SUPPLEMENTARY MATERIAL

Bioavailability of dietary (poly)phenols following acute ingestion of an enriched drink by ileostomists

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1 Experimental

1.1 HPLC-PDA-MS^n analysis of urine

Samples were analyzed with the instrumentation and conditions described in the text and used in an earlier report on bioavailability of the polyphenol-rich drink using healthy subjects with a functioning colon.[1] A 250 x 4.6 mm i.d 4 □m Synergi RP-Max HPLC column (Phenomenex, Macclesfield, UK) was used at a flow rate of 1 mL/min with three different mobile phase gradients used previously to analyse the urine of healthy volunteers. These were:

1) a 66 min gradient of 10-30% acetonitrile in 0.1 % aqueous formic acid for the analysis of (epi)catechin, phloretin, (epi)gallocatechin and hesperetin metabolites in urine.

2) a 45 min gradient of 5-27% acetonitrile in 0.5 % aqueous acetic acid for the analysis of gallic acid metabolites in urine.

3) a 40 min gradient of 5-16 % acetonitrile in 0.5 % aqueous acetic acid for the analysis of ferulic acid and caffeic acid metabolites.

Each urine sample was analyzed in three MS different conditions:

A) a two segment selected ion monitoring (SIM) method for flavan-3-ol and phloretin metabolites at *m/z* 545, 465, 383 and 369 from 0-40 min and at *m/z* 529 and 449 from 40 to 60 min.

B) a two segment selected reaction monitoring (SRM) method for hesperetin and (epi)gallocatechin metabolites at *m/z* 495, 481 and 399 from 0-30 min and at *m/z* 557 and 477 from 30-66 min.

C) a one segment SIM method to detect acid metabolites at *m/z* 263 and 259 for 45 min.

D) a two segment SIM and SRM method for ferulic acid and caffeic acid metabolites at *m/z* 275, 273, 261, 259, 250 and 195 for 40 min.

Data on metabolite levels are presented as mean values, in either nmoles or □moles ± standard error (SE) (n =6).

1.2 HPLC-PDA-MS^n analysis of ileal fluid

The ileal extracts were analyzed using the following HPLC mobile phase conditions:

4) a 60 min gradient of 20-55% acetonitrile in 0.1 % aqueous formic acid for the analysis of hesperetin and phloretin metabolites.

5) a 60 min gradient of 5-35% acetonitrile in aqueous 0.1% formic acid for the analysis of original compounds present in the drink.

6) a 60 min gradient of 4-25% acetonitrile in 0.1% aqueous formic acid for the analysis of metabolites of flavan-3-ols and gallic acid.

7) a 60 min gradient of 5-50% acetonitrile in 0.1% aqueous formic acid for the analysis of other flavan-3-ol metabolites.

8) a 40 min gradient of 5-16% acetonitrile in 0.5% aqueous acetic acid for the analysis of caffeoylquinic acid metabolites.

Gradients 4-7 were use with a 250 x 4.6 mm i.d 4 □m Synergi RP-Max column and gradient 8 with a 250 x 4.6 mm i.d. Synergi 4 □m Polar-RP column (Phenomenex)

After passing through the flow cell of the PDA detector the column eluate was directed to a LCQ Advantage ion trap mass spectrometer fitted with an electrospray interface (Thermo Electron Corporation). SIM and/or SRM were used for quantification. Each sample was analyzed using seven different conditions:

E) Gradient **6** with two segment SIM for flavan-3-ol metabolite ions at *m/z* 495, 481 and 465 for the first 25 min and *m/z* 399, 383 and 369 for the next 35 min.

F) Gradient **7** with two segment SIM for flavan-3-ols metabolite ions at *m/z* 545 and 385 for the first 23 min and *m/z* 537, 521, 551 and 535.

G) Gradient **5** with two segment SRM for original compounds in the drink and *O*-methyl gallic acid-O-sulfate at *m/z* 345, 263, 249 and 169 for the first 21 min and at *m/z* 579, 457, 441, and 435 for 21-60 min.

H) Gradient **5** with two segment SRM for additional original drink compounds at *m/z* 577, 353, 305 and 289 for 0-28 min and at *m/z* 609 for 28-60 min.

I) Gradient **4** with two SRM for phloretin and its metabolites at *m/z* 567 and 449 for 0-17.5 min and at *m/z* 529, 353 and 273 for 17.7-55 min.

J) Gradient **8** with two segment SIM for caffeic metabolites at *m/z* 433, 273, 259 and 179 for the first 20 min and at *m/z* 193 for 20-50 min.

K) Gradient **8** with one segment SIM for other caffeic metabolites at m/z 275, 261, 250 and 195.

L) Gradient **5** with PDA detection at 520 nm and positive ion SIM at *m/z* 655 and 625 for anthocyanins.

All quantitative estimates were based on SIM or SRM or 520 nm peak areas with data expressed, as equivalents of the corresponding aglycone or conjugate, in nmoles as mean values ± S.E. (n =6).

2 RESULTS

2.1 Qualitative analysis of the P-R drink, urine and ileal fluid

The HPLC-MS^3-based identification (poly)phenolics and their metabolites in urine and ileal fluid after consumption of the P-R drink are presented in Tables 1-6. Retention times of the same components in the different matrices differs due to the use of different in HPLC mobile phase gradients (see Experimental). Table 1 lists the original drink components that were detected unmodified in ileal fluid. Data on metabolites of these compounds that were also identified in ileal fluid and/or urine are presented in Tables 2-6 with 25 flavan-3-ol metabolites listed in Table 2, eight dihydrochalcone metabolites in Table 3, six metabolites of 5-*O*-caffeoylquinic acid in Table 4, four hesperetin-based metabolites in Table 5 and two gallic acid metabolites in Table 6. The spectrum of metabolites that were identified is in keeping with those detected in previous

studies in which green tea, coffee, orange juice and apple cider were fed individually to human volunteers.[2-8]

3 References

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Table 1. Identification of original drink components present in ileal fluid based on HPLC retention times and mass spectrometric fragmentation patterns.

Original compounds	R_t (min)	$[M-H]^-$ *(m/z)*	MS^2 *(m/z)*
Flavan-3-ols			
(+)-Catechin	17.9	289	
(−)-Epicatechin	22.9	289	245
(+)-Gallocatechin	10.5	305	261, 221
(−)-Epigallocatechin	15.7	305	261, 221
(−)-Epigallocatechin-3-*O*-gallate	24.6	457	331, 305
(−)-Epicatechin-3-*O*-gallate	33.1	441	305
Dihydrochalcones			
Phloretin-2′-*O*-(2′′-*O*-xylosyl)glucoside	10.8	567	273, 167
Phloretin-*O*-(*O*-xylosyl)hexoside	11.4	567	273, 167
Phloretin-2'-*O*-glucoside	43.0	435	273
Chlorogenic acid			
5-*O*-Caffeoylquinic acid	19.6	353	191
Flavanones			
Naringenin-7-*O*-rutinoside	37.2	579	459, 271
Hesperetin-7-*O*-rutinoside	40.4	609	301
Anthocyanins			
Peonidin-3,5-*O*-diglucoside	13.6	625^+	463, 301
Malvidin-3,5-*O*-diglucoside	14.2	655^+	493, 331
Procyanidins			
Procyanidin dimer	15.9	577	425, 289
Procyanidin dimer	21.4	577	425, 289
Phenolic acid			
Gallic acid	6.6	169	125

$^+$ - $[M+H]^+$

Table 2 Identification of flavan-3-ol metabolites in ileal fluid and urine based on HPLC retention times and mass spectrometric fragmentation patterns.*

Flavan-3-ol metabolites	Urine R_t (min)	Ileal fluid R_t (min)	$[M-H]^-$ *(m/z)*	MS^2 *(m/z)*	MS^3 *(m/z)*
(Epi)gallocatechin-*O*-glucuronide	6.7	18.7	481	305	261, 221, 219, 179, 165, 125
(Epi)catechin-*O*-glucuronide	12.9	22.4	465	289	245, 205, 179
(Epi)catechin-*O*-glucuronide-*O*-sulfates	8.0, 14.6	n.d.	545	289	245, 205 179
4′-*O*-Methyl-(epi)gallocatechin-*O*-glucuronide	9.7	23.7	495	319	304, 301, 275, 260, 235, 233, 137
(Epi)catechin-*O*-sulfate	15.3, 18.1, 22.7	28.9, 32.5, 36.7	369	289	245, 205, 179
O-Methyl-(epi)catechin-*O*-sulfates	22.7, 25.3, 28.1, 30.3, 33.0	38.6, 39.8, 41.4, 45.3, 47.6, 50.4	383	303	285, 259, 244, 219, 217
O-Methyl-(epi)gallocatechin-*O*-sulfates	20.1, 21.4	35.1, 36.3	399	319	304, 301, 275, 260, 235, 233, 137
(Epi)gallocatechin-*O*-sulfates	n.d.	14.8, 15.7, 19.2	385	305	179, 221, 219, 261
(Epi)gallocatechin-3-*O*-gallate-*O*-sulfate	n.d.	31.7	537	457	331, 305, 169
(Epi)catechin-3-*O*-gallate-*O*-sulfate	n.d.	40.9	521	441	289, 331, 169
O-Methyl-(epi)gallocatechin-3-*O*-gallate-*O*-sulfates	n.d.	39.3, 39.5	551	471	319, 169
O-Methyl-(epi)catechin-3-*O*-gallate-*O*-sulfates	n.d.	44.3, 49.3	535	455	303, 285, 169

* n.d. - not detected

Table 3 Identification of dihydrochalcone metabolites in ileal fluid and urine based on HPLC retention times and mass spectrometric fragmentation patterns.*

Dihydrochalcones metabolites	Urine R_t (min)	Ileal fluid R_t (min)	$[M-H]^-$ *(m/z)*	MS^2 *(m/z)*	MS^3 *(m/z)*
Phloretin-2′-*O*-glucuronide	41.5	14.9	449	273	167
Phloretin-*O*-glucuronide	43.4	15.8	449	273	167
Phloretin-*O*-glucuronide-*O*-sulfates	47.5, 48.9, 51.7	18.6, 23.1	529	353	273, 167
Phloretin	n.d.	31.0	273	167	167
Phloretin-*O*-sulphate	n.d.	34.1, 39.2	353	273	167

* n.d. - not detected

Table 4 Identification of 5-*O*-caffeoylquinic acid metabolites in ileal fluid and urine based on HPLC retention times and mass spectrometric fragmentation patterns.*

Chlorogenic acid metabolites	Urine R_t (min)	Ileal fluid R_t (min)	$[M-H]^-$ *(m/z)*	MS^2 *(m/z)*	MS^3 *(m/z)*
Dihydrocaffeic acid-3-*O*-sulfate	8.6	n.d	261	181	137, 119
Caffeic acid-3-*O*-sulfate	11.6	11.5	259	179	135
Ferulic acid-4-*O*-sulfate	13.8	13.7	273	193	178, 149, 134
Caffeic acid	n.d.	15.4	179	135	
Feruloylglycine	19.3	19.6	250	206, 191, 177, 149, 100	
Ferulic acid	n.d.	30.0	193	178, 149, 134	

* n.d. - not detected

Table 5 Identification of flavanone metabolites in urine based on HPLC retention times and mass spectrometric fragmentation patterns.*

Flavanone metabolites	Urine R_t (min)	Ileal fluid R_t (min)	$[M-H]^-$ *(m/z)*	MS^2 *(m/z)*	MS^3 *(m/z)*
Hesperetin-7-*O*-glucuronide	42.0	n.d.	477	301	
Hesperetin-*O*-glucuronides	44.4, 44.8	n.d.	477	301	
Hesperetin-*O*-glucuronide-*O*-sulfate	44.2	n.d.	557	301	

* n.d. - not detected

Table 6 Identification of gallic acid metabolites in urine based on HPLC retention times and mass spectrometric fragmentation patterns.*

Gallic acid & flavanone metabolites	Urine R_t (min)	Ileal fluid R_t (min)	$[M-H]^-$ *(m/z)*	MS^2 *(m/z)*	MS^3 *(m/z)*
O-Methyl gallic acid-*O*-sulfates	18.6, 21.3	n.d.	263	183	169, 139, 123

* n.d. - not detected