

SUPPLEMENTARY MATERIAL

Strawberry, Blueberry, and Strawberry-Blueberry Blend Beverages Prevent Hepatic Steatosis in Obese Rats by Modulating Key Genes Involved in Lipid Metabolism

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Table S1. Primer sequences, annealing temperature, and NCBI reference sequence.

Transcript	Sequence	Annealing temperature (Tm)	NCBI reference sequence
<i>Fasn</i>	Fw: CCATTCCATTGCCCTAGCC Rv: GTAACACATGCTGCTAAACGA	60 °C	NM_017332.2
<i>Acaca</i>	Fw: AGGAAGATGGTGTCCGCTCTG Rv: GGGGAGATGTGCTGGGTCAT	56 °C	NM_022193.2
<i>Acadm</i>	Fw: GCAGAGAAGAAGGGTGATGAA Rv: ACCGCTGACCCATGTTAG	60 °C	NM_016986.2
<i>Cpt1</i>	Fw: GATGCTTGACAGGTGGTTG Rv: CTTGTGGGCTTAGGGATGTT	65 °C	XM_039102321.1
<i>Cd36</i>	Fw: CTTGGATGTGGAACCCATAACT Rv: CAGGCCAGGAGCTTATT	60 °C	XM_039108092.1
<i>Fatp5</i>	Fw: CCATTCCATTGCCCTAGCC Rv: AGTCCTGGATGAAATC	60 °C	XM_008772501.3

Table S2. Urinary polyphenol metabolites and antioxidant capacity of high fat and fructose diet-fed rats supplemented with strawberry, blueberry, and strawberry-blueberry blend beverages.

Tentative identification	Rt (min)	Molecular formula	Expected mass (Da)	Observed mass (Da)	Mass error (ppm)	Adduct	Concentration ($\mu\text{g}/\text{mg}$ of creatinine)				
							SD	HFFD	HFFD+SB	HFFD+BB	HFFD+SBB
<i>Benzoic acid metabolites</i>											
Hippuric acid	3.37	C ₉ H ₉ NO ₃	179.0582	178.0504	-3.4285	[M-H] ⁻	0.051 ± 0.007 ^{b†}	0.025 ± 0.004 ^b	0.035 ± 0.006 ^{b†}	0.096 ± 0.012 ^{a†}	0.043 ± 0.010 ^{b†}
Vanillic acid sulfate	3.93	C ₈ H ₈ O ₇ S	247.9991	246.9929	4.3453	[M-H] ⁻	ND	ND	0.040 ± 0.004	ND	ND
Hydroxyhippuric acid	4.96	C ₉ H ₉ NO ₄	195.0532	194.0460	0.8356	[M-H] ⁻	ND	ND	0.012 ± 0.002	ND	ND
<i>Cinnamic acid metabolites</i>											
Caffeic acid glucuronide	2.56	C ₁₅ H ₁₆ O ₁₀	356.0743	355.0675	1.3356	[M-H] ⁻	ND	ND	0.002 ± 0.000 ^a	0.004 ± 0.001 ^a	ND
Ferulic acid sulfate	2.88	C ₁₀ H ₁₀ O ₅ S	274.0147	273.0061	-4.8980	[M-H] ⁻	ND	ND	0.194 ± 0.082 ^a	0.132 ± 0.020 ^a	0.044 ± 0.004 ^a
Coumaric acid sulfate	3.12	C ₉ H ₈ O ₆ S	244.0042	242.9963	-2.4014	[M-H] ⁻	ND	ND	0.022 ± 0.002	ND	ND
Caffeic acid sulfate	3.80	C ₉ H ₈ O ₇ S	259.9991	258.9917	-0.3509	[M-H] ⁻	ND	ND	0.025 ± 0.002 ^a	0.005 ± 0.000 ^a	ND
Dihydrocoumaric acid	3.81	C ₉ H ₁₀ O ₃	166.0630	165.0555	-1.2603	[M-H] ⁻	ND	ND	0.116 ± 0.009 ^a	0.036 ± 0.007 ^a	ND
Coumaric acid	4.23	C ₉ H ₈ O ₃	164.0473	163.0397	-2.2924	[M-H] ⁻	0.015 ± 0.001 ^{b†}	0.005 ± 0.001 ^c	0.018 ± 0.001 ^{a†}	0.008 ± 0.001 ^{d†}	0.011 ± 0.002 ^{c†}
Dimethylellagic acid	9.35	C ₁₆ H ₁₀ O ₈	330.0376	329.0318	4.5590	[M-H] ⁻	ND	ND	0.011 ± 0.001	ND	ND
<i>Ellagitannin metabolites</i>											
Dimethylulorithin D sulfate	3.53	C ₁₅ H ₁₂ O ₉ S	368.0202	367.0136	1.8186	[M-H] ⁻	ND	ND	0.004 ± 0.001	ND	ND
Methylulorithin D glucuronide	3.77	C ₂₁ H ₂₂ O ₁₁	450.1162	449.1098	2.0200	[M-H] ⁻	ND	ND	0.086 ± 0.0006	ND	ND
Urolithin C	4.71	C ₁₃ H ₈ O ₅	244.0372	243.0300	0.3044	[M-H] ⁻	ND	ND	0.196 ± 0.011	ND	ND
Methylulorithin A	5.18	C ₁₄ H ₁₀ O ₄	242.0579	241.0503	-1.3359	[M-H] ⁻	ND	ND	0.026 ± 0.004	ND	ND
Urolithin B glucuronide	6.62	C ₁₉ H ₁₆ O ₉	388.0794	387.0738	4.1636	[M-H] ⁻	ND	ND	2.910 ± 0.171	ND	ND
Urolithin A	9.32	C ₁₃ H ₈ O ₄	228.0423	227.0347	-1.0854	[M-H] ⁻	ND	ND	8.251 ± 0.496 ^a	ND	2.326 ± 0.246 ^b
Urolithin B	11.20	C ₁₃ H ₈ O ₃	212.0473	211.0404	1.4379	[M-H] ⁻	ND	ND	5.936 ± 0.416 ^a	ND	1.816 ± 0.140 ^a
<i>Flavanol metabolites</i>											
(Epi)-catechin glucuronide	1.14	C ₂₁ H ₂₂ O ₁₂	466.1111	465.1033	-1.2718	[M-H] ⁻	ND	ND	ND	0.013 ± 0.000 ^a	0.010 ± 0.004 ^a
Methyl-(epi)-catechin glucuronide	2.94	C ₂₂ H ₂₄ O ₁₂	480.1268	479.1196	0.2521	[M-H] ⁻	0.002 ± 0.000 ^{ab†}	0.002 ± 0.000 ^b	0.044 ± 0.007 ^{ab†}	0.055 ± 0.004 ^{a†}	0.051 ± 0.006 ^{a†}
<i>Flavonol metabolites</i>											
Dimethyl quercetin	11.12	C ₁₇ H ₁₄ O ₇	330.0740	329.0681	4.2260	[M-H] ⁻	0.312 ± 0.025 ^{a†}	0.197 ± 0.034 ^{ab}	0.313 ± 0.022 ^{a†}	0.084 ± 0.007 ^{b†}	0.212 ± 0.019 ^b
<i>Flavone metabolites</i>											
Apigenin diglucuronide	2.94	C ₂₇ H ₂₆ O ₁₇	622.1170	621.1094	-0.5710	[M-H] ⁻	0.027 ± 0.004 ^{a†}	0.005 ± 0.002 ^b	0.024 ± 0.002 ^{ab†}	0.026 ± 0.002 ^{a†}	0.026 ± 0.002 ^{ab†}
Apigenin glucuronide	4.44	C ₂₁ H ₁₈ O ₁₁	446.0849	445.0757	-4.3859	[M-H] ⁻	7.378 ± 0.570 ^{ab}	6.371 ± 1.234 ^b	9.137 ± 0.298 ^{a†}	6.744 ± 1.075 ^b	7.766 ± 0.909 ^{ab†}
<i>Gallic acid metabolites</i>											
Methylpyrogallol sulfate	3.77	C ₇ H ₈ O ₆ S	220.0042	218.9965	-1.7285	[M-H] ⁻	ND	ND	0.006 ± 0.001	ND	ND
<i>Isoflavone metabolites</i>											
Dihydrodaidzein glucuronide	4.24	C ₂₁ H ₂₀ O ₁₀	432.1056	431.0965	-4.2433	[M-H] ⁻	0.403 ± 0.022 ^b	0.379 ± 0.099 ^b	0.480 ± 0.020 ^{ab}	0.531 ± 0.090 ^{a†}	0.483 ± 0.031 ^{ab}
Dihydrodaidzein	4.67	C ₁₅ H ₁₂ O ₄	256.0736	255.0664	0.5466	[M-H] ⁻	0.014 ± 0.001 ^{a†}	0.005 ± 0.001 ^b	0.013 ± 0.001 ^{a†}	0.011 ± 0.002 ^{ab†}	0.013 ± 0.002 ^{ab†}
Genistein glucuronide	5.20	C ₂₁ H ₁₈ O ₁₁	446.0849	445.0760	-3.7303	[M-H] ⁻	0.631 ± 0.020 ^a	0.473 ± 0.093 ^{ab}	0.437 ± 0.015 ^{ab}	0.416 ± 0.054 ^{ab}	0.432 ± 0.020 ^b
Glycitein glucuronide	5.52	C ₂₂ H ₂₀ O ₁₁	460.1006	459.0916	-3.7453	[M-H] ⁻	2.374 ± 0.763 ^{ab}	2.553 ± 0.356 ^b	2.680 ± 0.278 ^{ab}	3.064 ± 0.374 ^{ab†}	3.169 ± 0.131 ^{a†}
Equol glucuronide	5.69	C ₂₁ H ₂₂ O ₉	418.1264	417.1173	-4.3003	[M-H] ⁻	12.105 ± 0.583 ^a	9.953 ± 1.917 ^a	10.587 ± 0.691 ^a	9.586 ± 1.650 ^a	9.520 ± 1.838 ^a
Hydroxyequol	8.13	C ₁₅ H ₁₄ O ₄	258.0892	257.0808	-4.3725	[M-H] ⁻	0.018 ± 0.001 ^{a†}	0.011 ± 0.001 ^{b†}	0.015 ± 0.003 ^{ab†}	0.013 ± 0.001 ^{a†}	0.026 ± 0.040 ^{ab†}
Hydroxyglycitein	8.59	C ₁₆ H ₁₂ O ₆	300.0634	299.0552	-3.0659	[M-H] ⁻	0.042 ± 0.002 ^{a†}	0.032 ± 0.003 ^b	0.037 ± 0.002 ^{ab†}	0.038 ± 0.003 ^{ab†}	0.034 ± 0.004 ^b
Hydroxygenistein	9.16	C ₁₅ H ₁₀ O ₆	286.0477	285.0396	-2.9990	[M-H] ⁻	0.011 ± 0.001 ^{a†}	0.008 ± 0.001 ^b	0.008 ± 0.001 ^{ab}	0.011 ± 0.002 ^a	0.011 ± 0.002 ^{ab†}
Equol	11.22	C ₁₅ H ₁₄ O ₃	242.0943	241.0862	-3.3874	[M-H] ⁻	0.051 ± 0.003 ^{a†}	0.032 ± 0.003 ^b	0.037 ± 0.002 ^{ab}	0.040 ± 0.005 ^{ab†}	0.037 ± 0.004 ^{b†}
Methylequol	11.42	C ₁₆ H ₁₆ O ₄	272.1049	271.0976	0.1402	[M-H] ⁻	0.004 ± 0.001 ^{a†}	0.003 ± 0.000 ^b	0.003 ± 0.000 ^{ab†}	0.004 ± 0.001 ^{a†}	0.003 ± 0.000 ^{a†}
Hydroxydaidzein	11.62	C ₁₅ H ₁₀ O ₅	270.0528	269.0451	-1.7580	[M-H] ⁻	12.948 ± 1.043 ^{bc}	11.437 ± 2.001 ^c	12.847 ± 1.431 ^{bc}	14.747 ± 2.123 ^{b†}	17.477 ± 1.764 ^{a†}
<i>Lignans</i>											
Enterodiol	9.09	C ₁₈ H ₂₂ O ₄	302.1518	301.1458	4.1634	[M-H] ⁻	ND	ND	0.016 ± 0.001 ^{ab}	0.007 ± 0.003 ^b	0.027 ± 0.003 ^a
Enterolactone sulfate	9.89	C ₁₈ H ₁₈ O ₇ S	378.0773	377.0705	1.2812	[M-H] ⁻	0.234 ± 0.006 ^{ab}	0.153 ± 0.013 ^{ab}	0.349 ± 0.018 ^{ab}	0.084 ± 0.011 ^b	0.102 ± 0.009 ^{ab}

Enterolactone	11.18	C ₁₈ H ₁₈ O ₄	298.1205	297.1132	-0.1737	[M-H] ⁻	4.262 ± 0.193 ^{a,b†}	2.983 ± 0.300 ^b	3.120 ± 0.246 ^b	9.023 ± 0.443 ^{a†}	7.597 ± 1.055 ^{a†}
<i>Other polyphenols</i>											
Catechol sulfate	2.22	C ₆ H ₆ O ₅ S	189.9936	188.9858	-2.8666	[M-H] ⁻	ND	ND	0.305 ± 0.024	ND	ND
Catechol	2.38	C ₆ H ₆ O ₂	110.0368	109.0297	2.1231	[M-H] ⁻	ND	ND	0.012 ± 0.001	ND	ND
<i>Phenylacetic acid metabolites</i>											
Hydroxyphenylacetic acid	2.75	C ₈ H ₈ O ₃	152.0473	151.0394	-4.4361	[M-H] ⁻	ND	ND	0.089 ± 0.007 ^a	0.079 ± 0.008 ^a	ND
Methoxyphenylacetic acid	4.55	C ₉ H ₁₀ O ₃	166.0630	165.0555	-1.0746	[M-H] ⁻	0.154 ± 0.010 ^{ab}	0.136 ± 0.023 ^{ab}	0.37 ± 0.009 ^{a,b†}	0.064 ± 0.007 ^{a†}	0.268 ± 0.041 ^{b†}
<i>Phenylpropanoic acid metabolites</i>											
Dihydroferulic acid sulfate	3.47	C ₁₀ H ₁₂ O ₇ S	276.0304	275.0223	-2.7228	[M-H] ⁻	0.053 ± 0.005 ^{a†}	0.016 ± 0.005 ^d	0.021 ± 0.003 ^{cd}	0.026 ± 0.003 ^{a†}	0.042 ± 0.005 ^{b†}
Phenylpropionic acid	4.39	C ₉ H ₁₀ O ₂	150.0681	149.0607	-0.4333	[M-H] ⁻	ND	ND	0.030 ± 0.003	ND	ND
Dihydroferulic acid	5.00	C ₁₀ H ₁₂ O ₄	196.0736	195.0662	-0.4924	[M-H] ⁻	ND	ND	0.085 ± 0.006	ND	ND
<i>Phenylvaleric acid metabolites</i>											
Dihydroxyphenylvaleric acid	3.93	C ₁₁ H ₁₄ O ₄	210.0892	209.0823	1.9848	[M-H] ⁻	ND	ND	0.045 ± 0.002 ^b	0.133 ± 0.010 ^a	ND
Dihydroxyphenylvaleric acid sulfate	10.98	C ₁₁ H ₁₄ O ₇ S	290.0460	289.0388	0.2431	[M-H] ⁻	0.027 ± 0.004 ^{bcd†}	0.012 ± 0.004 ^d	0.054 ± 0.004 ^{ab†}	0.051 ± 0.005 ^{bc†}	0.022 ± 0.003 ^{cd†}
<i>Valerolactone metabolites</i>											
Dihydroxyphenylvalerolactone sulfate	3.18	C ₁₁ H ₁₂ O ₇ S	288.0304	287.0238	2.3654	[M-H] ⁻	ND	ND	0.259 ± 0.018 ^a	0.212 ± 0.029 ^{ab}	0.121 ± 0.013 ^b
Dihydroxyphenylvalerolactone	3.24	C ₁₁ H ₁₂ O ₄	208.0736	207.0672	4.3184	[M-H] ⁻	ND	ND	0.055 ± 0.005 ^{ab}	0.142 ± 0.035 ^a	0.047 ± 0.009 ^b
Methoxyhydroxyphenylvalerolactone	11.43	C ₁₂ H ₁₄ O ₄	222.0892	221.0819	0.0663	[M-H] ⁻	1.898 ± 0.108 ^a	1.745 ± 0.140 ^a	1.652 ± 0.059 ^{ab†}	1.201 ± 0.097 ^{b†}	0.676 ± 0.039 ^{b†}
Urinary antioxidant capacity											
Folin-Ciocalteu reducing capacity assay (mg GAE/mg creatinine)							1.2 ± 0.4 ^b	0.8 ± 0.4 ^b	0.8 ± 0.3 ^b	2.3 ± 0.7 ^a	0.9 ± 0.5 ^b
DPPH [•] radical scavenging assay (mg TE/mg creatinine)							4.8 ± 2.4 ^b	1.8 ± 0.4 ^c	2.3 ± 0.9 ^{bc}	8.3 ± 2.4 ^a	3.8 ± 1.8 ^{bc}
ABTS [•] radical scavenging assay (mg TE/mg creatinine)							2.8 ± 1.7 ^b	1.7 ± 0.9 ^b	6.1 ± 3.0 ^a	5.7 ± 2.9 ^a	4.0 ± 2.9 ^a

Data are showed as mean ± standard deviation of ten replicates. Different letters indicate significant ($p<0.05$) differences between samples by Tukey's or Friedman's test. [†]Indicate significant ($p<0.05$) difference as compared to the HFFD group by Dunnet's or Wilcoxon test. SD: standard diet; HFFD: high fat and fructose diet; SB: strawberry beverage; BB: blueberry beverage; SBB: strawberry-blueberry beverage; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid); GAE: gallic acid equivalent; TE: trolox equivalent; Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

Table S3. Top 25 loading values of component 1 from the sparse Partial Least Square-Discriminant Analysis of urinary polyphenol metabolites of high fat and fructose diet-fed rats supplemented with strawberry, blueberry, and strawberry-blueberry blend beverages.

Urinary metabolites	Loadings 1	
	Component 1	Component 2
Catechol sulfate	0.2710	0
Urolithin B glucuronide	0.2696	0
Urolithin C	0.2694	0
Dihydroferulic acid	0.2689	0
Methylurolithin A	0.2658	0
Methylpyrogallol sulfate	0.2656	0
Hydroxyhippuric acid	0.2656	0
Caffeic acid sulfate	0.2526	0
Methylurolithin D glucuronide	0.2237	0
Vanillic acid sulfate	0.2207	0
Catechol	0.2197	0
Dimethylellagic acid	0.2189	0
Urolithin A	0.2034	0.0769
Dihydrocoumaric acid	0.1923	0
Hydroxydaidzein	0.1794	0
Phenylpropionic acid	0.1640	0
Dimethylurolithin D sulfate	0.1639	0
Coumaric acid sulfate	0.1601	0
Urolithin B	0.1524	0.0654
Enterolactone	0.1137	0.2547
Hydroxyphenylacetic acid	0.0986	0.0184
Dihydroxyphenylvalerolactone sulfate	0.0450	0.2139
Enterolactone sulfate	0.0249	0.0526
Ferulic acid sulfate	0.0087	0.1636
Methoxyphenylacetic acid	0.0041	0

Data was normalized by sum, square root transformed, and auto scaled.

Table S4. Top 25 loading values of component 2 from the sparse Partial Least Square-Discriminant Analysis of urinary polyphenol metabolites of high fat and fructose diet-fed rats supplemented with strawberry, blueberry, and strawberry-blueberry blend beverages.

Urinary metabolites	Loadings 1	
	Component 1	Component 2
Methyl-(epi)-catechin glucuronide	0	0.4208
Methoxyhydroxyphenylvalerolactone	0	0.3746
Equol glucuronide	0	0.3099
Dimethyl quercetin	0	0.3034
Dihydroxyphenylvalerolactone	0	0.2936
Hydroxyglycitein	0	0.2594
Enterolactone	0.1137	0.2547
(Epi)-catechin glucuronide	0	0.2362
Enterodiol	0	0.2338
Dihydroxyphenylvalerolactone sulfate	0.0450	0.2139
Apigenin glucuronide	0	0.2123
Ferulic acid sulfate	0.0087	0.1636
Dihydroxyphenylvaleric acid	0	0.1463
Dihydrodaidzein glucuronide	0	0.0938
Urolithin A	0.2034	0.0769
Urolithin B	0.1524	0.0654
Glycitein glucuronide	0	0.0545
Enterolactone sulfate	0.0249	0.0526
Caffeic acid glucuronide	0	0.0470
Dihydroferulic acid sulfate	0	0.0448
Dihydroxyphenylvaleric acid sulfate	0	0.0382
Coumaric acid	0	0.0269
Hydroxyphenylacetic acid	0.0986	0.0184
Equol	0	0.0112
Methylequol	0	0.0097

Data was normalized by sum, square root transformed, and auto scaled.

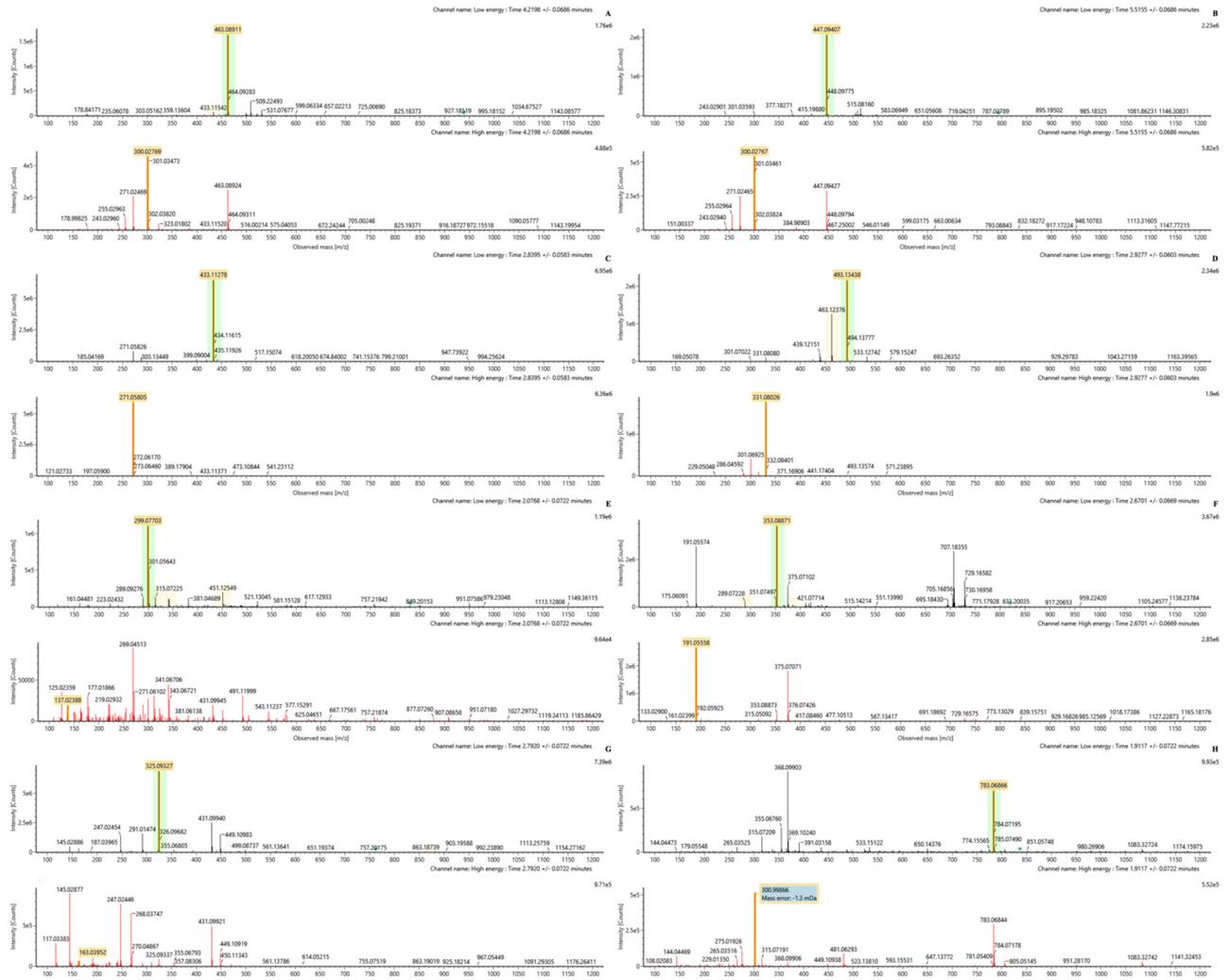


Figure S1. High resolution MS^{E} spectra at high (superior) and low (inferior) collision energy of the major polyphenols identified in berry fruit beverages: (A) myricetin rhamnoside, (B) quercetin rhamnoside, (C) pelargonidin hexoside, (D) malvidin hexoside, (E) hydroxybenzoic acid hexoside, (F) caffeoylquinic acid isomer II, (G) coumaric acid hexoside, and (H) pedunculagin.