Electronic supplementary material (ESI)

Purified recombinant enzymes efficiently hydrolyze conjugated urinary (poly)phenols metabolites

Jacob Lessard-Lord[a,b,c], Pier-Luc Plante[a,b] and Yves Desjardins[a,b,c*]

Affiliations

a) Institute of Nutrition and Functional Foods (INAF), Faculty of Agriculture and Food Sciences, Laval University, Québec, QC, Canada
b) Nutrition, Health and Society Centre (NUTRISS), INAF, Laval University, Québec, QC, Canada
c) Department of Plant Science, Faculty of Agriculture and Food Sciences, Laval University, Québec, QC, Canada

*Corresponding author. *E-mail address:* yves.desjardins@fsaa.ulaval.ca

Supplemental Table S1: Identification of (poly)phenols in cranberry extract by UPLC-QToF

Class	Identification	RT (min)	$[M-H]^-$	Molecular formula	Error (ppm)	MS^2 fragments (Relative intensity)	ID level[a]
Flavan-3-ols	Catechin	7.39	289.0711	C15H14O6	-0.35	203.0706 (100) ; 245.0814 (57)	1
	Epicatechin	10.81	289.0715	C15H14O6	1.04	245.0814 (100)	1
	Procyanidin A2	17.54	575.1177	C30H24O12	-1.22	285.0251 (100) ; 289.0676 (53) ; 449.0878 (29) ; 539.0981 (19) ; 407.0771 (19)	1
	Trimer PACs A-Type (1x A) - 1	15.18	863.1815	C45H36O18	-1.67	575.1187 (100) ; 449.0852 (46) ; 289.0691 (34)	2
	Trimer PACs A-Type (1x A) - 2	17.97	863.1820	C45H36O18	-1.05	575.1187 (100) ; 449.0852 (30) ; 289.0691 (29)	2
	Procyanidin B2	9.52	577.1348	C30H26O12	0.35	289.0717 (100) ; 407.0768 (87) ; 425.0872 (30) ; 245.0814 (23)	1
	Trimer PACs B-Type - 1	14.20	865.1975	C45H38O18	-1.18	407.0761 (100) ; 289.0904 (67) ; 577.1392 (59)	2
	Trimer PACs B-Type - 2	12.64	865.1980	C45H38O18	-0.60	407.0710 (100) ; 289.0978 (72) ; 577.1395 (57)	2
Phenolic acids	p-Coumaric acid	11.49	163.0392	C9H8O3	-1.84	119.0483 (100)	1
	Coumaroyl-hexoside - 1	5.76	325.0916	C15H18O8	-2.21	119.0500 (100) ; 163.0395 (27)	2
	Coumaroyl-hexoside - 2	8.85	325.0924	C15H18O8	-1.41	145.0315 (100) ; 119.0504 (71) ; 117.0354 (42) ; 163.0403 (17)	2
	Coumaroyl-monotropein	17.71	535.1445	C25H28O13	-1.31	163.0350 (100) ; 147.0441 (67) ; 119.0466 (54) ; 191.0296 (20)	2
	Coumaroyl-dihydromonotropein	18.61	537.1607	C25H30O13	-0.19	163.0405 (100) ; 119.0502 (27) ; 123.0474 (22)	2
	Chlorogenic acid	8.08	353.0871	C16H18O9	-0.96	191.0561 (100)	1
	Caffeoyl-hexoside	7.36	341.0872	C15H18O9	-1.94	135.0423 (100) ; 179.0324 (45)	2
	Sinapoyl-hexoside	10.91	385.1126	C17H22O10	-3.62	1902.0269 (100) ; 205.0514 (70) ; 175.0040 (39)	2
	Vanilloyl-hexoside	8.62	329.0875	C14H18O9	-1.03	121.0289 (100)	2
	Feruloyl-hexoside	9.89	355.1027	C16H20O9	-2.12	160.0163 (100) ; 175.0396 (86) ; 132.0209 (29) ; 134.0368 (20) ; 193.0491 (9)	2
	3,4-dihydroxybenzoic acid	3.48	153.0184	C7H6O4	-2.61	109.0280 (100)	1
	Dihydroxybenzoic acid	4.32	153.0182	C7H6O4	-3.92	109.0279 (100)	2
	Hydroxybenzoyl-hexosyl-hexoside	9.25	461.1289	C19H26O13	-1.30	121.0274 (100)	2
Flavonols	Quercetin	23.46	301.0356	C15H10O7	2.66	151.0050 (100)	1
	Quercetin-3-galactoside	17.27	463.0883	C21H20O12	-2.53	300.0275 (100) ; 271.0247 (39) ; 255.0297 (16) ; 243.0292 (8)	1
	Quercetin-3-rhamnoside	19.80	447.0923	C21H20O11	-0.94	300.0280 (100) ; 271.0244 (37) ; 255.0272 (17) ; 243.0303 (7)	1
	Quercetin-pentoside - 1	18.30	433.0767	C20H18O11	-1.85	300.0293 (100) ; 271.0258 (42) ; 255.0318 (20) ; 243.0319 (10)	2
	Quercetin-pentoside - 2	18.70	433.0765	C20H18O11	-2.67	300.0284 (100) ; 271.0252 (56) ; 255.0298 (21) ; 243.0306 (12)	2
	Quercetin-pentoside - 3	19.24	433.0779	C20H18O11	1.85	300.0329 (100) ; 271.0289 (46) ; 255.0265 (22) ; 243.0275 (11)	2
	Quercetin-deoxyhexoside	21.56	447.0918	C21H20O11	-2.16	314.0479 (100) ; 271.0211 (62) ; 243.0363 (58) ; 285.0472 (39) ; 299.0133 (15)	2
	Quercetin-hydroxybenzoyl-hexoside	24.94	567.1136	C28H24O13	-0.53	300.0285 (100) ; 271.0280 (23) ; 255.0282 (8) ; 243.0245 (3)	2
	Isorhamnetin	26.67	315.0508	C16H12O7	-0.71	151.0060 (100) ; 163.0027 (58) ; 148.0164 (35)	1
	Myricetin	20.07	317.0302	C15H10O8	-0.86	151.0013 (100) ; 137.0282 (86) ; 178.9988 (38) ; 109.0270 (36)	1
	Myricetin-pentoside - 1	15.18	449.0719	C20H18O12	-1.52	316.0221 (100) ; 271.0240 (34) ; 287.0200 (23)	2
	Myricetin-pentoside - 2	16.36	449.0719	C20H18O12	-1.52	316.0219 (100) ; 271.0244 (38) ; 287.0189 (22)	2
	Myricetin-pentoside - 3	16.48	449.0718	C20H18O12	-1.69	316.0235 (100) ; 271.0238 (32) ; 287.0180 (21)	2
	Myricetin-hexoside	14.30	479.0822	C21H20O13	-2.15	316.0213 (100) ; 271.0258 (23) ; 287.0223 (13)	2
	Myricetin methyl	23.87	331.0454	C16H12O8	-1.55	151.0039 (100) ; 316.0238 (60) ; 107.0132 (28) ; 136.0170 (25) ; 271.0250 (17)	2
	Syringetin-hexoside	20.43	507.1137	C23H24O13	-0.39	344.0503 (100) ; 273.0387 (31) ; 301.0366 (20) ; 242.0424 (16)	2

[a] Identification levels were established according to Sumner *et al.*[1] Level 1 identifications were validated by comparing RT and MS^2 fragmentation spectra an authentic standard, while level 2 identifications were proposed according to exact mass, MS^2 fragmentation spectra and UV absorption spectra compared to the literature and online databases.

Supplemental Table S2: Daily dose of (poly)phenols provided by cranberry extract

Class	Compound	Daily dose (mg)		
Flavan-3-ols	Catechin	0.219	±	0.005
	Epicatechin	1.57	±	0.02
	Procyanidin A2	3.83	±	0.07
	Trimer PACs A-Type (1x A) – 1	1.16	±	0.03
	Trimer PACs A-Type (1x A) – 2	1.86	±	0.03
	Procyanidin B2	*Non detected*		
	Trimer PACs B-Type – 1	7.98	±	0.12
	Trimer PACs B-Type – 2	1.59	±	0.03
	Flavan-3-ols total *	**86.9**	**±**	**1.9**
Phenolic acids	p-Coumaric acid	1.54	±	0.02
	Coumaroyl-hexoside – 1	0.400	±	0.006
	Coumaroyl-hexoside – 2	0.218	±	0.004
	Coumaroyl-monotropein	0.446	±	0.007
	Coumaroyl-dihydromonotropein	0.905	±	0.011
	Chlorogenic acid	2.31	±	0.03
	Caffeoyl-hexoside	0.206	±	0.002
	Sinapoyl-hexoside	0.423	±	0.007
	Vanilloyl-hexoside	0.0640	±	0.0011
	Feruloyl-hexoside	0.251	±	0.004
	3,4-dihydroxybenzoic acid	*Non detected*		
	Dihydroxybenzoic acid	0.139	±	0.005
	Hydroxybenzoyl-hexosyl-hexoside	0.150	±	0.002
	Phenolic acids and derivatives total	**7.05**	**±**	**0.11**
Flavonols	Quercetin	3.48	±	0.05
	Quercetin-3-galactoside	0.418	±	0.006
	Quercetin-3-rhamnoside	0.940	±	0.013
	Quercetin-pentoside – 1	0.643	±	0.036
	Quercetin-pentoside – 2	0.422	±	0.009
	Quercetin-pentoside – 3	0.993	±	0.015
	Quercetin-deoxyhexoside	0.124	±	0.002
	Quercetin-hydroxybenzoyl-hexoside	0.335	±	0.005
	Isorhamnetin	0.267	±	0.004
	Myricetin	1.82	±	0.02
	Myricetin-pentoside – 1	0.104	±	0.002
	Myricetin-pentoside – 2	0.157	±	0.002
	Myricetin-pentoside – 3	0.385	±	0.008
	Myricetin-hexoside	0.174	±	0.003
	Myricetin methyl	0.149	±	0.002
	Syringetin-hexoside	0.232	±	0.004
	Flavonols total	**10.64**	**±**	**0.18**
Anthocyanins	Cyanidin 3-galactoside	0.0281	±	0.0007
	Cyanidin 3-glucoside	0.0461	±	0.0014
	Cyanidin 3-arabinoside	0.303	±	0.002
	Peonidin 3-galactoside	0.0374	±	0.0004
	Peonidin 3-glucoside	0.077	±	0.006
	Peonidin 3-arabinoside	0.2495	±	0.0002
	Anthocyanins total	**0.740**	**±**	**0.011**
	(Poly)phenols total	**105**	**±**	**2**

* Determined by phloroglucinolysis with a mean degree of polymerization of 6.

Supplemental Table S3: Concentration of deuterated internal standards in acetonitrile used to stop the enzymatic hydrolysis

Compound	Concentration (ppm)
3,4,5-trihydroxybenzoic-2,6-d_2 acid	5
Succinic acid-d_6	25
trans-Cinnamic-d_5 acid	25
L-Tryptophan-2′,4′,5′,6′,7′-d_5 (indole-d_5)	5
Glycocholic-2,2,4,4-d_4 acid	5
L-Leucine-d_7 (iso-propyl-d_7)	25
4-Hydroxybenzoic-2,3,5,6-d_4 acid	5
Methyl 4-hydroxybenzoate-2,3,5,6-d_4	5

Supplemental Table S4: Possible origin of the identified MPM in urine

Class	Metabolite[a]	Usual name / abbreviation[b]	Possible origin
PPAs	3-(3′-Hydroxyphenyl)propanoic acid	3-HPPA	Flavan-3-ols[2], anthocyanins[3], dihydrochalcone[3], flavanones[4], phenolic acids[3] , phenylalanine[5,c] and tyrosine[6,c]
	3-(3′,4′-Dihydroxyphenyl)propanoic acid	3,4-DHPPA	
PVAs	5-(3′-Hydroxyphenyl)valeric acid	3-HPVA	Flavan-3-ols[2]
	3-(3′,4′-Dihydroxyphenyl)valeric acid	3,4-DHPVA	
PVLs	5-(3′-Hydroxyphenyl)-γ-valerolactone	3-HPVL	Flavan-3-ols[2]
	5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone	3,4-DHPVL	
(Epi)catechin	Catechin		Oligomeric flavan-3-ols[2], monomeric flavan-3-ols[d]
	Epicatechin		
Cinnamic acids	3′,4′-Dihydroxycinnamic acid	Caffeic acid	Chlorogenic acids and cinnamic acids[e]
	4′-Hydroxy-3′-methoxycinnamic acid	Ferulic acid	
	4′-Hydroxy-3′,5′-dimethoxycinnamic acid	Sinapic acid	Cinnamic acids[e]
Urolithins	3,8-Dihydroxy-urolithin	Urolithin A	Ellagic acids and ellagitannins[7]
	3,9-Dihydroxy-urolithin	Isourolithin A	
	3-Hydroxy-urolithin	Urolithin B	
Lignans metabolites	Enterolactone		Lignan[4]
	Enterodiol		
Isoflavones metabolites	O-desmethylangolensin		Isoflavone[3]

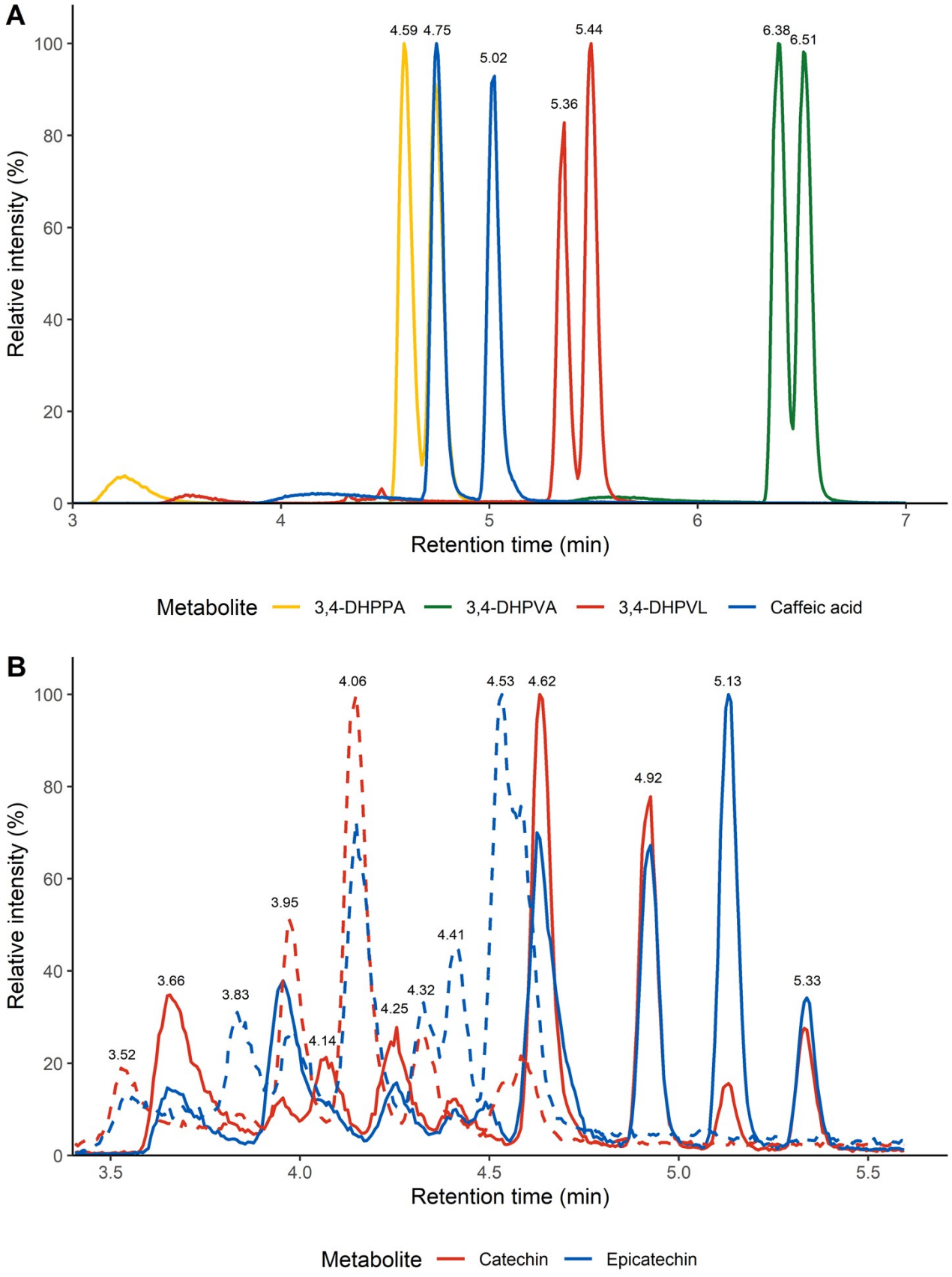
[a] Metabolites were named according to the standardized nomenclature proposed by Kay *et al.*[8] [b] The usual names and abbreviations cited in this column will be used in the manuscript for clarity and conciseness. [c] Only 3-HPPA can originate from aromatic amino acids metabolism. [d] (Epi)catechin can originate from absorption of intact dietary monomeric flavan-3-ols. [e] Cinnamic acids can originate from absorption of intact dietary cinnamic acids.

Supplemental figures legends

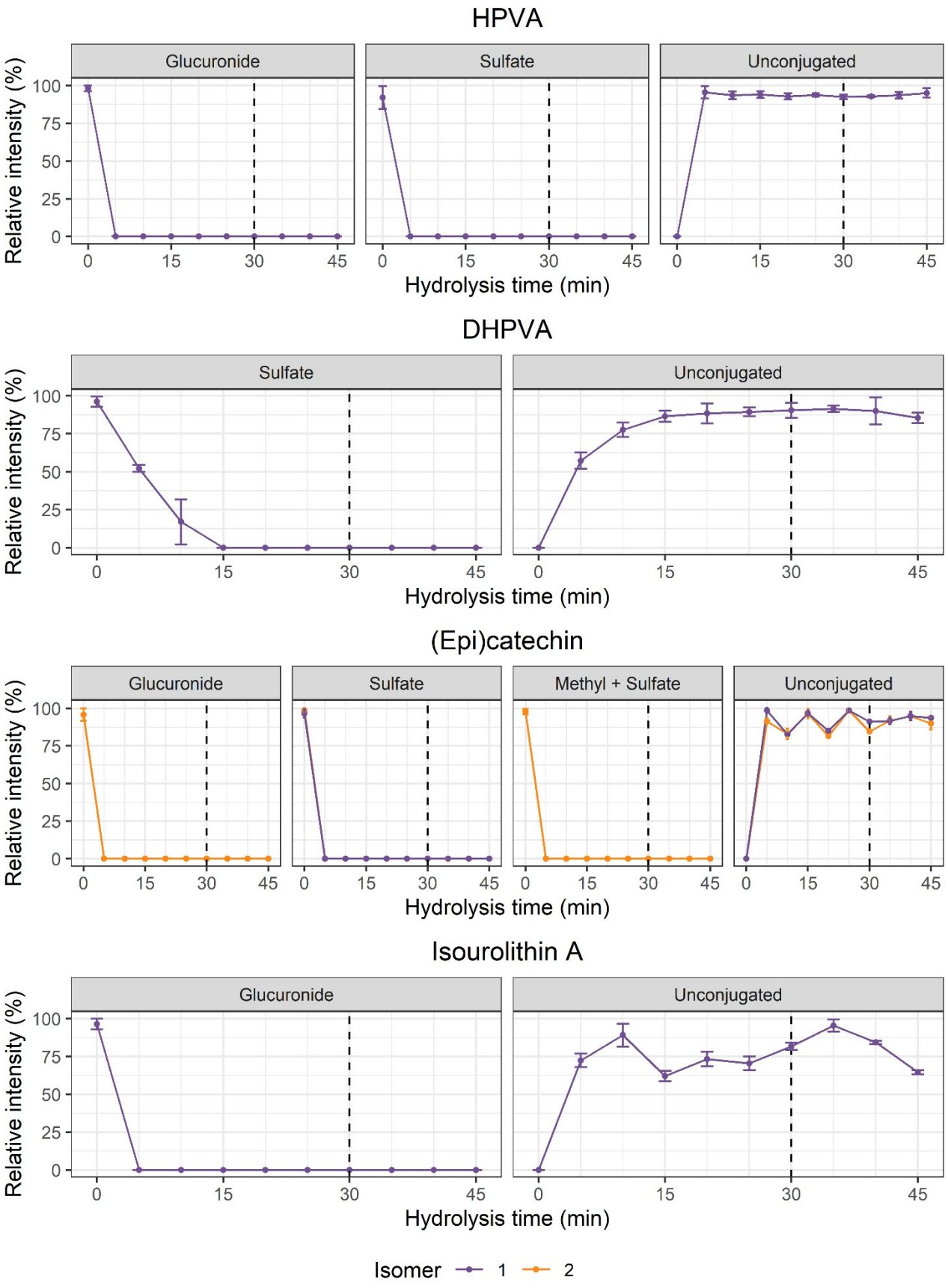
Supplemental Figure S1: Extracted ion chromatograms of multihydroxylated sulfated MPM obtained from rapid synthesis. (A) Dihydroxylated MPM (3,4-DHPPA, 3,4-DHPVA, 3,4-DHPVL and caffeic acid. (B) Multihydroxylated MPM (catechin and epicatechin). Extracted ion chromatograms of monosulfated compounds are represented with a full line, while disulfated compounds are represented with a dashed line.

Supplemental Figure S2: Kinetic of degradation of conjugated MPM and release of unconjugated MPM during enzymatic hydrolysis (continuation of Figure 1). Relative intensity of each MPM is expressed as mean of triplicate ± SD at each time point. Different color is used when there are multiple isomers for a specific form. For (epi)catechin unconjugated graph, epicatechin is represented by purple line and points, while catechin is represented by yellow line and points. The vertical dashed line at 30 min represents the hydrolysis time required for complete hydrolysis of all conjugated MPM included in this study.

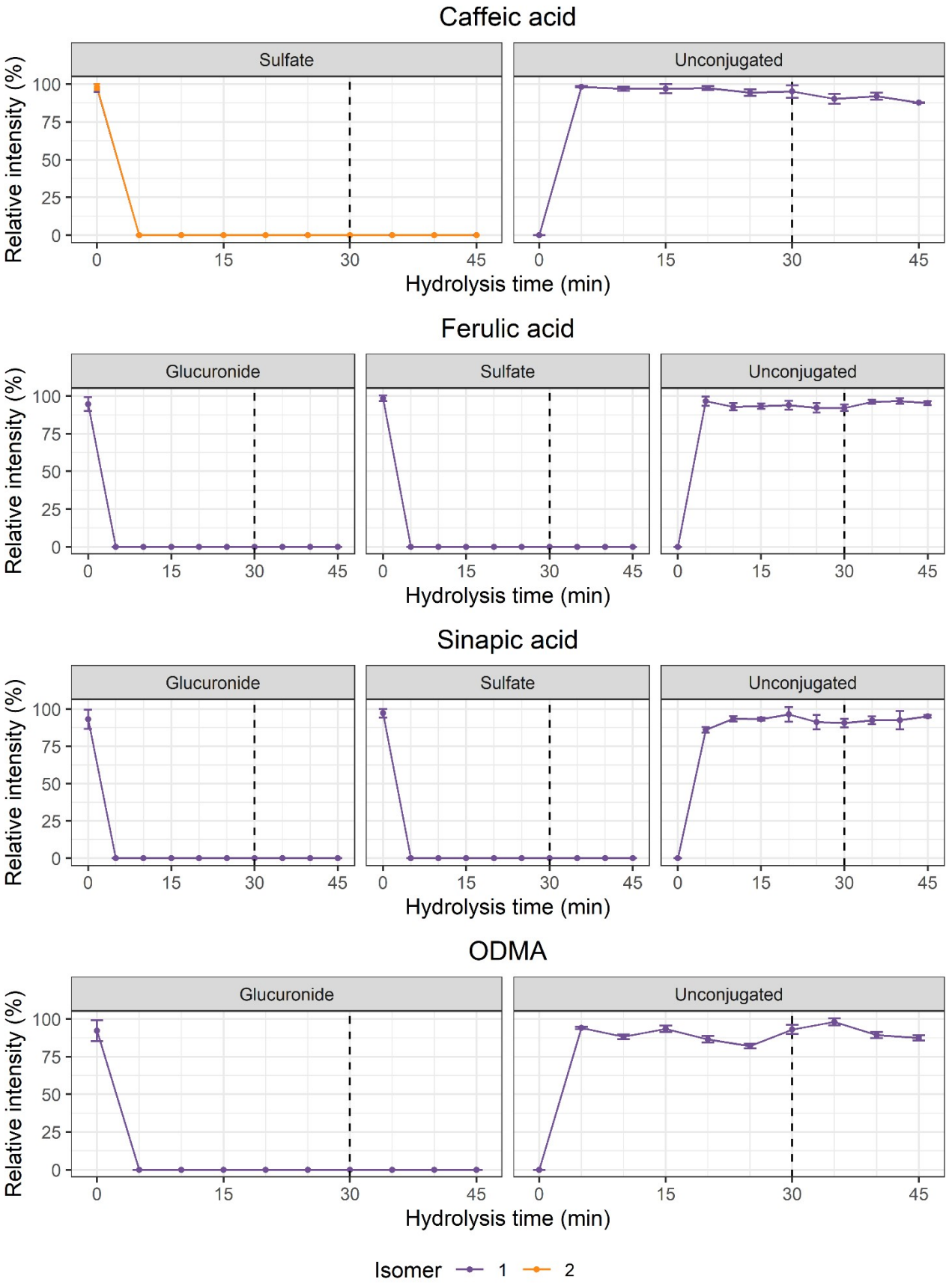
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S2 (continued)



Bibliography

1. L. W. Sumner, A. Amberg, D. Barrett, M. H. Beale, R. Beger, C. A. Daykin, T. W. M. Fan, O. Fiehn, R. Goodacre, J. L. Griffin, T. Hankemeier, N. Hardy, J. Harnly, R. Higashi, J. Kopka, A. N. Lane, J. C. Lindon, P. Marriott, A. W. Nicholls, M. D. Reily, J. J. Thaden and M. R. Viant, Proposed minimum reporting standards for chemical analysis, *Metabolomics*, 2007, **3**, 211-221.
2. P. Mena, L. Bresciani, N. Brindani, I. A. Ludwig, G. Pereira-Caro, D. Angelino, R. Llorach, L. Calani, F. Brighenti, M. N. Clifford, C. I. R. Gill, A. Crozier, C. Curti and D. Del Rio, Phenyl-γ-valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: synthesis, analysis, bioavailability, and bioactivity, *Natural Product Reports*, 2019, **36**, 714-752.
3. D. Del Rio, A. Rodriguez-Mateos, J. P. E. Spencer, M. Tognolini, G. Borges and A. Crozier, Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases, *Antioxid. Redox Signal.*, 2013, **18**, 1818-1892.
4. C. Morand, B. De Roos, M. T. Garcia-Conesa, E. R. Gibney, R. Landberg, C. Manach, D. Milenkovic, A. Rodriguez-Mateos, T. Van de Wiele and F. Tomas-Barberan, Why interindividual variation in response to consumption of plant food bioactives matters for future personalised nutrition, *Proceedings of the Nutrition Society*, 2020, **79**, 225-235.
5. Q. Zhang, Y. Zhang, L. Zeng, G. Chen, L. Zhang, M. Liu, H. Sheng, X. Hu, J. Su, D. Zhang, F. Lu, X. Liu and L. Zhang, The Role of Gut Microbiota and Microbiota-Related Serum Metabolites in the Progression of Diabetic Kidney Disease, *Frontiers in Pharmacology*, 2021, **12**.
6. G. Di Pede, P. Mena, L. Bresciani, T. M. Almutairi, D. Del Rio, M. N. Clifford and A. Crozier, Human colonic catabolism of dietary flavan-3-ol bioactives, *Molecular Aspects of Medicine*, 2022, DOI: https://doi.org/10.1016/j.mam.2022.101107, 101107.
7. J. C. Espín, A. González-Sarrías and F. A. Tomás-Barberán, The gut microbiota: A key factor in the therapeutic effects of (poly)phenols, *Biochem. Pharmacol.*, 2017, **139**, 82-93.
8. C. D. Kay, M. N. Clifford, P. Mena, G. J. McDougall, C. Andres-Lacueva, A. Cassidy, D. Del Rio, N. Kuhnert, C. Manach, G. Pereira-Caro, A. Rodriguez-Mateos, A. Scalbert, F. Tomás-Barberán, G. Williamson, D. S. Wishart and A. Crozier, Recommendations for standardizing nomenclature for dietary (poly)phenol catabolites, *The American Journal of Clinical Nutrition*, 2020, **112**, 1051-1068.