Supplementary Material

The *In Vitro* Simulated Gastrointestinal Digestion Affects the Bioaccessibility and Bioactivity of *Beta vulgaris* Constituents

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Table S1. Phases of the method used for simulated gastrointestinal digestion of beetroot.

Phases	Conditions
Oral phase (GP)	The sample was mixed with simulated salivary fluid (1:1) and amylase (75 U/mL) at pH 7 for 2 min.
Gastric phase (GP)	The oral bolus was mixed with simulated gastric fluid (1:1), pepsin (2,000 U/mL) and gastric lipase (60 U/mL) at pH 3 for 2 h.
Intestinal phase (IP)	The gastric chime was mixed with simulated intestinal fluid (1:1) and pancreatin (Trypsin activity 100 U/mL) at pH 7 for 2 h.
Digested (D) sample	The mixture was centrifugated at 4500 rpm for 30 min and then filtered through a 1 μm glass-fiber membrane.

Table S2. Method, equipment and analytical conditions used in the analysis of minerals, organic acids and betacyanins.

	Minerals	Organic acids	Betacyanins
Method	Inductively coupled plasma optical emission spectroscopy (ICP-OES).	Ultra-fast liquid chromatography with photodiode array detection (UFLC-PDA).	High-performance liquid chromatograph with diode array detection/electrospray ionization mass spectrometry (HPLC-DAD-ESI/MS).
Equipment & Analytical Conditions	An inductively coupled plasma optical emission spectrometer (700 Series ICP-OES; Agilent Technologies, Santa Clara, United States) equipped with an axial viewing and a charge-coupled device detector with a radiofrequency generator of 40 MHz, plasma gas flow rate of 15 L/min, auxiliary gas flow rate of 1.5 L/min, power of 1 kW and nebulizer gas (One Neb 2) pressure of 200 kPa.	An UFLC system (Shimadzu Corporation, Kyoto, Japan) with photodiode array detection (PDA) equipped with a SphereClone reverse-phase C18 column (5 μm particle size, 250 × 4.6 mm) (Phenomenex, Torrance, CA) thermostated at 35 °C was used for compounds separation. The mobile phase consisted of a sulfuric acid solution (3.6 mM) at a flow rate of 0.8 mL/min.	An HPLC (Dionex Ultimate 3000 HPLC, Thermo Scientific, San Jose, CA, USA) system and a Linear Ion Trap LTQ XL MS (Thermo Finnigan, San Jose, CA, USA) MS equipped with an ESI source. Separation was performed on a Waters Spherisorb S3 ODS-2 C18 column (3 μm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) thermostatted at 35 °C. The solvents ((A) 0.1% trifluoroacetic acid (TFA) in water, (B) acetonitrile) were used as folows: 10% B for 3 min, from 10 to 15% B for 12 min, 15% B for 5 min, from 15 to 18% B for 5 min, from 18 to 30% B for 20 min, from 30 to 35% B for 5 min, and from 35 to 10% B for 10 min.

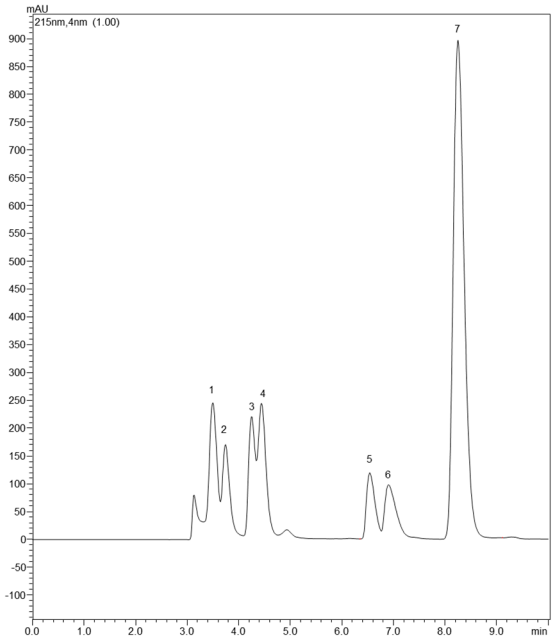
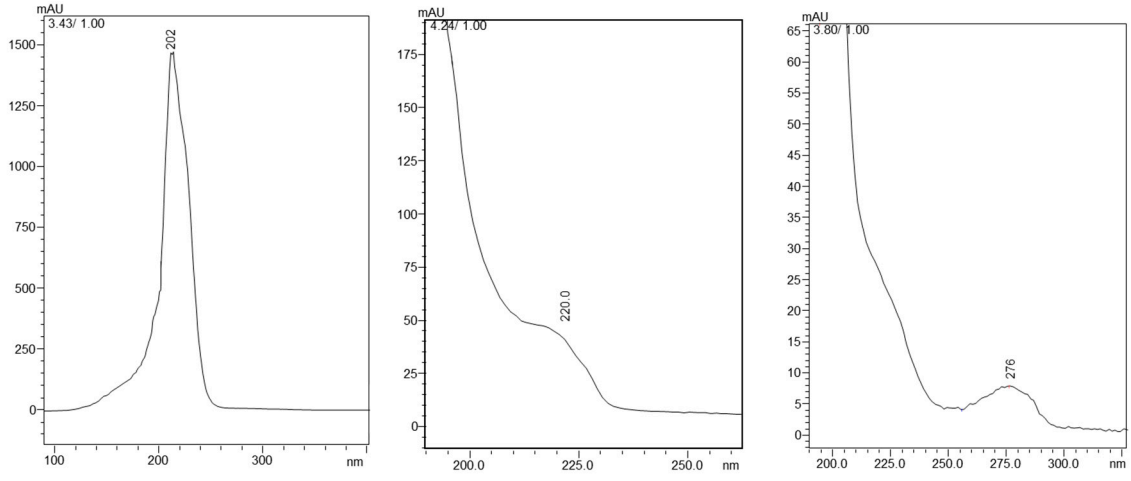


Figure S1. UFLC profile of commercial organic acid standards recorded at 215 nm. Peak identification: 1- oxalic acid; 2- quinic acid; 3- malic acid; 4- shikimic acid; 5- citric acid; 6- succinic acid; and 7- fumaric acid.



A B C

Figure S2. Maximum UV-Vis absorption spectrum (recorded at 215 nm) of oxalic acid (A), quinic acid (B), and malic acid (D).

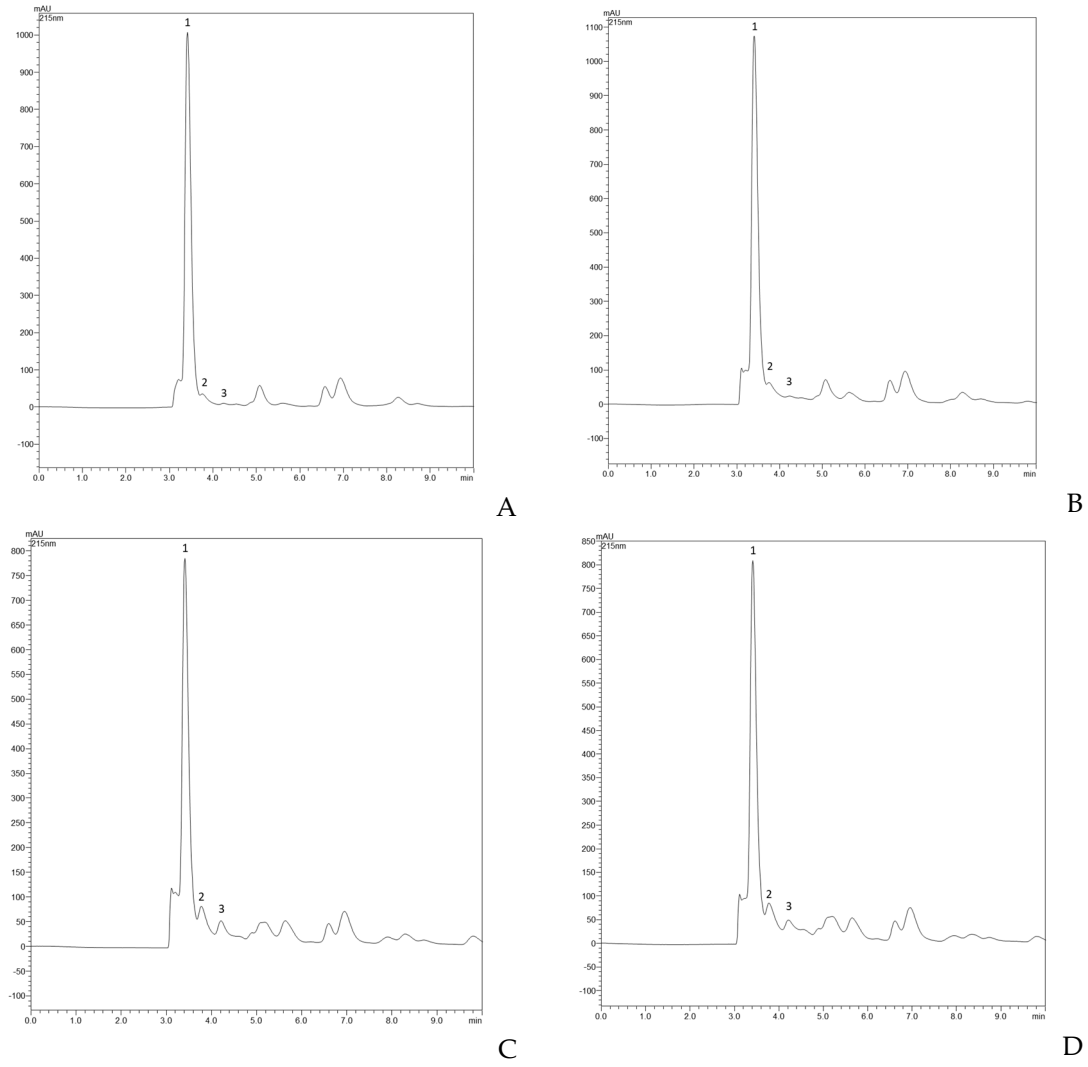


Figure S3. UFLC organic acid profile of beetroot (A), gastric phase (B), intestinal phase (C), and digested (D) sample recorded at 215 nm. Peak identification: 1- oxalic acid; 2- quinic acid; and 3- malic acid.

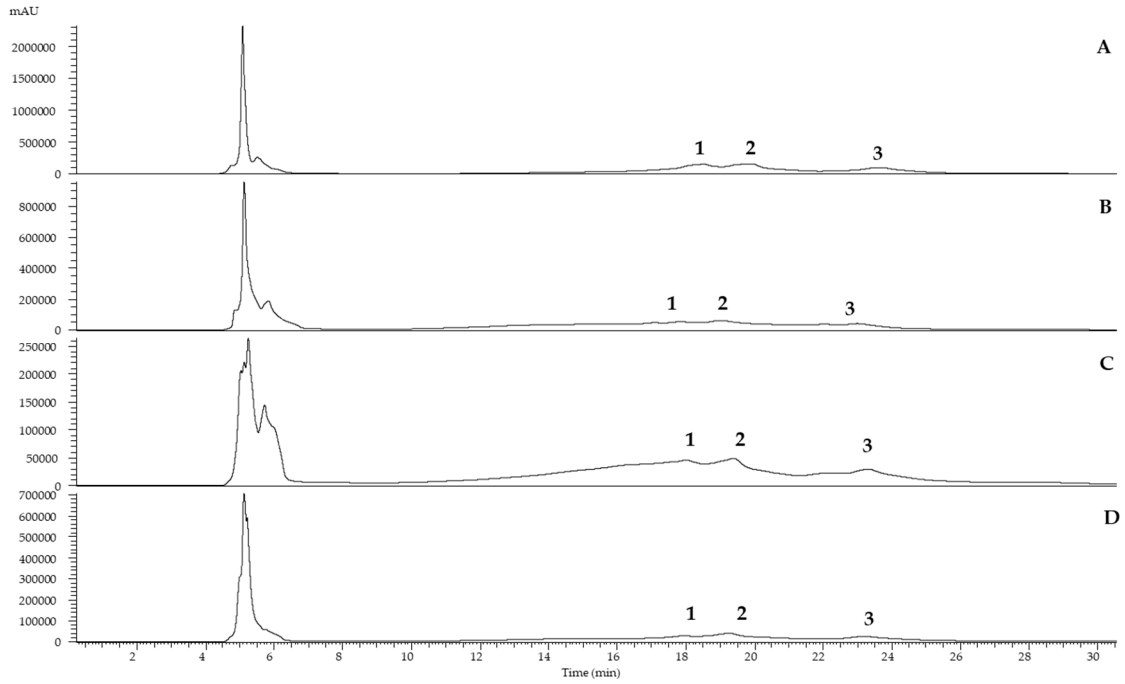


Figure S4. HPLC chromatographic profile of betacyanins in beetroot (A), gastric phase (B), intestinal phase (C), and digested (D) sample recorded at 535 nm. Peak identification: 1- betanidin-5-*O*-glucoside (betanin); 2- isobetanidin-5-*O*-glucoside (isobetanin); and 3-14,15-dehydrobetanin (neobetanin).

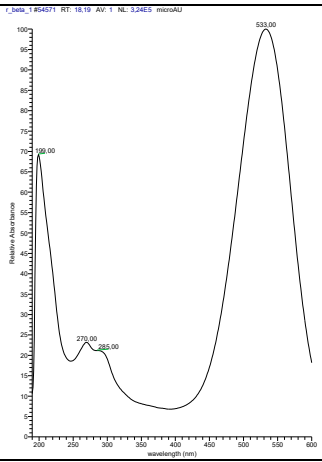
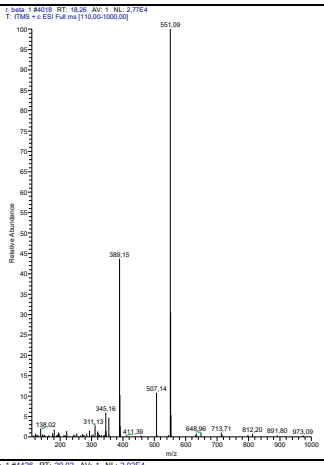
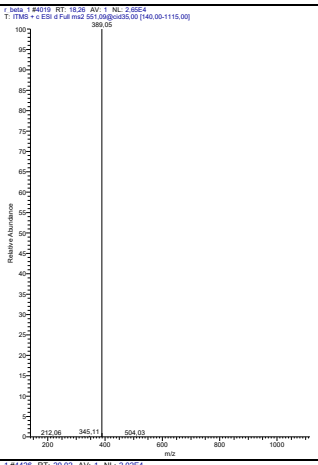
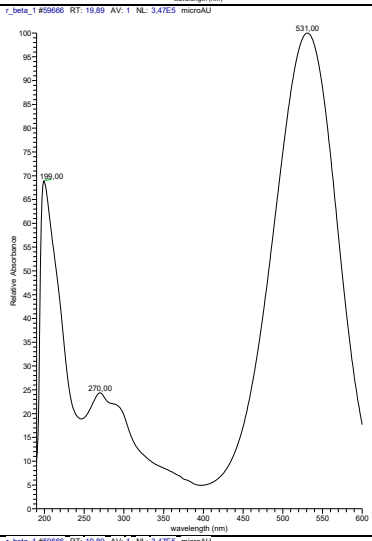
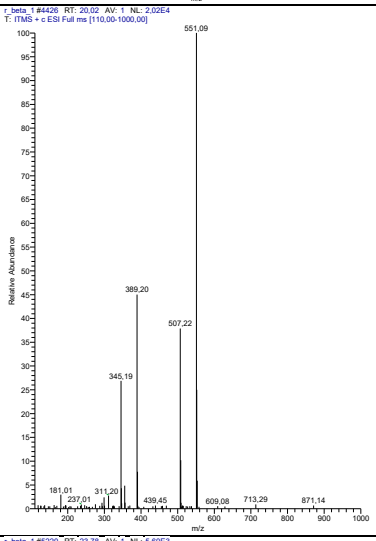
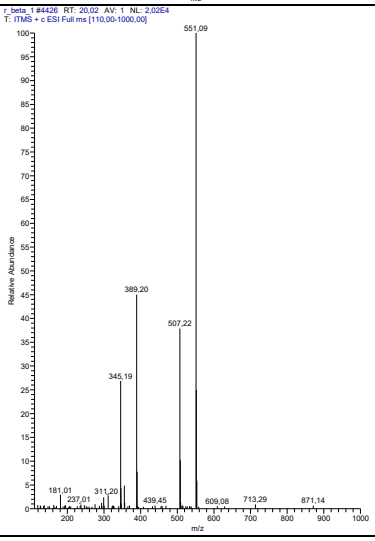
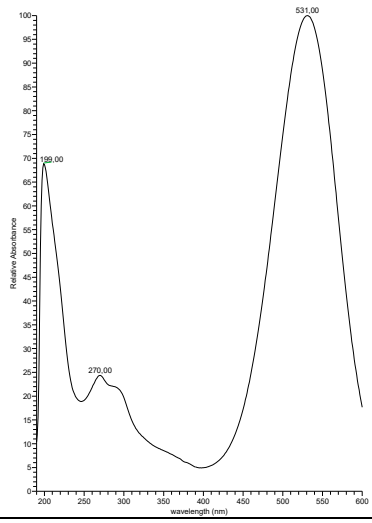
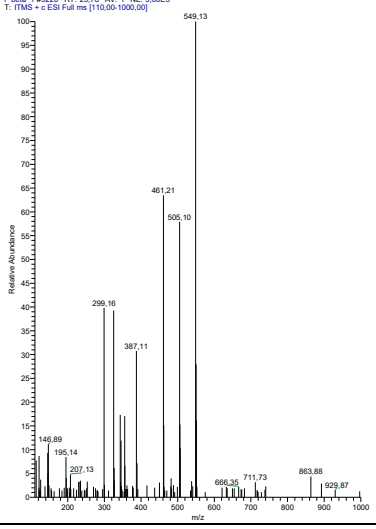
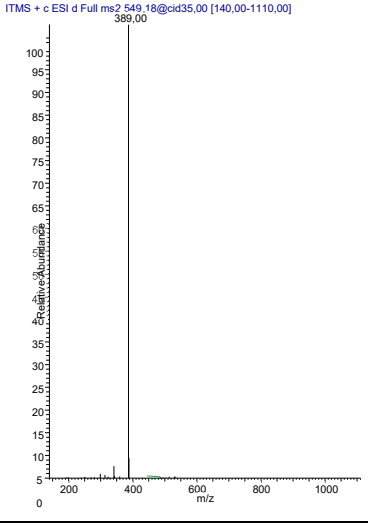
Peak	UV-Vis Spectra	Mass Spectra	
		Full MS	MS²
1			
2			
3			

Figure S5. Maximum absorption spectrum (recorded at 535 nm) and mass spectrum (full MS and MS²), obtained by HPLC-DAD-ESI/MS^n, of the three betacyanins identified in the beetroot samples. Peak identification: 1- betanidin-5-*O*-glucoside (betanin); 2-isobetanidin-5-*O*-glucoside (isobetanin); and 3- 14,15-dehydrobetanin (neobetanin).