The effect of a shading mesh on the metabolic, nutritional and defense profiles of greenhouse harvested organic tomato fruits and leaves revealed by NMR metabolomics

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Analysis of fatty acids content. The fatty acid content and profile in tomato samples were determined by gas chromatography (Agilent Technologies 6890 N Series Gas Chromatograph, Santa Clara, CA, USA) after direct transesterification as described by Rodríguez-Ruiz et al.[1]

Analysis of carotenoids content. The analysis of carotenoids was performed as described by Cerón-García et al.[2] Briefly, 20 mg of dry tomato was placed in glass Pyrex tubes and 1 ml of monophasic tricomponent solution was added. The tricomponent solution was composed of ethanol:hexane:water in a proportion of 77:17:6 v/v/v and contained 0–60% d.w. potassiumhydroxide (KOH) ((g KOH/g dry biomass) × 100).). The tube was submerged in a water bath with a preset temperature of 45 ºC, where it was left for 5 min. After this, the tube was taken out and vortexed for 30 s and left to cool for 1 h at room temperature. Subsequently, it was centrifuged at 12000 rpm for 2 min (Mini Spin Plus, Eppendorf) and the supernatant transferred into a vial. The carotenoid content and profile were determined using a photodiode-array HPLC (HPLC-DAD) apparatus (Shimadzu SPDM10AV). All the measurements were carried out by duplicate.

a) b)

Figure S1. (**a**) Organic tomato variety DELYCA; (**b**) image of the shading mesh (polypropylene, gray, 50% reduction in light intensity) applied in an area of the plantation.

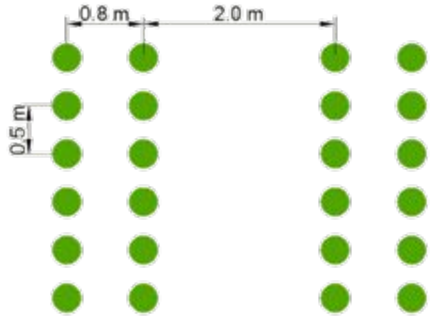

Figure S2. Scheme of the greenhouse: the crop was planted in paired lines, spaced 0.8 **m** part, the separation between lines was 2 m and the separation between plants was 0.5 m. Each line contained 16 plants. The planting framework used is 1.43 plants m^{-2}.

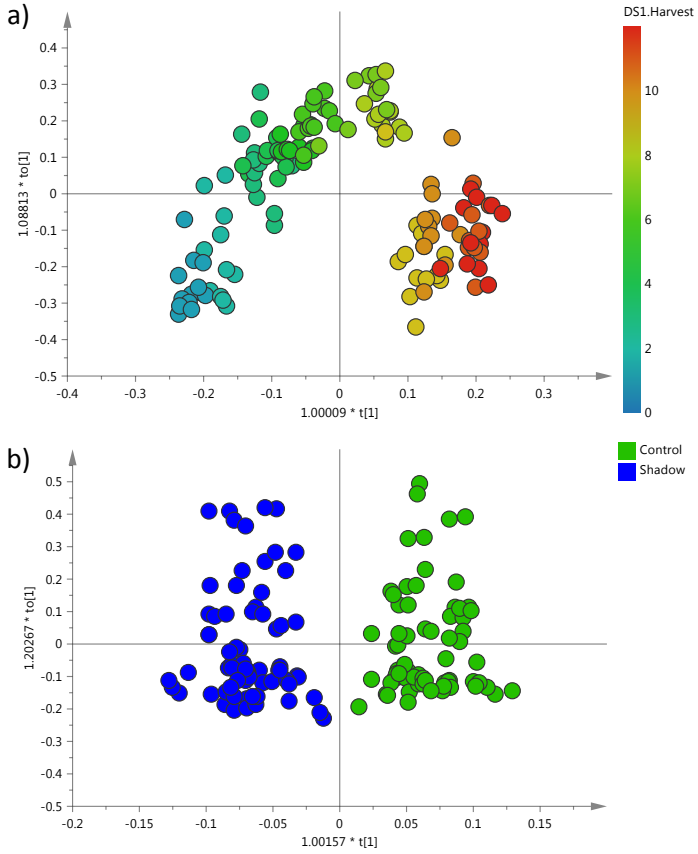

Figure S3. (a) OPLS and (b) OPLS-DA scores plots applied to ^{1}H NMR data of a total of 144 CD_3OD: D_2O KH_2PO_4 buffer (80:20, v/v) extracts of tomatoes to discriminate tomatoes according to harvest date and shading regime, respectively. Both models were used to build the SUS-plot presented in Figure 5. Scaling was done to pareto. (a): R^2X =0.862, R^2Y=0.974, Q^2 = 0.961, *p* (CV-ANOVA) = 0 (< 0.00001); (b): R^2X =0.897, R^2Y=0.880, Q^2 = 0.768, *p* (CV-ANOVA) = 2.66×10^{-30}.

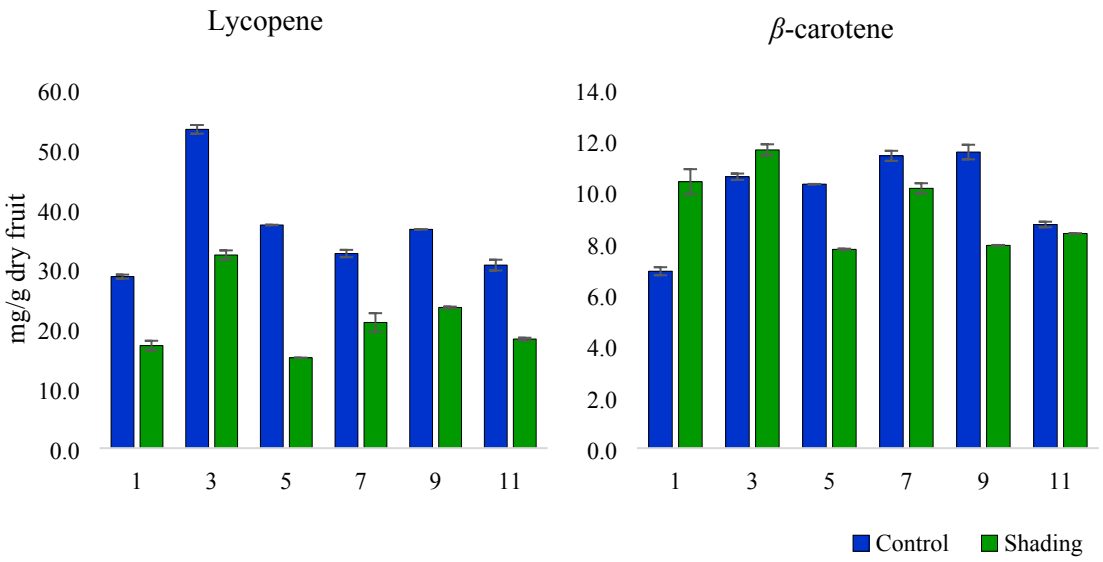

Figure S4. HPLC-DAD quantification of lycopene and *β*-carotene for shaded and non-shaded (control) tomatoes collected every two weeks.

Table S1. Chemical shifts (ppm), multiplicity and coupling constants (Hz) for the metabolites identified on methanol and phosphate buffer (80:20, v/v) extracts of tomato fruits

	Metabolite	Peak assignments
Amino acids		
1	Valine	1.01 (d, *J* = 7.2 Hz), 1.06 (d, *J* = 7.1 Hz)
2	Isoleucine	0.95 (t, *J* = 7.4 Hz), 1.02 (d, *J* = 7.2 Hz)
3	Leucine	0.97 (d, *J* = 6.3 Hz), 0.98 (d, *J* = 6.3 Hz)
4	Threonine	1.33 (d, *J* = 6.7 Hz)
5	Alanine	1.47 (d, *J* = 7.2 Hz)
6	GABA	1.91 (quint, *J* = 7.3 Hz), 2.39 (t, *J* = 7.3 Hz), 2.99 (t, *J* = 7.3 Hz)
7	Lysine	1.51 (m), 1.72 (m), 1.92 (m)
8	Arginine	1.69 (m), 1.88 (m)
9	Glutamate	2.07 (m), 2.09 (m), 2.50 (m)
10	Glutamine	2.15 (m), 2.48 (m)
11	Aspartate	2.69 (dd, *J* = 17.5, 9.0 Hz), 2.87 (dd, *J* = 17.5, 3.8 Hz)
12	Asparagine	2.80 (dd, *J* = 17.0, 8.7 Hz), 2.94 (dd, *J* = 17.0, 3.9 Hz)
13	Tyrosine	6.80 (d, *J* = 8.5 Hz), 7.15 (d, *J* = 8.5 Hz)
14	Phenylalanine	7.29 (m), 7.32 (m), 7.36 (m)
15	Tryptophan	7.06 (m), 7.14 (m), 7.23 (s), 7.40 (d, *J* = 8.0 Hz), 7.70 (d, *J* = 8.0 Hz)
16	Histidine	7.27 (d, *J* = 1.2 Hz), 8.28 (d, *J* = 1.2 Hz)
Organic acids		
17	Acetate	1.98 (s)
18	Malate	2.57 (dd, *J* = 16.0, 7.6 Hz), 2.79 (dd, *J* = 16.0, 5.0 Hz), 4.32 (dd, *J* = 7.6, 5.0 Hz)
19	Citrate	2.70 (d, *J* = 15.7 Hz), 2.80 (d, *J* = 15.7 Hz)
20	Fumarate	6.66 (s)
21	Formate	8.41 (s)
Sugars		
22	Fructose	4.05 (m)
23	Glucose	4.52 (d, *J* = 8.3 Hz), 5.14 d, *J* = 3.7 Hz)

24	Galactose	4.52 (d, *J* = 7.8 Hz), 5.28 (d, *J* = 3.7 Hz)
25	Sucrose	5.41 (d, *J* = 3.7 Hz)
Nucleosides/tides		
26	Adenosine	5.99 (d, *J* = 6.4 Hz), 8.20 (s), 8.33 (s)
27	Uridine	5.79 (d, *J* = 8.1 Hz), 5.89 (d, *J* = 4.7 Hz), 7.97 (d, *J* = 8.1 Hz)
28	Adenosine-like	6.10 (d, *J* = 5.9 Hz), 8.22 (s), 8.55 (s)
29	Uridine-like	5.87 (d, *J* = 7.6 Hz), 8.04 (d, *J* = 8.2 Hz)
Phenylpropanoids and phenolic compounds		
30	Cinnamic acid derivative 1	6.31 (d, *J* = 15.8 Hz), 7.58 (d, *J* =15.8 Hz)
31	Cinnamic acid derivative 2	6.33 (d, *J* = 15.7 Hz), 7.53 (d, *J* =15.7 Hz)
32	Cinnamic acid derivative 3	6.38 (d, *J* = 15.6 Hz), 7.61 (d, *J* =15.6 Hz)
33	Cinnamic acid derivative 4	6.40 (d, *J* = 15.8 Hz), 7.64 (d, *J* =15.8 Hz)
34	Rutin	6.27 (d, *J* =1.8 Hz), 6.48 (d, *J* =1.8 Hz), 6.90 (d, *J* =8.7 Hz), 7.63 (dd, *J* = 8.7, 2.0 Hz), 7.66 (d, *J* = 2.0 Hz)
35	Quercetin-like	6.25 (d, *J* =2.0 Hz), 6.46 (d, *J* =2.0 Hz), 6.87 (d, *J* =8.4 Hz), 7.54 (dd, *J* = 8.4, 2.1 Hz), 7.67 (d, *J* = 2.1 Hz)
Others		
36	Ascorbate	4.68 (d, *J* = 2.2 Hz)
37	Choline	3.21 (s)
38	Trigonelline	8.08 (dd, *J* = 8.0; 6.1 Hz), 8.86 (d, *J* = 6.1 Hz), 8.89 (d, *J* = 8.0 Hz), 9.17 (s)
39	Nicotinurate	8.31 (m), 9.03 (m), 9.38 (s)
40	1-Methylnicotinamide	9.59 (s), 9.33 (m), 9.04 (m)
41	Sterols	0.65-0.75 (s)
42	Fatty acids*	0.87 (–C**H**$_3$, for all FA except n-3), 0.96 (–C**H**$_3$, for n-3), 1.24-1.36 (–(C**H**$_2$)n–), 1.60 (–C**H**$_2$–CH$_2$–COOR), 2.04 (–C**H**$_2$–CH=CH–, for UFA), 2.34 (–C**H**$_2$–COOR), 2.78 (=CH–C**H**$_2$–CH=, for PUFA), 5.34 (–C**H**=C**H**–, for UFA)

* characterization of fatty acids by GC-FID is presented in Table S2. Acronyms: FA: fatty acids, UFA, unsaturated fatty acids, PUFA, polyunsaturated fatty acids, n-3: omega-3.

Table S2. GC-FID fatty acid profile and concentration regarding dried tomato

Fatty acid		Conc. (mg/ g dried tomato)	%
C8:0	Caprylic acid	7.9 ± 3.3	5.2
C10:0	Capric acid	9.7 ± 3.4	6.5
C12:0	Lauric acid	4.8 ± 2.4	3.2
C16:0	Palmitic acid	25.8 ± 5.2	17.1
C18:0	Stearic acid	8.9 ± 2.1	5.9
C16:1n7	Palmitoleic acid	2.0 ± 1.0	1.3
C18:1n9	Oleic	19.3 ± 7.4	12.8
C18:2n6	Linoleic	63.0 ± 9.9	41.8
C18:3n3	α-Linolenic	9.3 ± 3.8	6.2

Table S3. Chemical shifts (ppm), multiplicity and coupling constants (Hz) for the metabolites identified on methanolic extracts of tomato leaves (EtOAc fractions)

	Assigned metabolites	Peak assignments
1	Quercetin-like	6.19 (d, *J* =2.1 Hz), 6.38 (d, *J* =2.1 Hz), 6.85 (d, *J* =8.3 Hz), 7.61 (dd, *J* = 8.3, 2.2 Hz), 7.64 (d, *J* = 2.2 Hz)
2	Cinnamic acid	6.27 ppm (d, *J* = 15.8 Hz), 7.54 ppm (d, *J* = 15.8 Hz)
3	Trigonelline	8.01 (dd, *J* = 7.3.0; 5.9 Hz), 8.82 (d, *J* = 5.9 Hz), 8.87 (d, *J* = 7.3 Hz), 9.17 (s)
4	Formate	8.70 (s)
5	Phenylalanine	7.27 (m), 7.30 (m)
6	Tyrosine	6.71 (d, *J* = 8.5 Hz), 7.03 (d, *J* = 8.5 Hz)
7	Tryptophan	7.05 (m), 7.21 (m), 7.22 (s), 7.37 (d, *J* = 8.0 Hz), 7.63 (d, *J* = 8.0 Hz)

REFERENCES

[1] Rodríguez-Ruiz, J.; Belarbi, E.-H.; Sánchez, J. L. G.; Alonso, D. L. Rapid simultaneous lipid extraction and transesterification for fatty acid analyses. *Biotechnol. Tech.* **1998**, *12* (9), 689-691.

[2] Cerón-García, M. C.; González-López, C. V.; Camacho-Rodríguez, J.; López-Rosales, L.; García-Camacho, F.; Molina-Grima, E. Maximizing carotenoid extraction from microalgae used as food additives and determined by liquid chromatography (HPLC). *Food Chem.* **2018**, *257*, 316-324.