampling II	26.07.2016	Havran	Boritov	36.55	27.87	1.12	7.70	0.94
Sampling III	02.10.2016	Havran	Boritov	34.60	27.83	3.00	7.71	0.99
Sampling I	30.06.2016	Unikat	Boritov	32.87	38.06	0.98	7.65	1.22
Sampling II	26.07.2016	Unikat	Boritov	37.63	24.07	1.44	6.72	0.77
Sampling III	02.10.2016	Unikat	Boritov	36.60	23.40	3.16	5.25	0.80
Sampling IV	29.01.2017	Unikat	Boritov	36.85	28.41	11.84	7.03	2.01
Sampling I	30.06.2016	Vinar	Boritov	34.01	40.26	0.18	11.45	0.50
Sampling II	26.07.2016	Vinar	Boritov	38.28	26.24	1.23	9.67	0.88
Sampling III	02.10.2016	Vinar	Boritov	36.44	27.97	2.61	7.19	0.92
Sampling IV	29.01.2017	Vinar	Boritov	38.29	24.11	13.70	5.73	1.54
Sampling I	27.06.2016	Germidour	Bratkovice	35.89	22.81	0.64	2.04	0.71
Sampling I	27.06.2016	Theador	Bratkovice	32.65	23.12	0.72	2.53	0.69
Sampling II	10.08.2016	Bjetin	Doksy	35.79	27.29	1.67	4.58	0.70
Sampling II	10.08.2016	Havran	Doksy	37.15	24.34	1.17	6.97	0.54
Sampling II	10.08.2016	Rusak	Doksy	39.64	27.49	2.12	5.01	0.54
Sampling II	10.08.2016	Rusak*	Doksy	40.94	32.58	3.35	6.39	0.73
Sampling II	10.08.2016	Tantal	Doksy	35.19	24.64	2.50	1.37	0.67
Sampling II	10.08.2016	Unikat	Doksy	41.38	25.01	1.89	3.00	0.62
Sampling II	01.08.2016	Dukat	Olbramovice	41.27	13.05	2.09	4.69	0.43
Sampling I	19.06.2016	Lukan	Olbramovice	39.14	16.21	0.45	1.97	0.57
Sampling II	01.08.2016	Lukan	Olbramovice	39.36	13.73	2.01	2.51	0.44
Sampling III	02.10.2016	Lukan	Olbramovice	36.86	13.26	2.19	1.17	0.50
Sampling IV	29.01.2017	Lukan	Olbramovice	34.17	15.42	8.48	2.16	0.98
Sampling I	19.06.2016	Tantal	Olbramovice	32.87	18.41	0.39	0.79	0.58
Sampling II	01.08.2016	Tantal	Olbramovice	38.49	15.85	1.67	1.05	0.51
Sampling III	02.10.2016	Tantal	Olbramovice	41.17	15.09	3.49	1.83	0.61
Sampling I	19.06.2016	Unikat	Olbramovice	37.62	14.43	0.31	2.60	0.56
Sampling II	01.08.2016	Unikat	Olbramovice	39.05	12.93	1.00	1.91	0.43
Sampling III	02.10.2016	Unikat	Olbramovice	38.24	16.62	3.18	3.44	0.66
Sampling IV	29.01.2017	Unikat	Olbramovice	37.03	11.74	9.92	2.25	0.85
Sampling I	19.06.2016	Vekan	Olbramovice	37.63	10.97	0.17	2.41	0.43
Sampling II	01.08.2016	Vekan	Olbramovice	39.32	15.02	1.89	3.32	0.46
Sampling III	02.10.2016	Vekan	Olbramovice	38.27	12.58	3.53	3.68	0.50
Sampling I	27.06.2016	Dukat	Slapanice	33.01	35.45	1.37	7.15	1.09
Sampling II	07.08.2016	Dukat	Slapanice	41.61	21.76	1.03	4.60	0.74
Sampling III	03.10.2016	Dukat	Slapanice	39.86	18.99	2.65	4.24	0.82

Sampling	Sampling date	Cultivar	Locality, postal code	Dry weight (%)	Aliin (g/kg dw)	Isoaliin (g/kg dw)	Methiin (g/kg dw)	Propiin (g/kg dw)
Sampling I	27.06.2016	Rusak	Slapanice	38.48	29.24	1.07	5.60	0.91
Sampling II	07.08.2016	Rusak	Slapanice	40.60	18.81	0.82	4.18	0.60
Sampling III	03.10.2016	Rusak	Slapanice	39.81	15.78	1.60	2.73	0.62
Sampling IV	29.01.2017	Rusak	Slapanice	38.28	22.00	11.27	3.79	1.47
Sampling I	27.06.2016	Unikat	Slapanice	34.36	36.14	1.19	5.95	1.25
Sampling II	07.08.2016	Unikat	Slapanice	38.07	22.41	1.34	5.12	0.76
Sampling III	03.10.2016	Unikat	Slapanice	37.65	15.89	2.26	3.89	0.70
Sampling IV	29.01.2017	Unikat	Slapanice	35.89	20.01	10.94	4.56	1.28
Sampling I	27.06.2016	Vinar	Slapanice	34.33	25.43	1.43	6.25	0.79
Sampling II	07.08.2016	Vinar	Slapanice	39.33	22.30	0.92	6.60	0.69
Sampling III	03.10.2016	Vinar	Slapanice	38.60	19.29	1.93	4.37	0.75
Sampling II	25.07.2016	Eden rose	Vitov	35.64	19.08	0.77	2.83	0.65
Sampling II	25.07.2016	Fhermidrome	Vitov	35.86	13.85	0.19	0.94	0.46
Sampling II	25.07.2016	Germidour	Vitov	37.70	14.17	0.31	0.44	0.43
Sampling II	25.07.2016	Goulurose	Vitov	33.49	20.71	1.91	3.93	0.64
Sampling II	25.07.2016	Theador	Vitov	33.71	16.48	0.31	0.90	0.55
Sampling IV	29.01.2017	Theador	Vitov	35.93	9.18	2.32	0.45	0.56

In gray: Sample subset, involving four cultivars from three growing localities present in all samplings

*****original Rusak, of which Dukat and Unikat were bred

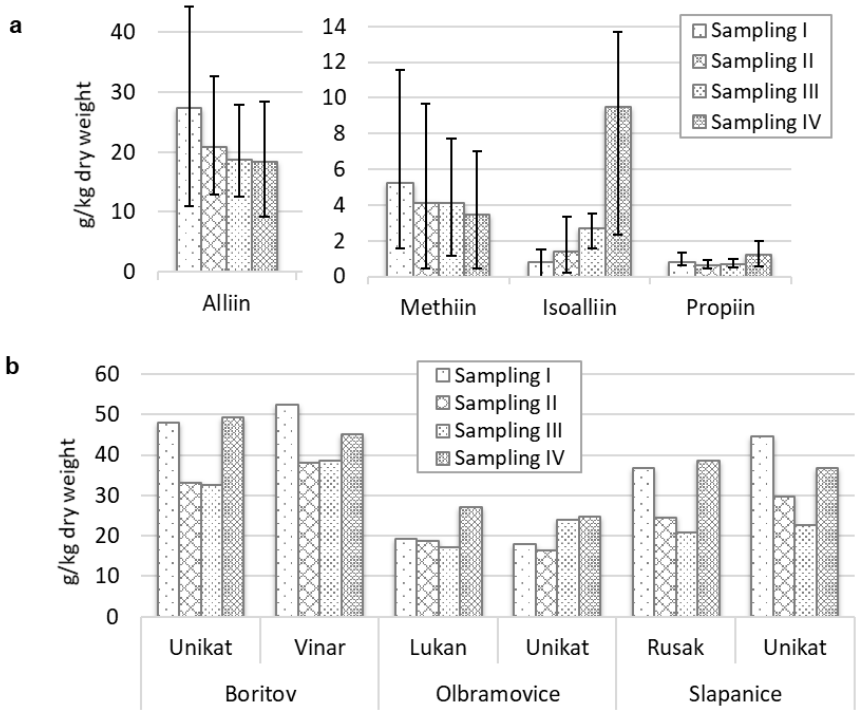

Fig. S2 Changes in S-alk(en)yl-L-cysteine sulfoxide (ACSO) content in garlic samples over the sampling period
a – Average content of ACSOs in all samplings, error bars represent maximum and minimum determined values and show a high natural variability between samples of different cultivar and growing locality;
b – Total content of ACSOs in sample subset over the sampling period. Notice a different pattern for samples of same cultivar grown at different localities (Unikat)
Sampling I - bulbs harvested one month before achieving harvest maturity (in late June); *Sampling II* - bulbs harvested mature and then dried under ambient conditions for two weeks (July/August); *Sampling III* - bulbs from *Sampling II* stored for two months at 4 °C (early October); and *Sampling IV* - bulbs from *Sampling II* stored for six months at 4 °C (end of January)

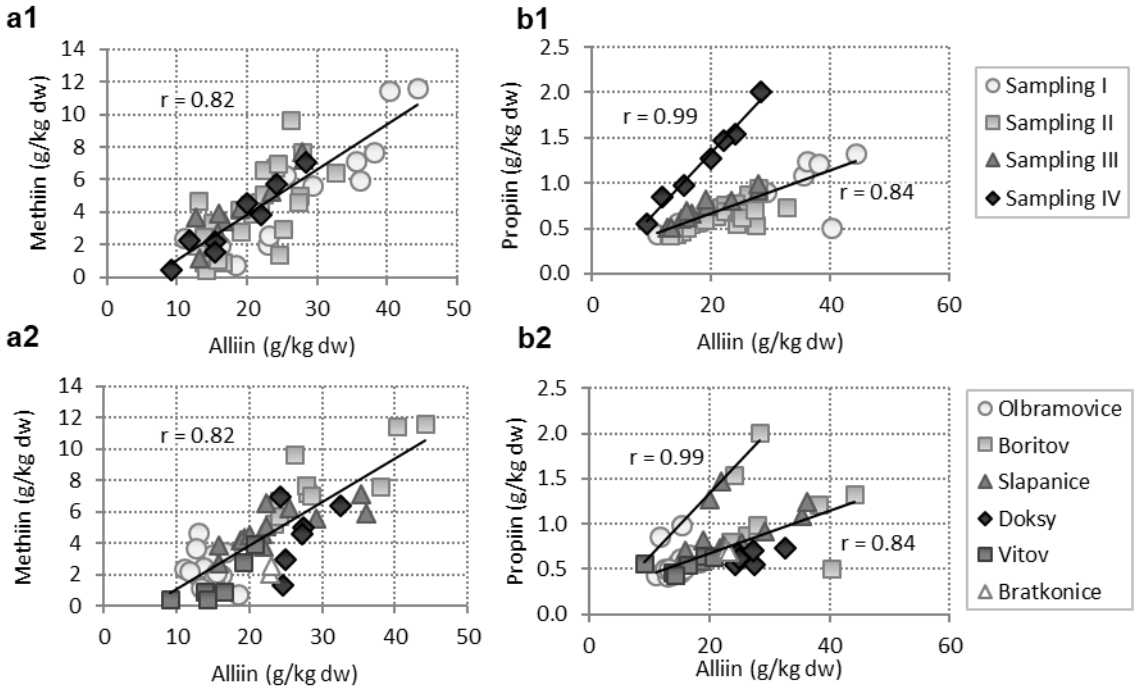

Fig. S3 Correlation between the determined content of individual S-alk(en)yl-L-cysteine sulfoxides (ACSOs) in all garlic samples

a – Alliin and methiin, linear regression for all samples;

b – Alliin and propiin, linear regression for Sampling IV (after storage at 4 °C for six months) separated from Sampling I-III (one month before harvest maturity/two weeks after harvest/after two-month storage);

1 - Coloured by samplings;

2 - Coloured by localities

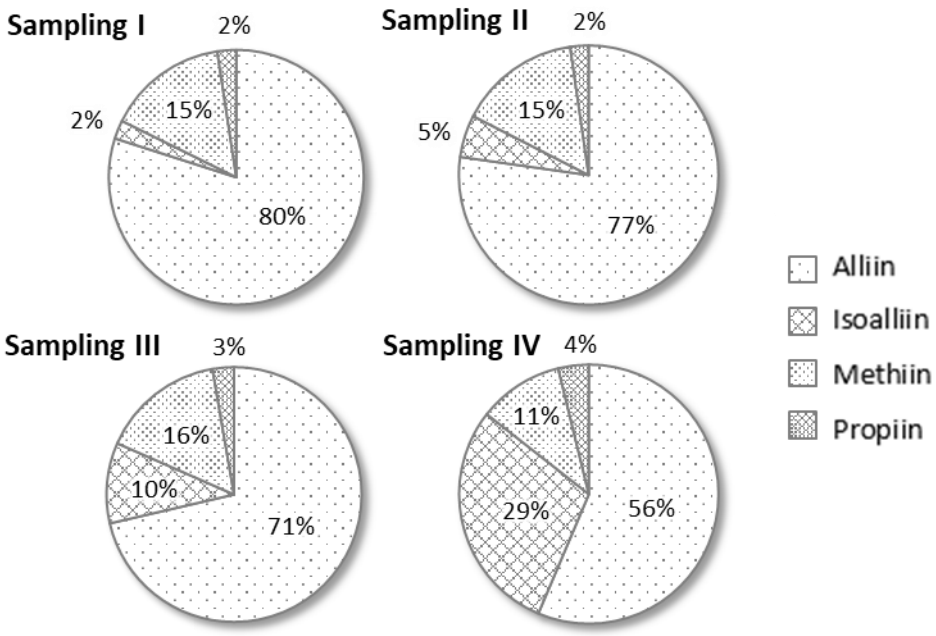

Fig. S4 Relative composition (pattern) of the S-alk(en)yl-L-cysteine sulfoxides (ACSOs) in each sampling, mean values of all samples
Sampling I – bulbs harvested one month before achieving harvest maturity (in late June);
Sampling II – bulbs harvested mature and then dried under ambient conditions for two weeks (July/August);
Sampling III – bulbs from *Sampling II* stored for two months at 4 °C (early October); and
Sampling IV – bulbs from *Sampling II* stored for six months at 4 °C (end of January)

Table S4 Compounds to be included to the LC-MS/MS method.

Compound	Abbreviation	Ion type	MRM* quantitative transition m/z	MRM* qualitative transition m/z
γ-L-glutamyl-S-methyl-L-cysteine sulfoxide	γ-GluMethiin	$[M+H]^+$	281 > 216	281 > 129
γ-L-glutamyl-S-allyl-L-cysteine sulfoxide	γ-GluAlliin	$[M+H]^+$	307 > 217	307 > 129
γ-L-glutamyl-S-(prop-1-en-1-yl)-L-cysteine sulfoxide	γ-GluIsoalliin	$[M+H]^+$	307 > 217	307 > 129
γ-L-glutamyl-S-propyl-L-cysteine sulfoxide	γ-GluPropiin	$[M+H]^+$	309 > 216	309 > 129
γ-L-glutamyl-S-methyl-L-cysteine	γ-GMCys	$[M+H]^+$	265 > 119	265 > 135
γ-L-glutamyl-S-allyl-L-cysteine	γ-GACys	$[M+H]^+$	291 > 144	291 > 72
γ-L-glutamyl-S-propyl-L-cysteine	γ-GPCys	$[M+H]^+$	293 > 146	293 > 163
γ-L-glutamyl-S-(prop-1-en-1-yl)-L-cysteine	γ-G-1-PeCys	$[M+H]^+$	291 > 84	291 > 161

*Multiple reaction monitoring

Figure S5 Chromatograms of γ-L-glutamyl peptides and their sulfoxides. For abbreviations, see Table S4.