

Analytical Platform 3 (amino acid profiling) was conducted by the Nikolau group at the W.M. Keck Metabolomics Research Laboratory, Iowa State University. Plant material (25 mg) was homogenized with 0.5 mL 10% (w/v) trichloroacetic acid, spiked with 5 nmol Norvaline as an internal standard. Extracts were centrifuged (16,000 x g for 5 min) and the supernatants were transferred to a 2 mL glass vial. Amine-containing analytes were derivatized with propyl chloroformate (Kugler et al., 2006) and analyzed by GC-FID (EZ:faast™ GC-FID kit; KGO-7165, Phenomenex, Torrance, CA, USA).

GC-FID analyses were performed on an Agilent 6890 GC equipped with an Agilent 7863 G2613A autosampler and flame ionization detector controlled by MSD Chemstation (D.00.01) software. Hydrogen and synthetic air were used as auxiliary gasses for the FID detector. Analytes were separated at a flow rate of 1.2 mL/min using He as carrier gas, and using a thermal gradient that started at 110°C (1 min), and then ramped to 290°C at a rate of 30°C/min. Data were acquired MSD Chemstation (D.00.01) software. Analytes were identified based on comparison of their retention time to standard mixtures provided in kit. Quantification was obtained by using the TIC response of the standard mixture, raw data were exported to Microsoft Excel and peak areas normalized to tissue mass and the internal standard.

Kugler F, Graneis S, Schreiter PP, Stintzing FC, Carle R (2006) Determination of free amino compounds in betalainic fruits and vegetables by gas chromatography with flame ionization and mass spectrometric detection. *J Agric Food Chem* **54**: 4311-4318