

Analytical Platform 6 (chlorophyll and carotenoid profiling; ISO1 protocol) was conducted by the Lange group at Washington State University. Plant material (approximately 100 mg) was sequentially extracted with three aliquots of 2 mL chloroform/methanol (2:1; v/v), containing 2 mg/L ubiquinone-10 as an internal standard. Each extraction included a 15-min incubation at room temperature, and the three organic extracts were combined in a glass vial wrapped with aluminum foil to avoid light exposure. The pooled organic phase was extracted with 1.5 mL solution of 1 M NaCl, 50 mM Tris-HCl (pH 7.5), and the vials were centrifuged (3,000 g for 2 min) to achieve phase separation. The (lower) organic phase was transferred to a new tube (kept in darkness) and the solvent was evaporated to dryness. Analytes were dissolved in 200 μ L ethyl acetate, the mixture was filtered through a 0.45 μ m nylon syringe filter and was transferred to a brown 2 mL autosampler glass vial with a 100 μ L conical glass insert.

HPLC/DAD/MS analyses were performed using an Agilent Series 1100 HPLC system (including a G1315B diode array detector) coupled to an Agilent G2445D LC/MSD Trap SL mass spectrometer. Samples were loaded (injection volume 20 μ L) onto a ProntoSil C30 column (250 x 4.6 mm; 5 μ m particle size; Bischoff Chromatography distributed via MAC-MOD Analytical, Chadds Ford, PA) equipped with a guard column of the same stationary phase material. The mobile phase consisted of (A) methanol, (B) water/methanol (20:80; v:v) containing 0.2 % ammonium formate, and (C) tert-butyl methyl ether, and the following gradient was used at a flow rate of 1 mL/min (Fraser et al., 2000): 11 min of isocratic gradient of 95% A/5% B, a step change to 90% A/ 5% B/5 % C, a linear gradient (15 min) to 30% A/5% B/65% C, hold at this condition for 5 min, followed by a conditioning phase to return to the initial conditions. UV/VIS absorbance was monitored at 275, 287, 460 and 655 nm, and spectra were recorded from 250 to 750 nm in 2 nm increments. Analytes were identified and quantified by comparison of retention times and UV/VIS spectra with those of corresponding reference standards. To ensure proper peak assignments, eluting analytes from random samples were also ionized using atmospheric pressure chemical ionization and mass traces were acquired by single-ion monitoring in positive ion mode to detect the following mass ion transitions: m/z 864 \rightarrow 680 (ubiquinone-10; internal standard), m/z 538 \rightarrow 444 (*alpha*- and *beta*-carotene), m/z 569 \rightarrow 551 (lutein), 894 \rightarrow 615 (chlorophyll *a*), m/z 907 \rightarrow 629 (chlorophyll *b*), m/z 872 \rightarrow 593 (pheophytin *a*). The probe voltage was set to 4.0 kV, the capillary voltage was at 2200 V, the gas temperature was 350 $^{\circ}$ C, and the nebulizer gas flow was 9.0 L/min. Raw data were exported to Microsoft Excel and peak areas normalized to tissue mass and internal standard using Microsoft Access. To ensure low background signals a blank injection was performed after every 10 samples. Prior to sample analyses, and then after every 20 samples, a standard mix was run to evaluate the reproducibility of the analyses.

Fraser PD, Elisabete M, Pinto S, Holloway DE, Bramley PM (2000)
Application of high-performance liquid chromatography with photodiode array detection to the metabolic profiling of plant isoprenoids. *Plant Journal* **24**: 551-558

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