

Analytical Platform 5 (Targeted Phytosterol and Tocopherol Profiling; PS1 protocol) was conducted by the Lange group at Washington State University. Plant material (approximately 100 mg) was homogenized (Ball Mill MM301, Retsch, Haan, Germany) in the presence of liquid nitrogen and analytes were extracted at 75°C for 60 min with 4 mL chloroform/methanol (2:1, vol:vol; containing 1.25 mg/L epi-cholesterol as an internal standard). Extracts were kept at room temperature for at least 1 h, solvents were evaporated to dryness (EZ2-Bio, GeneVac, Ipswich, UK), and the remaining residue was saponified at 90 °C for 60 min in 2 mL 6 % (w/v) KOH in methanol. Upon cooling to room temperature, 1 mL n-hexanes and 1 mL H<sub>2</sub>O were added, and the mixture was shaken vigorously for 20 sec. Following centrifugation (3000 x g for 2 min) to separate the phases, the hexane phase was transferred to a 2 mL glass vial, and the aqueous phase was re-extracted with 1 mL n-hexanes as above, centrifuged as above, and the hexane phase added to the 2 mL glass vial containing the hexane phase from the first extraction. The combined hexane phases were evaporated to dryness using a gentle stream of nitrogen, 50 µL of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) were added to the residue, the sample was shaken vigorously for 20 sec, and the mixture transferred to a 2 mL autosampler glass vial with a 100 µL conical glass insert. After capping the vial, the reaction mixture was incubated at room temperature for at least 5 min.

GC-MS analyses were performed on an Agilent 6890N GC coupled to an Agilent 5973 inert MSD detector. Samples were loaded (injection volume 1 µL) with a LEAP CombiPAL onto a HP-5MS fused silica column (30 m x 250 µm; 0.25 µm film thickness). The temperatures of the injector and MSD interface were both set to 280°C. Analytes were separated at a flow rate of 1 mL/min using He as carrier gas and using a thermal gradient starting at 170°C (1.5 min), which was ramped first to 280°C at 37°C/min and then to 300°C at 1.5°C, where it was held for 5.0 min. Eluents were fragmented in electron impact mode with an ionization voltage of 70 eV. Data were acquired using MSD ChemStation (Revision D.01.02.SP1) software. Background was subtracted and peaks were deconvoluted using AMDIS (Automatic Mass Spectral Deconvolution and Identification Software). Analytes were identified based on their mass fragmentation patterns by comparison with those of authentic standards using the NIST Mass Spectral Search Program (Version D.05.00). Peak areas were obtained from the Total Ion Chromatogram (TIC) for all detectable peaks with a phytosterol mass fragmentation signature. The quantification of tocopherols (note that only  $\alpha$ -tocopherol accumulated to quantifiable levels in present set of samples) the Extracted Ion Chromatogram (EIC) at m/z 502 (detecting the trimethylsilyl derivative) was used. 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 (followed by a shortened thermal gradient) was performed after each sample run. Prior to sample analyses, and then after every 20 samples, a standard mix was run to evaluate the reproducibility of the analyses.