

Supplementary Information

Extraction of phytochemicals from the pomegranate (*Punica granatum L.*, *Punicaceae*) by reverse iontophoresis

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Time (min)	% B (C18)	%B (HILIC)
0.0	5	100
1.0	20	-
4.0	45	-
4.5	95	-
6.5	95	-
7.0	5	-
8.0	95	-
10.0	95	-
10.5	5	-
11.5	-	30
12.0	-	100
12.5	5	-
15.0	-	100

Supplementary Table S1:
Chromatographic separation schedules.

Supplementary Table S2: LC-MS operational conditions for the two analysis methods employed.

Parameter	C18 method	HILIC method
Injection volume (μL)	5	5
Mobile phase flow rate (mL/min)	0.4	0.5
Gas temperature ($^{\circ}\text{C}$)	250	300
Drying gas flow rate (L/min)	12	13
Nebulising gas pressure (bar)	3.1	2.1
Sheath gas temperature ($^{\circ}\text{C}$)	350	350
Sheath gas flow rate (L/min)	12	12
VCap voltage (V)	3500	1500
Fragmentor voltage (V)	125	100
Skimmer voltage (V)	45	40
MS/MS scan segment collision energies (eV)	0, 20, 40	0, 20, 40

Supplementary Table S3: Summary of mass spectrometric data for bioactive compounds generated from *ex vivo* and *in vivo* RI experiments with their relevant physicochemical parameters. Putative hits were qualified based on precursor ppm mass error (within 5 ppm) with at least two curated fragment ions within 10 ppm mass error.

RT (min)	HPLC column	Putative phytochemical name (CAS number)	pK _a (s)	Charge at pH 7.4	Metabolite Class	Molecular Formula	ES(-/+) theor. m/z	ES(-/+) found m/z	Adduct	m/z error (ppm)	MS/MS ES(-) fragment ions
0.87*	C18	Citric acid [†] (320-77-4)	3.1 4.8 6.4 ^a	-3	Organic acid	C ₆ H ₈ O ₇	191.0192	191.0199	[M-H] ⁻	3.7	57.0346 67.0189 85.0295 87.0088 111.0088 154.9986 173.0092
6.97	C18	Luteolin 7-β-rutinoside ^{†§} (20633-84-5)	8.3 ^b	0	Flavonoid glycoside	C ₂₇ H ₃₀ O ₁₅	593.1506	593.1513	[M-H] ⁻	1.2	227.0383 255.0302 284.0302 285.04 286.0427 327.0510
4.98	C18	(-)-Epicatechin [†] (490-46-0)	8.3 (1)	0	Flavonoid	C ₁₅ H ₁₄ O ₆	289.0712	289.0718	[M-H] ⁻	2.1	109.0295 179.0350 203.0714 205.0506 245.0819
6.60	C18	Rutin [†] (115888-40-9)	6.4 ^b	-1	Flavonoid glycoside	C ₂₇ H ₃₀ O ₁₆	609.1456	609.1457	[M-H] ⁻	0.2	178.9986 300.0334 301.0412
6.70*	C18	Ellagic acid ^{†‡§} (476-66-4)	5.4 6.8 (2)	-2	Tannin	C ₁₄ H ₆ O ₈	300.9984	300.9987	[M-H] ⁻	1.0	257.0086 284.9994
9.49*	HILIC	Histidine [§] (71-00-1)	1.8 6.0 9.2 ^a	0	Amino acid	C ₆ H ₉ N ₃ O ₂	156.0773	156.0772	[M+H] ⁺	-0.6	81.0448 82.0527 93.045 83.0601 110.0714
10.42*	HILIC	Lysine [§]	2.2	+1	Amino acid	C ₆ H ₁₄ N ₂ O ₂	147.1133	147.1129	[M+H] ⁺	-2.7	56.0495

		(923-27-3)	9.0 10.5 ^a								84.0808
9.88*	HILIC	Arginine [§] (74-79-3)	2.2 9.0 12.5 ^a	+1	Amino acid	C ₆ H ₁₄ N ₄ O ₂	175.1195	175.1193	[M+H] ⁺	-1.1	60.0570 70.0651 116.0706 130.0975
8.88*	HILIC	Aspartic acid [§] (56-84-8)	1.9 3.7 9.6 ^a	-1	Amino acid	C ₄ H ₇ NO ₄	134.0450	134.0453	[M+H] ⁺	-2.2	46.0286 70.0288 74.0238 116.0341
9.36*	HILIC	Glutamylglutamic acid [§] (3929-61-1)	2.2 4.3 9.7 ^{ad}	-2	Dipeptide	C ₁₀ H ₁₆ N ₂ O ₇	277.1035	277.1041	[M+H] ⁺	2.2	84.0450 130.0496 148.06
0.81	C-18	Malic acid [§] (6915-15-7)	3.5 5.0 ^c	-2	Organic acid	C ₄ H ₆ O ₅	133.0137	133.0144	[M-H] ⁻	5.3	71.0139 72.9930 115.0033
7.13	C-18	Abscisic acid [§] (7773- 56-0)	4.7 ^b	-1	Phytohormone	C ₁₅ H ₂₀ O ₄	263.1283	263.1288	[M-H] ⁻	1.9	153.0919 203.1077 219.1386

^a <https://www.rsc.org/merck-index>

^b <https://hmdb.ca/metabolites>

^c <https://pubchem.ncbi.nlm.nih.gov>

^d pK_a values based on those of glutamic acid

*Verified with analytical reference standard

† RI extraction (isolated peel) confirmed from unknown cultivar (Israel)

‡ RI extraction *in fructo* confirmed from Hicaz (Turkey)

§ RI extraction *in fructo* confirmed from Aco (South Africa)

|| RI extraction *in fructo* confirmed from Wonderful (Peru)

Supplementary Table S4: Intra-fruit variability in two Hicaz (Turkey) pomegranates

Peel section	Ellagic acid concentration in dry peel ($\mu\text{g}/\text{mg}$)	
	Experiment P1A & P1B	Experiment P2A & P2B
Top	2.62 (\pm 0.19)	1.53 (\pm 0.10) ^b
Middle	1.96 (\pm 0.22) ^{a,b}	1.57 (\pm 0.04) ^b
Bottom	2.72 (\pm 0.20)	1.94 (\pm 0.06)

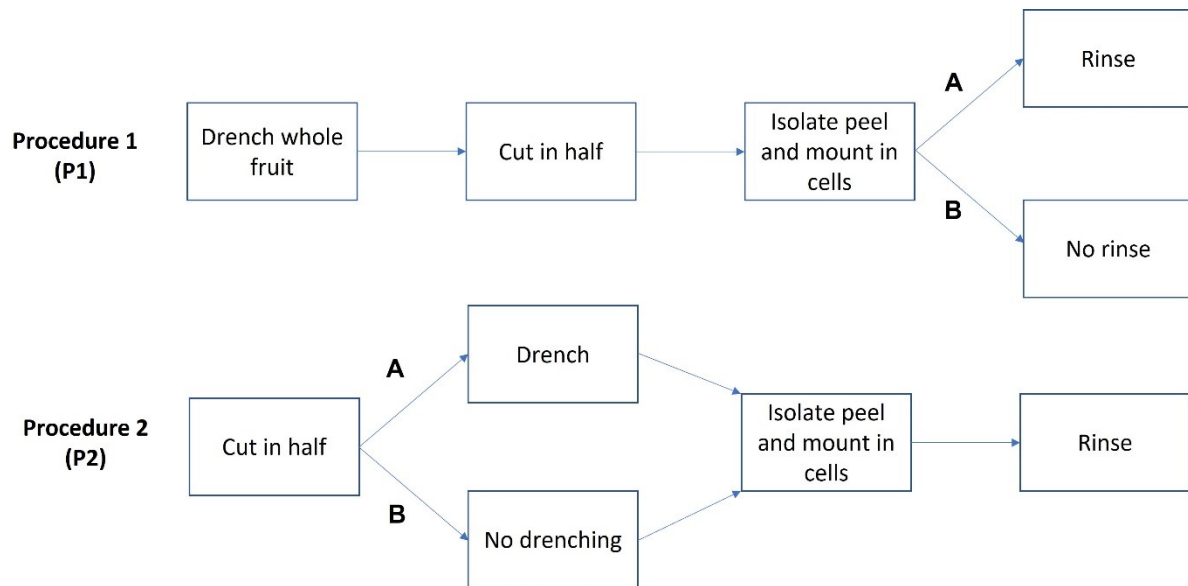
^a Significantly different to Top

^b Significantly different to Bottom

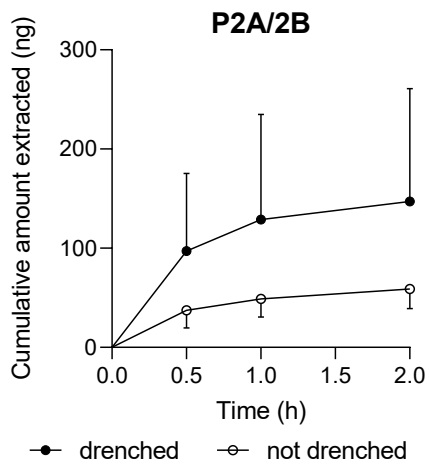
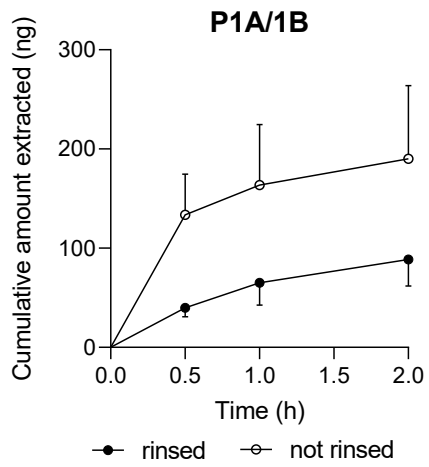
Supplementary Table S5: Measured parameters from the reverse iontophoresis extraction of ellagic acid (EA) on intact pomegranates

Cultivar	% loss on drying	pH of dry peel suspension	Ellagic acid in dry peel ($\mu\text{g}/\text{mg}$)	EA extracted in 1 st hr (ng)	EA extracted in 2 nd hr (ng)
Aco (South Africa)	69	3.7	3.78 (\pm 1.70)	2060 (\pm 1001)	1321 (\pm 1069)
Wonderful (Peru)	63	3.4	1.09 (\pm 0.04)	53 (\pm 33)	58 (\pm 32)

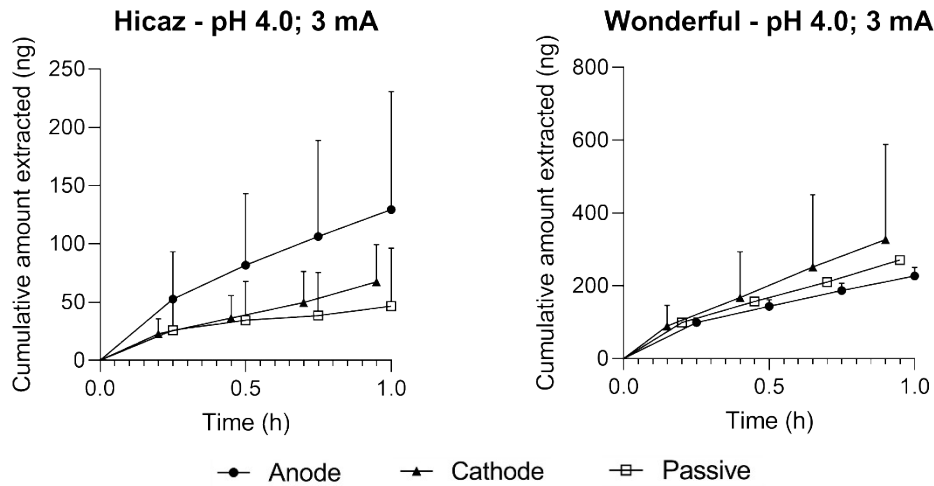
Supplementary Figure S1: Details of the four peel preparation procedures examined.



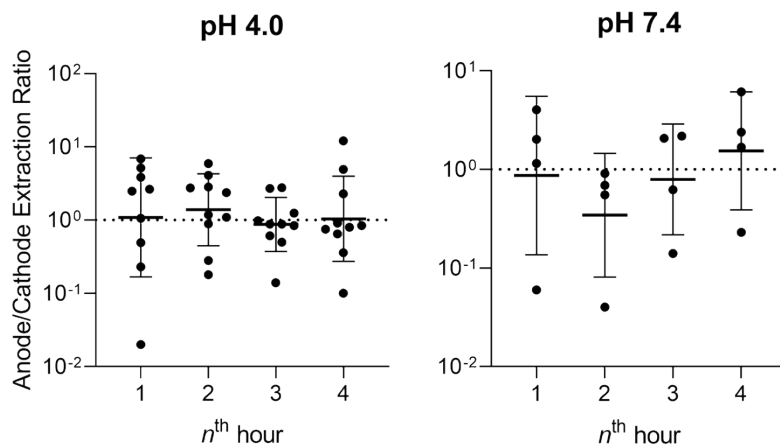
Supplementary Figure S2: Cumulative passive extraction of ellagic acid following [left panel] P1A and P1B, and [right panel] P2A and P2B (mean \pm SD; $n = 5$ except for P2B for which $n = 6$).



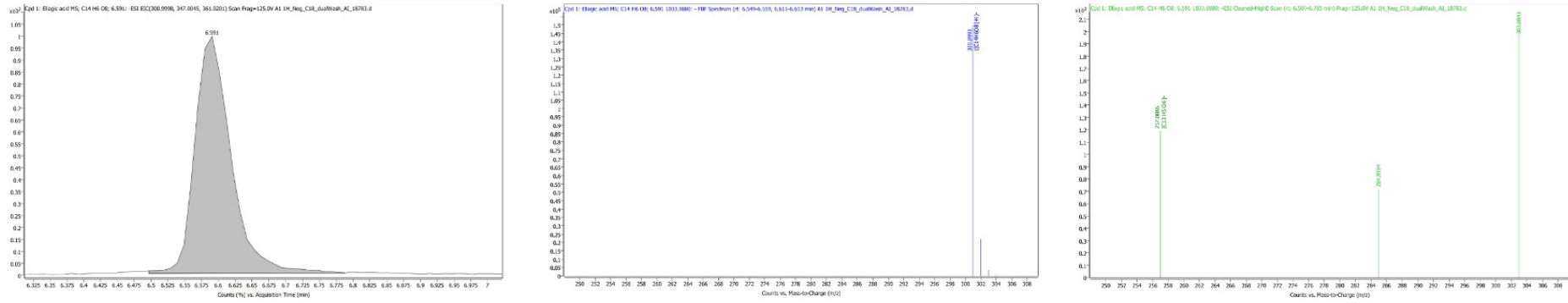
Supplementary Figure S3: Cumulative reverse iontophoretic extraction of ellagic acid at pH 4.0 from the isolated peels of Hicaz and Wonderful pomegranates at currents 3 mA current. Extraction to anode (closed circles, $n = 4$ or 5), cathode (closed triangles, $n = 5$) and passively (open squares, $n = 1$ or 3) (mean \pm SD). Data points are slightly offset for clarity.



Supplementary Figure S4: Pooled anode-to-cathode extraction ratios of paracetamol as a function of the time of current (0.2 mA) application at pH 4.0 and pH 7.4 (geometric mean \pm SD; $n \geq 9$ and $n = 5$, respectively).



Supplementary Figure S5: Representative ellagic acid analysis of a RI extracted anodal sample from an intact Aco pomegranate *in fructo* after 1 h of current passage. Left panel - extracted ion chromatogram (EIC). Middle panel - molecular ion $[M-H]^-$ MS/MS spectrum. Right panel - MS/MS spectrum.



Supplementary Figure S6: Representative histidiine analysis of a RI extracted cathodal sample from an intact Aco pomegranate *in fructo* after 1 h of current passage. Left panel - extracted ion chromatogram (EIC). Middle panel - molecular ion $[M+H]^+$ MS/MS spectrum. Right panel - MS/MS spectrum.

