Quantification of urinary phenyl-γ-valerolactones and related valeric acids in human urine after consumption of apples.

Andrea Anesi [1], Pedro Mena [2], Achim Bub [3], Marynka M. Ulaszewska [1], Daniele Del Rio [4,5,] Sabine E. Kulling [6] and Fulvio Mattivi [1,7]

[1] Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via Mach 1, 38010 San Michele all 'Adige, TN, Italy;
[2] Human Nutrition Unit, Department of Food & Drug, University of Parma, Parma, Italy
[3] Department of Physiology and Biochemistry of Nutrition, Max Rubner-Institut, Karlsruhe, Germany
[4] School of Advanced Studies on Food and Nutrition, and Microbiome Research Hub, University of Parma, Parma, Italy
[5] Human Nutrition Unit, Department of Veterinary Medicine, University of Parma, Parma, Italy
[6] Department of Safety and Quality of Fruit and Vegetables, Max Rubner-Institut, Karlsruhe, Germany
[7] University of Trento, Department of Physics, Bioorganic Chemistry Laboratory, Via Sommarive 14, 38123 Povo, TN, Italy

***** Correspondence: fulvio.mattivi@unitn.it; Tel.: +39-0461-615-259 (F.M.)

SUPPLEMENTARY MATERIAL

Table S1. Flavan-3-ol composition of study apples as determined by UHPLC-ESI-QqQ-MS/DAD following the procedure described in [35].

Compound	µmol in 400 g of Elstar apples
Free catechin	86.57 ± 4.68
Free epicatechin	688.85 ± 29.96
Free gallocatechin	0.17 ± 0.03
Free epigallocatechin	0.00 ± 0.00
Free catechin gallate	0.18 ± 0.00
Catechin terminal units	633.01 ± 35.64
Epicatechin, terminal units	2262.56 ± 58.30
Gallocatechin, terminal units	1.19 ± 0.20
Epigallocatechin, terminal units	20.30 ± 2.83
Catechin gallate, terminal units	1.46 ± 0.23
Catechin+epicatechin + phloroglucinol	14863.06 ± 615.46
Epigallocatechin + phloroglucinol	450.69 ±45.26
Epicatechin gallate + phloroglucinol	17.66 ± 4.57
Procyanidin B1	176.35 ± 11.90
Procyanidin B2	1882.18 ± 62.13
Total flavan-3-ol monomers	563.04 ± 18.65
Total dimeric PACs	2058.53 ± 55.28
Total oligomeric PACs (terminal + superior units)	2922.50 ± 121.74
Total flavan-3-ols	5756.81 ± 154.10
Mean degree of polymerization (mDP)	8.5 ± 0.11

Table S2. Standard compounds used for PVL and PVA quantitation, their retention times, linearity range, curve equation and R^2. The last column reports the detected compounds that were quantified using the specified calibration curve.

Standard compound	RT (min)	Range	Equation	R^2	Curve used for the calibration of
5-phenyl-γ-VL-3′-sulfate	2.88	100 μm – 10 nm	46343.1x-111.7	0.997	
5-phenyl-γ-VL-3′-glucuronide	2.10	50 μm – 100 nm	2932.73x-159.2	0.994	5-phenyl-γ-VL-methoxy-glucuronide
5-(5′-hydroxyphenyl)-γ-VL-3′-sulfate	2.28	100 μm – 50 nm	20490.2x-397.5	0.997	
5-(3′-hydroxyphenyl)-γ-VL-4′-sulfate	2.66	100 μm – 5 nm	36220.5x-67.3	0.994	• 5-phenyl-γ-VL-methoxy-sulfate (1, 2) • 5-phenyl-γ-VL-sulfate-glucuronide • 4-hydroxy-5-(hydroxyphenyl)-VA-sulfate • 4-hydroxy-5-phenyl-VA-methoxy sulfate
5-(5’-hydroxyphenyl)-γ-VL-3’-glucuronide	1.76	20 μm – 50 nm	4154.85x+9.1	0.994	• 5-(hydroxyphenyl)-y-VL-glucuronide (3′,4′ isomer) • 4-hydroxy-5-(hydroxyphenyl)-VA-glucuronide

Table S3. Quantification of selected PVLs and PVAs (μmol) at different time points in urine of 11 study subjects

	5-phenyl-γ-VL-3′-sulfate					
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	0.001896	0.005282	0.000421	0.05835	2.006898	0.094276
S2	0.03518	0.147459	0.602618	0.972602	4.36228	2.214342
S3	0.02827	0.028659	0.017262	0.226868	0.873558	1.250919
S4	0.004746	0.000032	3.462032	1.904976	2.936	2.538965
S5	0.000528	0.000595	0.063616	1.083779	1.630233	0.17082
S6	0.000258	0.001044	0.000494	0.09581	1.147661	5.777099
S7	5.39E-05	0.000359	0.15984	0.223338	1.757376	1.168908
S8	0.005938	0.000225	0.000685	0.010384	0.18391	0.355999
S9	0.000292	0.004717	0.896566	0.974883	4.19083	2.539058
S10	0.131511	0.046062	0.117525	0.342695	12.90533	10.54295
S11	0.621251	0.85352	1.400177	4.855904	35.06078	10.43365

	5-(3′-hydroxyphenyl)-γ-VL-4′-sulfate					
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	0.02476	0.218044	0.875363	7.36701	69.18813	1.382005
S2	0.433188	0.824707	4.077125	10.26854	43.08864	9.487121
S3	2.389781	2.513285	2.566494	35.3891	87.89143	37.29121
S4	0.197995	1.179064	38.33872	33.60361	83.63832	17.58936
S5	0.169905	0.230921	8.083883	34.19901	57.88222	3.727891
S6	0.003048	0.059827	0.346346	2.269014	14.94503	22.58768
S7	0.006197	0.3072	3.719	4.128896	30.55488	19.07525
S8	0.270251	0.13424	0.338459	6.013964	63.36023	21.8509
S9	0.250094	0.723092	9.021644	28.72569	108.4844	44.84253
S10	0.561869	0.148446	1.060775	3.383349	38.68688	22.3959
S11	0.084806	8.47762	0.835449	2.26336	13.54982	6.195584

	5-phenyl-γ-VL-3′-glucuronide					
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	0.001964	0.002332	0.004603	0.007364	0.019516	0.012213
S2	0.001289	0.003191	0.003498	0.004787	0.009819	0.014361
S3	0.00043	0.001191	0.005155	0.003314	0.01031	0.008039
S4	0.001718	0.001964	3.020332	0.976824	0.23856	0.017552
S5	0.005769	0.006505	0.008715	0.38076	0.011722	0.00896
S6	0.002823	0.003559	0.005401	0.005401	0.010678	3.674319
S7	0.000589	0.003928	0.006812	0.005216	0.011783	0.009574
S8	0.00178	0.002455	0.007487	0.0027	0.01301	0.017552
S9	0.003191	0.003253	0.093645	0.553014	3.159665	2.46089
S10	0.00135	0.003314	0.006137	0.002087	2.321258	0.424995
S11	0.036309	0.2769	0.496619	2.922992	19.62698	2.240441

	5-(hydroxyphenyl)-γ-VL-glucuronide (3', 4' isomer)					
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	0.001031	0.001224	0.002416	1.40919	18.12369	0.006411
S2	0.032314	0.001675	0.915221	4.984805	16.04832	1.830641
S3	0.524528	0.451734	0.402675	22.59619	59.57633	9.613632
S4	0.089586	0.167208	55.3492	56.06874	60.7852	1.936506
S5	0.003028	0.003415	4.287868	39.34235	39.9838	0.004703
S6	0.001482	0.001868	0.002835	0.002835	2.451617	7.498286
S7	0.000309	0.002062	0.298895	0.458979	3.069216	0.989859
S8	0.000934	0.001289	0.00393	1.734337	70.63951	9.064842
S9	0.001675	0.001707	4.811635	52.34109	178.57	39.86912
S10	0.043115	0.00174	0.003222	0.383571	12.99443	3.183838
S11	0.002513	4.19602	0.004703	0.235264	2.34657	0.122741

	5-phenyl-γ-VL-methoxy-sulfate (1)					
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	0.00019	0.000226	0.036388	0.060405	0.211891	0.001183
S2	0.010092	0.025077	0.030156	0.045002	0.109692	0.117977
S3	0.006061	0.010872	0.043145	0.071688	0.211798	0.120101
S4	0.014827	0.01575	0.088939	0.064769	0.189576	0.146875
S5	0.045186	0.00063	0.071593	0.063559	0.138642	0.072828
S6	0.000273	0.000345	0.000523	0.041961	0.085895	0.105492
S7	5.71E-05	0.00038	0.055356	0.0428	0.100771	0.08612
S8	0.013857	0.000238	0.000725	0.023727	0.16368	0.154805
S9	0.000309	0.025398	0.050381	0.058743	0.180073	0.124164
S10	0.010747	0.000321	0.000595	0.016431	0.154535	0.154933
S11	0.000464	0.046526	0.070069	0.031141	0.135533	0.103944

	5-phenyl-γ-VL-methoxy-sulfate (2)					
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	0.015576	0.000226	0.037539	0.076671	0.365271	0.095734
S2	0.010609	0.025258	0.040604	0.081333	0.283852	0.150228
S3	0.008639	0.016461	0.047737	0.179906	0.594649	0.265678
S4	0.013559	0.015732	0.202006	0.148295	0.429664	0.188145
S5	0.000559	0.00063	0.106283	0.216948	0.418132	0.094239
S6	0.000273	0.028104	0.000523	0.045298	0.107036	0.148069
S7	5.71E-05	0.00038	0.053336	0.044194	0.126662	0.108861
S8	0.013826	0.000238	0.000725	0.031477	0.329464	0.196975
S9	0.024808	0.025893	0.056388	0.166272	0.664131	0.364758
S10	0.011477	0.025893	0.049018	0.027363	0.203094	0.187993
S11	0.000464	0.053128	0.000868	0.035752	0.162196	0.109163

	5-phenyl-γ-VL-methoxy-glucuronide					
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	0.017907	0.001224	0.043238	0.06711	0.559736	0.118012
S2	0.016287	0.027196	0.030553	0.132337	0.292064	0.119141
S3	0.011719	0.016302	0.002706	0.171808	0.458543	0.140894
S4	0.038979	0.019275	0.360696	0.207036	0.374808	0.177713
S5	0.003028	0.003415	0.094998	0.266055	0.346121	0.097867
S6	0.022848	0.001868	0.002835	0.044878	0.121204	0.104871
S7	0.007217	0.002062	0.06153	0.002738	0.190003	0.122581
S8	0.046355	0.026933	0.00393	0.060273	0.727944	0.207343
S9	0.026031	0.001707	0.279299	0.23473	0.97854	0.442066
S10	0.22628	0.079542	0.166948	0.173655	0.379391	0.378929
S11	0.03736	0.219008	0.004703	0.038074	0.008698	0.117187

	5-phenyl-γ-VL-sulfate-glucuronide					
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	0.001031	0.02268	0.047154	0.311391	4.984531	0.142021
S2	0.02851	0.03095	0.177193	0.764917	4.978764	0.847724
S3	0.23553	0.155001	0.12365	1.029977	6.872132	3.879614
S4	0.022404	0.051838	3.810523	7.499738	18.10053	4.435338
S5	0.050762	0.003415	0.350744	2.502551	7.160838	0.599538
S6	0.001482	0.035132	0.043496	0.249407	2.190234	4.348229
S7	0.000309	0.002062	0.160892	0.285536	2.883341	2.156298
S8	0.019026	0.019218	0.08228	0.359855	6.050061	3.042189
S9	0.026663	0.051125	0.849024	3.184713	17.86215	8.934781
S10	0.034613	0.033988	0.056788	0.192339	1.643909	1.133559
S11	0.043097	0.961322	0.094896	0.186642	1.369184	0.543123

	4-hydroxy-5-(hydroxyphenyl)-VA-sulfate					
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	0.00019	0.000226	0.000446	0.000713	4.666094	0.001183
S2	0.000125	0.000309	0.000339	0.000464	0.000951	0.001391
S3	0.182602	0.15471	0.000499	1.546209	5.576214	3.142657
S4	0.000166	0.00019	0.000666	4.30416	0.001902	1.830615
S5	0.000559	0.00063	0.000844	2.063894	0.001135	0.000868
S6	0.000273	0.000345	0.000523	0.000523	0.637188	1.210109
S7	5.71E-05	0.00038	0.00066	0.000505	0.001141	1.815021
S8	0.000172	0.000238	0.000725	0.000262	5.20036	2.184254
S9	0.010426	0.000315	0.000493	0.000499	7.163422	0.000874
S10	0.000131	0.000321	0.058025	0.000202	0.001605	0.001724
S11	0.000464	0.000476	0.000868	0.00038	0.292073	0.001266

	4-hydroxy-5-phenyl-VA-methoxy-sulfate					
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	0.00019	0.000226	0.000446	0.000713	0.001891	0.001183
S2	0.000125	0.000309	0.000339	0.000464	0.000951	0.001391
S3	0.000777	0.000115	0.000499	0.004577	0.017472	0.013919
S4	0.000166	0.00019	0.000666	0.000428	0.001902	0.0017
S5	0.000559	0.00063	0.000844	0.005187	0.001135	0.000868
S6	0.000273	0.000345	0.000523	0.000523	0.001034	0.001118
S7	5.71E-05	0.00038	0.00066	0.000505	0.001141	0.011076
S8	0.000172	0.000238	0.000725	0.000262	0.016218	0.0017
S9	0.000309	0.000315	0.000493	0.000499	0.02192	0.000874
S10	0.000131	0.000321	0.000595	0.000202	0.001605	0.001724
S11	0.000464	0.000476	0.000868	0.00038	0.001605	0.001266

	4-hydroxy-5-(hydroxyphenyl)-VA-glucuronide					
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	0.001031	0.001224	0.002416	0.003866	0.010244	0.006411
S2	0.000677	0.001675	0.001836	0.002513	0.005154	0.007538
S3	0.000226	0.005936	0.002706	0.001931	0.104034	0.091242
S4	0.000902	0.001031	0.003608	0.148464	0.010309	0.009213
S5	0.003028	0.003415	0.004575	0.002448	0.006153	0.004703
S6	0.001482	0.001868	0.002835	0.002835	0.19227	0.006056
S7	0.000309	0.002062	0.003576	0.002738	0.006185	0.005026
S8	0.000934	0.001289	0.017873	0.001417	0.00683	0.009213
S9	0.001675	0.001707	0.002674	0.002706	0.004413	0.004736
S10	0.000709	0.00174	0.003222	0.001095	0.008698	0.009342
S11	0.002513	0.002577	0.004703	0.178592	0.367875	0.418758

Table S4. Peak areas of epicatechin phase II metabolites at different time points in urine of 11 study participants

Epicatechin sulfate						
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	1326389	6729630	7627886	4545819	537949.1	115695.8
S2	2286928	4686064	4759275	2693558	522643.9	47355.99
S3	12001577	47765606	23652766	19061541	3626151	486581.6
S4	3381769	11963453	8665182	3894692	735901.3	225699.5
S5	203828.6	4514361	7777964	5027105	1117218	333298.3
S6	36209.58	13144646	15101821	11624780	2639002	364616
S7	7942834	10757225	11869435	9368591	3299.233	111671.6
S8	366346.4	8557358	11639119	10917464	980372.2	87007.98
S9	286249.8	5551614	12297950	9452829	2835509	334020.5
S10	1457951	6334921	13595429	14717488	878015.3	143436.4
S11	872249.3	6761057	10234210	8407972	1080709	218703.1

Epicatechin methoxy-sulfate						
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	264155.2	1505928	1715618	756096.5	842118.3	155791.6
S2	515779.8	1604495	1794357	721462.2	1040562	113782.8
S3	2228138	8786534	5138701	4409302	1465165	99307.92
S4	690347.8	2486397	2201221	1080031	751369.8	196323.9
S5	241809.9	1220005	2273699	1693201	329099.7	379075.3
S6	95482.43	3308303	4418853	2910722	687244.1	197620.7
S7	1669724	2199023	2670830	1726327	62038.97	139673.9
S8	423632.2	1720478	2936943	2295600	405058	364021.1
S9	418943.1	1388609	3588964	3329649	865319	352424
S10	447708.8	1596924	3185476	3001613	268254.5	273954.2
S11	245223.5	1957469	2759612	2162724	478304.9	410378.1

Epicatechin glucuronide						
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	202881.9	1629378	2359769	1499848	309913.7	77646.22
S2	228722.1	633382.3	743255.2	528876.8	174653.8	0
S3	1558598	5973485	3321729	4855303	1043087	139266.5
S4	399656	1849520	1877834	1034390	225865.7	52267.37
S5	17571.76	623071	1121546	1151800	243468.1	70097.7
S6	5260.954	2847977	3788255	3382646	1297641	301090.7
S7	668327.6	1157062	1566698	1297940	0	43100.36
S8	76165.32	2498802	4169011	6193930	986075	149360.9
S9	14363.95	1822636	5357244	5290114	2265415	211945
S10	350289.2	1848168	3813223	4550370	442151.6	96873.25
S11	151091.6	1443595	1762950	2093796	475948.4	117135.1

Epicatechin methoxy-glucuronide						
	0-1 H	**1-2 H**	**2-4 H**	**4-6 H**	**6-12 H**	**12-24 H**
S1	53325.73	187265.3	410527.9	263698.4	44379.53	3334.106
S2	57420.7	144792.3	174158.7	143130.9	64053.84	5234.604
S3	206971.1	642872.9	545616.8	606339.6	139712.8	8155.154
S4	79783.13	256745.1	235160.9	202879.7	49920.92	9343.942
S5	7575.494	75754.94	174440.7	197368.9	35440.03	2925.878
S6	42551.95	425519.5	732121.4	698675.9	286720.2	157462.7
S7	91762.97	124026.5	226577.2	226080.1	0	47443.82
S8	11057.8	370447.6	764561.8	953267.9	289455.2	106957.7
S9	12971.22	320977.4	917894.5	967904.3	445749.4	101725.8
S10	62764.87	269044.5	527606.6	627855.8	122608.4	32897.62
S11	27498.78	232632.1	371709.3	431238.5	160841	91763.81

Table S5. Composition of the Keto-Drink. Ingredients: water, rapeseed oil, pea protein, dietary fibre: corn dextrin, medium-chain triglycerides (MCT), calcium phosphate, magnesium hydroxide, chromium chloride, ferrous gluconate, potassium chloride, potassium iodate, potassium lactate, copper gluconate, manganese sulphate, sodium selenate, zinc gluconate, sodium L-ascorbate, D-biotin, cyanocobalamin, menaquinone, nicotinamide, calcium D-pantothenate, phylloquinone, folic acid, pyridoxine hydrochloride, retinyl acetate, riboflavin, DL-alpha tocopheryl acetate, cholecalciferol, vitamin E rich oil from palm fruit/annatto, choline tartrate, L-carnitine, maltodextrin, aroma, emulsifier: mono and diglycerides of edible fatty acids. Details on the composition of the Keto-Drink (neutral taste) are available online at the homepage of the manufacturer at: https://www.keto-drink.de/en/keto-drink-neutral-taste/

Nutrition Facts

Nutrients	**per 100 ml**	**per drink (250 ml)**
Energy	828 kJ	2,070 kJ
Energy	200 kcal	500 kcal
Fat	16.8 g	42 g
of which saturated fatty acids	4.6 g	11.5 g
of which mono-unsaturated fatty acids	8.5 g	21.25 g
of which polyunsaturated fatty acids	3.7 g	9.25 g
Carbohydrate	1.6 g	4 g
of which sugars	0.2 g	0.5 g
Fibre	3.1 g	7.75 g
Protein	7.4 g	18.5 g
Salt	0.23 g	0.575 g
Vitamins	**per 100 ml**	**per drink (250 ml)**
Vitamin A	174 µg	435 µg
Vitamin D	4 µg	10 µg
Vitamin E (α-tocopherol equivalent)	7.1 mg	17.75 mg
of which tocopherols	8.9 mg	22.25 mg
of which tocotrienols	10.5 mg	26.25 mg
Vitamin K	11.2 µg	28 µg
Vitamin C	8.6 mg	21.5 mg
Thiamin	0.2 mg	0.5 mg
Riboflavin	0.2 mg	0.5 mg
Niacin (NE)	2.6 mg	6.5 mg
Vitamin B6	0.3 mg	0.75 mg
Folic acid	44 µg	110 µg
Vitamin B12	0.6 µg	1.5 µg
Biotin	8.9 µg	22.25 µg
Pantothenic acid	1.2 mg	3 mg
Minerals	**per 100 g**	**per drink (250 ml)**
Sodium	92 mg	230 mg
Potassium	285 mg	712.5 mg
Chloride	130 mg	325 mg
Calcium	98 mg	245 mg
Phosphorus	92 mg	230 mg
Magnesium	28 mg	70 mg
Iron	3.4 mg	8.5 mg
Zinc	2.1 mg	5.25 mg
Copper	210 µg	525 µg
Manganese	0.3 mg	0.8 mg
Selenium	15 µg	37.5 µg
Chromium	9.2 µg	23 µg
Molybdenum	11 µg	27.5 µg
Iodine	48.0 µg	120 µg
Other ingredients	**per 100 ml**	**per drink (250 ml)**
MCT-Fat	3.5 g	8.75 g
Omega-3 fatty acid	1 g	2.5 g
Omega-6 fatty acid	2.6 g	6.5 g
Choline	81 mg	203 mg
L-carnitine	40 mg	100 mg
Lactate	1.3 g	3.25 g

Table S6. Mass spectrometry characteristic of (epi)catechin metabolites in urine samples after apple intake. For each metabolite is reported a) retention time (min), b) the theoretical formula, molecular weight (MW) and the identification level, c) ESI polarity, d) *m/z* of molecular ion and fragments with their relative intensities, e) the annotation and f) the fragments obtained in MS/MS

Metabolite	Rt (min)	Formula MW ID level	ESI	*m/z* (relative intensity)	annotation	MS/MS fragments
(Epi)catechin-glucuronide	4.48	$C_{21}H_{22}O_{12}$ 466.1111 Level II	neg	**465.1007(100)** *466.1046(11)* *467.1094(1.3)* 487.0824(30) 289.0700(15)	$[M-H]^-$ $[M-2H+Na]^-$ $[M-H-GLC]^-$	**MS/MS² 465:** 289.0706(100) $[M-H-GLC]^-$ 327.0708(7) $[M-H-C_7H_7O_3]^-$ 245.0806(5)$[M-H-GLC-CO_2]^-$ 179.0346 (2) **MS/MS² 289:** 245.0818(100)
(Epi)catechin-sulfate	4.71	$C_{15}H_{13}O_9S$ 369.0280 Level II	neg	**369.0278(100)** *370.0312(18)* *371.0278(9)*	$[M-H]^-$	**MS/MS² 369:** 289.0707(100) $[M-H-SULF]^-$ 230.9963(65) $[M-H-C_7H_5O_3]^-$ 216.9801(10) $[M-H-C_8H_7O_3]^-$ 245.0812(10) $[M-H-SULF-CO_2]^-$
(Epi)catechin-methoxy glucuronide	4.86	$C_{22}H_{24}O_{12}$ 480.1268 Level II	neg	**479.1159(100)** *480.1168(14)* *481.1202(2)*	$[M-H]^-$	**MS/MS² 479:** 303.0851(100) $[M-H-GLC]$ 313.0536(50) $[M-H-C_9H_{10}O_3]^-$
			pos	481.1317(100) *482.1351(10)*	$[M+H]^+$	
(Epi)catechin-methoxy sulfate	5.25	$C_{16}H_{16}O_9S$ 384.0515 Level II	neg	**383.0438(100)** *384.0469(21)* *385.0436(9)*	$[M-H]^-$	**MS/MS² 383:** 303.0863(100) $[M-H-SO_3]$ 216.9807(61) $[M-H-C_9H_{10}O_3]^-$ 137.0242 (38) $[M-H-C_9H_{10}O_6S]^-$ 285.0750(6) $[M-H-SULF-H_2O]^-$

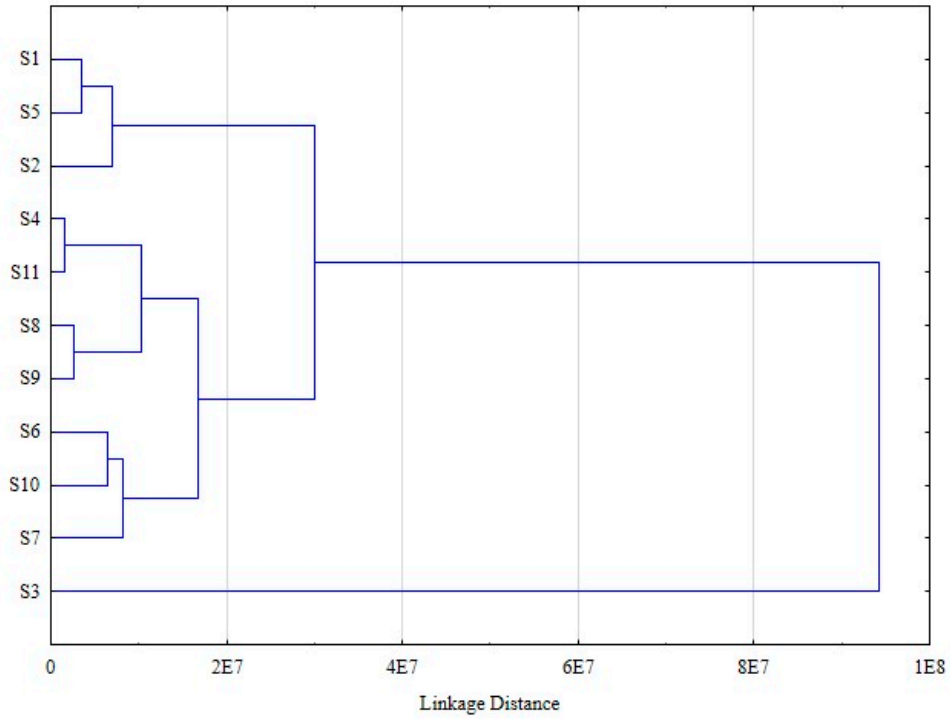

Figure S1: Cluster analysis based on percentages of excreted epicatechin phase II metabolites in the 11 subjects (complete linkage, Euclidean distance)