DeFuria et al., Supplemental Methods

Analysis of Blueberry Anthocyanins by LC / MS

Sample Preparation.
A freeze dried blueberry powder was obtained from the U.S. High bush Blueberry Council (USHBC) which was comprised of a 50/50 blend of the blueberry varieties Vaccinium ashei (Tifblue) and Vaccinium corymbosum (Rubel) designated 10506. Dried powder was stored at -80 °C until analysis.

Powder was dissolved to a concentration of 1 g dry powder/L of either 5% acetonitrile (Fisher Co., Fair Lawn, NJ) in water containing 1% formic acid (HFO) (Sigma Chemical Co., St. Louis, MO) or in methanol containing 1% formic acid. This solution was diluted in 5% acetonitrile in water containing 1% formic acid to produce a 0.1 g/L berry extract for injection on HPLC-MS/MS with a diode array detector (DAD) in line.

LC-MS Analysis.
LC/MS analysis was accomplished by modification of a previously reported method (1). Chromatographic separation of extracted anthocyanins was conducted using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) fitted with a Phenomenex Synergy Max-RP C18 analytical columns, 250 x 4.6 mm, 4 µm particle size. The columns were protected with a Phenomenex Max-RP cartridge. Anthocyanin separation was achieved using a gradient between 4.5% HFO in water (mobile phase A) to 4.5% HFO in 100% acetonitrile (mobile phase B) over an 80 min analytical run at a flow rate of 0.0003 L/min. The gradient profile was as follows: mobile phase B, 5% at 0 min, 12% at 12 min, 24% at 40 min, 40% between 45 and 50 min, 100% from 55 to 70 min, and then 5% from 75 to 80 min. Using a six-port valve, the eluant flowing from the analytical column was directed to waste until just before anthocyanins began to elute at 18 min. The flow was then redirected to an Agilent UV G1315A DAD, where absorbance was monitored between 250 and 700 nm. Once through the DAD, the eluant was directed to a Bruker Esquire ion trap MS/MS (Bruker Daltonics Inc., Billerica, MA). This MS unit was fitted with an electrospray interface and was operated in the positive ion mode with alternating MS and MS/MS scans from m/z 150 to 1000.

MS/MS scans of anthocyanins were compared with authentic anthocyanin standards that were obtained from either Polyphenols AS (Sandnes, Norway) or Extrasynthase (Genay, France). Eight pure anthocyanin standards were used including cyanidin 3-glucoside (Cyn glu), cyanidin 3-galactoside (Cyn gal), cyanidin 3-arabinoside (Cyn ara), delphinidin 3-glucoside (Del glu), peonidin 3-glucoside (Peo glu), peonidin 3-galactoside (Peo gal), peonidin 3-arabinoside (Peo ara), and malvidin 3-glucoside (Mal glu). MS data were handled using Bruker Daltronics Esquire LC 4.5 software for collection (Build 21) and analysis (Build 49). Compounds were identified by matching their HPLC retention time, UV absorption profile, m/z of their molecular ions, and their MS/MS fragmentation pattern with standards. Area counts of the intensity scans for the individual anthocyanin aglycone fragment obtained by MS/MS were collected for anthocyanins in the blueberry powder and standards. An equation for the standard curves was calculated by fitting a linear line using the least squares method. The resulting equation was used to calculate the value of anthocyanins in the blueberry powder.
Literature Cited