Methods

uHPLC analysis

uHPLC analysis of anthocyanins and conjugates was performed on Dionex Ultimate 3000 (Thermo Scientific; USA) equipped with a BDS Hypersil 150 x 4.6 mm i.d. (particle size 3 µm) reversed phase C18 column (Thermo Scientific; USA). Detection was carried out at 520 nm using a diode array detector (DAD). The solvents were (A) H₂O/HCOOH (9:1) and (B) H₂O/HCOOH/CH₃CN (6:1:3). The gradient consisted of 100-64% A for 40 min, followed by 10 min for cleaning with 100% solvent B and 10 min with initial conditions, at a flow rate of 1 mL/min.

LC-MS analysis

HPLC analysis of the anthocyanin metabolites was performed on a liquid chromatograph (Hewlett-Packard 1100 series) equipped with a BDS Hypersil 150 x 4.6 mm i.d. (particle size 3 µm) reversed phase C18 column (Thermo Scientific; USA), thermostatted at 35ºC. Solvents, gradient and flow rate were the same as for uHPLC. Double online detection was done in a photodiode spectrophotometer and by mass spectrometry. The mass detector was a Finnigan LCQ (Finnigan Corporation, San Jose, USA) equipped with an API source, using an electrospray ionization (ESI) interface. Both the auxiliary and the sheath gas were a mixture of nitrogen and helium. The capillary voltage was 3 V and the capillary temperature 190ºC. Spectra were recorded in positive ion mode. The mass spectrometer was programmed to do MS² scan events of the parent ions described in Table 1_ESI using relative collision energies of 45 and recorded between 135-2000.