

Supplementary Materials: Alterations in the Chemical Composition of Spinach (*Spinacia oleracea* L.) as Provoked by Season and Moderately Limited Water Supply in Open Field Cultivation

Christine Schlering ^{1,2,*}, Jana Zinkernagel ², Helmut Dietrich ¹, Matthias Frisch ³ and Ralf Schweiggert ¹

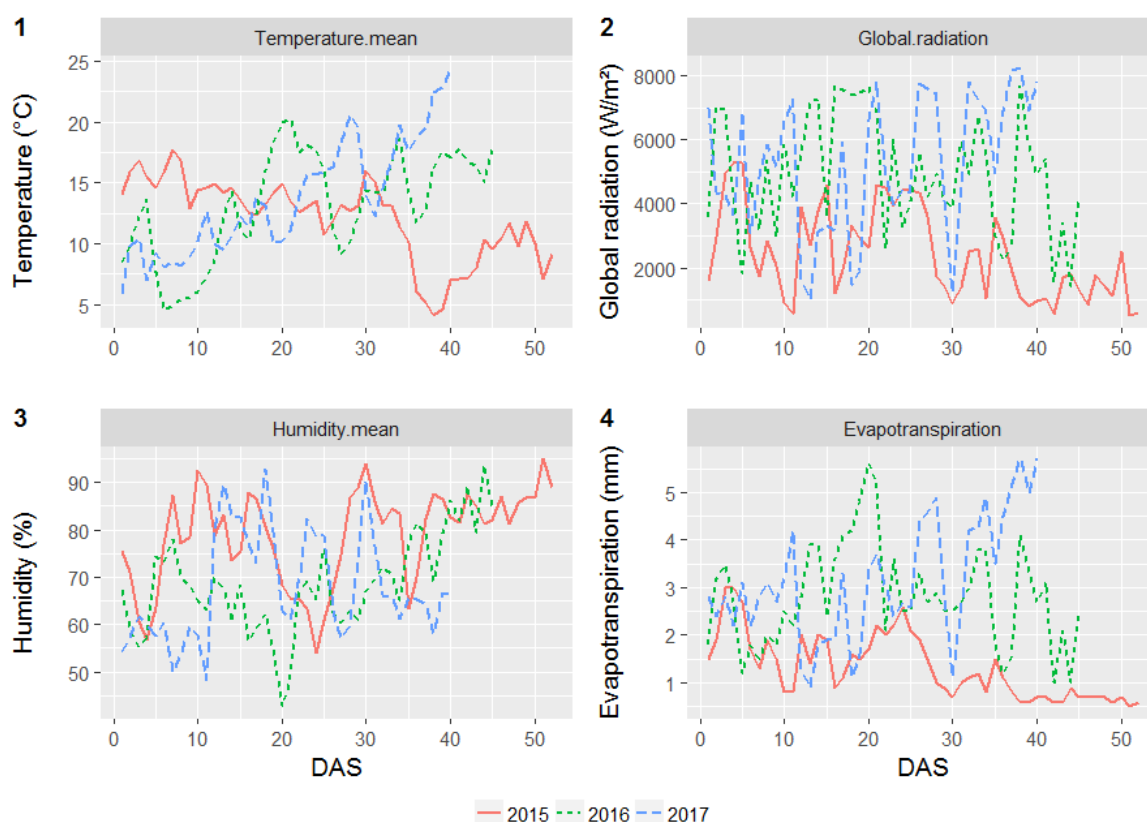


Figure S1–S4. Daily mean weather conditions during the different spinach cultivation periods (2015, 2016 and 2017): (1) Mean air temperature (°C), (2) global irradiation sum (W/m²), (3) daily mean relative humidity (%) and (4) daily mean evapotranspiration (mm). DAS: Days after sowing.

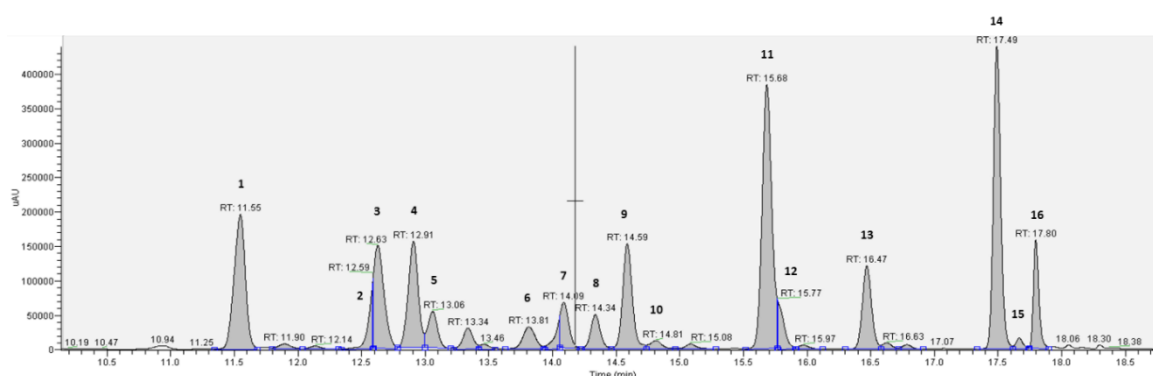


Figure S5. HPLC-DAD chromatogram of spinach flavonoids at 350 nm. Flavonoid peaks assigned by comparing retention times, UV/Vis-spectra and mass spectral data to those reported by the literature, as shown in Table S2.

Table S1. Proportion of single carotenoids on the total amount of carotenoids in spinach fresh biomass from three years (2015, 2016 and 2017). CTR: Control treatment with full water supply, RWS: Reduced water supply treatment.

	2015				2016				2017			
	CTR		RWS		CTR		RWS		CTR		RWS	
	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*
Violaxanthin	9.66	28.5%	10.05	28.6%	5.13	27.1%	5.99	27.6%	11.15	24.5%	12.09	23.6%
Neoxanthin	3.18	9.4%	3.28	9.4%	1.7	9.0%	1.86	8.6%	4.07	8.9%	4.55	8.9%
Lutein	11.52	34.0%	11.87	33.8%	5.83	30.8%	6.39	29.5%	15.91	34.9%	18	35.1%
β -Carotene	8.54	25.2%	8.88	25.3%	5.68	30.0%	6.73	31.0%	12.85	28.2%	14.81	28.9%
(9Z)- β -Carotene	0.98	2.9%	1	2.9%	0.6	3.2%	0.71	3.3%	1.55	3.4%	1.77	3.5%
Total carotenoids	33.88	100.0%	35.08	100.0%	18.94	100.0%	21.68	100.0%	45.53	100.0%	51.22	100.0%

*: abs.: absolute content in mg/100 g FM. rel.: relative content as percentage of total carotenoids.

Table S2. Peak assignment to flavonoid-compounds as detected in spinach from years 2015, 2016 and 2017 by comparing retention time (RT), UV/Vis-spectra and negative ion m/z and important MS/MS-fragments to the literature. An exemplary chromatogram is shown in Figure S5.

Peak	Identity	RT (min)	λ_{max} (nm)	[M-H] ⁻	Fragments	Reference
1	patuletin-3-O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-[β -D-apiofuranosyl-(1 $\rightarrow$ 2)]- β -D-glucopyranoside	11,55	258, 351	787	655, 439, 331	[1]
2	patuletin-coumaroyl-pentosyl-hexoside	12,59	260, 347	933	787, 331	*
3	patuletin-3-O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside	12,63	257, 351	655	493, 331	[1]
4	spinacetin-3-O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-[β -D-apiofuranosyl-(1 $\rightarrow$ 2)]- β -D-glucopyranoside	12,91	257, 351	801	669, 345	[2]
5	patuletin-3-O- β -D-(2''-feruloylglucopyranosyl-(1 $\rightarrow$ 6)-[β -D-apiofuranosyl-(1 $\rightarrow$ 2)]- β -D-glucopyranoside	13,06	258, 336	963	787, 655, 331	[2]
6	spinacetin-3-O- β -D-(2''- β -coumaroylglucopyranosyl-(1 $\rightarrow$ 6)-[β -D-apiofuranosyl-(1 $\rightarrow$ 2)]- β -D-glucopyranoside	13,81	260, 321	947	801, 345	[2]
7	spinacetin-3-O- β -D-(2''-feruloylglucopyranosyl-(1 $\rightarrow$ 6)-[β -D-apiofuranosyl-(1 $\rightarrow$ 2)]- β -D-glucopyranoside	14,09	258, 332	977	801, 669, 345	[2]
8	patuletin-feruloyl-dihexoside	14,34	258, 335	831	655, 331	*
9	spinacetin-3-O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside	14,59	257, 352	669	345	[1]
10	patuletin-coumaroyl-dihexoside	14,81	263, 347	801	655, 331	*
11	spinatoside	15,68	270, 340	521	345	[1]
12	spinacetin-3-O- β -D-(2''-feruloylglucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside	15,78	258, 336	845	669, 499, 345	[2]
13	jaceidin-4'-glucuronide	16,47	270, 340	535	359	[3]
14	5,3',4'-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4'- β -D-glucuronide	17,49	277, 341	519	343	[3]
15	Dihydroxy-methoxy-methylenedioxyflavone-hexuronide	17,68	277, 336	503	327	*
16	5,4'-dihydroxy-3,3'-dimethoxy-6:7-methylenedioxyflavone-4'- β -D-glucuronide	17,80	278, 341	533	357	[3]

* Tentatively identified

Table S3. Influence of reduced water supply on the dry biomass (DM)-related levels of single flavonoid compounds in spinach leaves from three years (2015, 2016 and 2017). Linear mixed model, t-test, *p*-values: <0.1, <0.05 (*), <0.01 (**) and <0.001 (***); CTR: Control treatment with full water supply, RWS: Reduced water supply treatment. Compound to peak number assignment is provided in Table S2.

	Spinach 2015			Spinach 2016			Spinach 2017			Spinach total		
	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value	CTR	RWS	<i>p</i> -value
Flavonoid-compound (peak) (mg g ⁻¹ DM)												
Peak no. 1	1.10	1.12	0.5686	1.82	1.93	0.2204	1.81	1.86	0.5432	1.58	1.64	0.2135
Peak no. 2	0.14	0.16	0.0671	0.16	0.17	0.2031	0.15	0.16	0.3165	0.15	0.16	0.0093 **
Peak no. 3	0.74	0.78	0.0616	0.71	0.77	0.2628	0.71	0.74	0.4890	0.72	0.76	0.5199
Peak no. 4	0.85	0.86	0.6606	0.43	0.46	0.2987	0.43	0.44	0.6033	0.57	0.59	0.1996
Peak no. 5	0.31	0.31	0.7926	0.79	0.88	0.0805	0.78	0.84	0.1886	0.63	0.68	0.0218 *
Peak no. 6	0.20	0.20	0.4150	0.06	0.08	0.0540	0.06	0.07	0.2892	0.11	0.12	0.0220 *
Peak no. 7	0.35	0.36	0.6685	0.28	0.30	0.3663	0.28	0.29	0.6084	0.30	0.32	0.2332
Peak no. 8	0.24	0.25	0.3165	0.41	0.45	0.1237	0.41	0.43	0.2799	0.35	0.38	0.1084
Peak no. 9	0.70	0.70	0.9376	0.20	0.21	0.5862	0.20	0.21	0.8180	0.37	0.37	0.6579
Peak no. 10	0.07	0.07	1.0000	0.06	0.07	0.4302	0.06	0.07	0.3250	0.06	0.07	0.2256
Peak no. 11	2.03	2.04	0.9337	1.31	1.40	0.0677	1.30	1.35	0.3518	1.55	1.60	0.1251
Peak no. 12	0.20	0.20	0.7827	0.15	0.15	0.7089	0.14	0.15	0.8560	0.16	0.17	0.7550
Peak no. 13	0.66	0.67	0.6975	0.12	0.12	0.6109	0.11	0.11	1.0000	0.30	0.30	0.5886
Peak no. 14	1.73	1.76	0.6358	3.01	3.17	0.3166	2.99	3.05	0.6723	2.58	2.66	0.2231
Peak no. 15	0.06	0.06	0.6109	0.10	0.10	1.0000	0.10	0.10	1.0000	0.09	0.09	0.8045
Peak no. 16	0.53	0.54	0.5948	0.30	0.30	1.0000	0.29	0.29	0.9444	0.37	0.38	0.7462
Total flavonoids	9.91	10.08	0.9490	9.91	10.56	0.1919	9.82	10.16	0.4886	9.88	10.27	0.1500

Table S4. Proportion of single flavonoids on the total amount of flavonoids in spinach dry biomass from three years (2015, 2016 and 2017). CTR: Control treatment with full water supply, RWS: Reduced water supply treatment. Compound to peak number assignment is provided in Table S2.

	Spinach 2015				Spinach 2016				Spinach 2017			
	CTR		RWS		CTR		RWS		CTR		RWS	
	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*	abs.*	rel.*
Peak no. 1	1.1	11.1%	1.12	11.1%	1.82	18.4%	1.93	18.3%	1.81	18.4%	1.86	18.3%
Peak no. 2	0.14	1.4%	0.16	1.6%	0.16	1.6%	0.17	1.6%	0.15	1.5%	0.16	1.6%
Peak no. 3	0.74	7.5%	0.78	7.7%	0.71	7.2%	0.77	7.3%	0.71	7.2%	0.74	7.3%
Peak no. 4	0.85	8.6%	0.86	8.5%	0.43	4.3%	0.46	4.4%	0.43	4.4%	0.44	4.3%
Peak no. 5	0.31	3.1%	0.31	3.1%	0.79	8.0%	0.88	8.3%	0.78	7.9%	0.84	8.3%
Peak no. 6	0.2	2.0%	0.2	2.0%	0.06	0.6%	0.08	0.8%	0.06	0.6%	0.07	0.7%
Peak no. 7	0.35	3.5%	0.36	3.6%	0.28	2.8%	0.3	2.8%	0.28	2.9%	0.29	2.9%
Peak no. 8	0.24	2.4%	0.25	2.5%	0.41	4.1%	0.45	4.3%	0.41	4.2%	0.43	4.2%
Peak no. 9	0.7	7.1%	0.7	6.9%	0.2	2.0%	0.21	2.0%	0.2	2.0%	0.21	2.1%
Peak no. 10	0.07	0.7%	0.07	0.7%	0.06	0.6%	0.07	0.7%	0.06	0.6%	0.07	0.7%
Peak no. 11	2.03	20.5%	2.04	20.2%	1.31	13.2%	1.4	13.3%	1.3	13.2%	1.35	13.3%
Peak no. 12	0.2	2.0%	0.2	2.0%	0.15	1.5%	0.15	1.4%	0.14	1.4%	0.15	1.5%
Peak no. 13	0.66	6.7%	0.67	6.6%	0.12	1.2%	0.12	1.1%	0.11	1.1%	0.11	1.1%
Peak no. 14	1.73	17.5%	1.76	17.5%	3.01	30.4%	3.17	30.0%	2.99	30.4%	3.05	30.0%
Peak no. 15	0.06	0.6%	0.06	0.6%	0.1	1.0%	0.1	0.9%	0.1	1.0%	0.1	1.0%
Peak no. 16	0.53	5.3%	0.54	5.4%	0.3	3.0%	0.3	2.8%	0.29	3.0%	0.29	2.9%
Total flavonoids	9.91	100.0%	10.08	100.0%	9.91	100.0%	10.56	100.0%	9.82	100.0%	10.16	100.0%

*: abs.: absolute content in mg/100 g DW. rel.: relative content as percentage of total flavonoids.

References

1. Aritomi, M.; Komori, T.; Kawasaki, T. Flavonol glycosides in leaves of *Spinacia oleracea*. *Phytochemistry* 1985, 25, 231–234, doi:10.1016/S0031-9422(00)94534-5.
2. Ferreres, F.; Castañer, M.; Tomás-Barberán, F.A. Acylated flavonol glycosides from spinach leaves (*Spinacia oleracea*). *Phytochemistry* 1997, 45, 1701–1705, doi:10.1016/S0031-9422(97)00244-6.
3. Aritomi, M.; Kawasaki, T. Three highly oxygenated flavone glucuronides in leaves of *Spinacia oleracea*. *Phytochemistry* 1984, 23, 2043–2047, doi:10.1016/S0031-9422(00)84967-5.

