Profiling of phenols in human faecal water after raspberry supplementation

Gordon McDougall[1], Chris Gill[2], Sheila Glidewell[1], Derek Stewart[1], Emma Coates[2], Jennifer Pearson[2], Qing Shen[3], Kieran Tuohy[3], Adele Boyd[2], Sumato Haldar[2] & Ian Rowland[3]

1 -Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, U.K.
2 -Northern Ireland Centre for Food and Health, Univeristy of Ulster, Coleraine, UK
3 -Department of Food Biosciences, University of Reading, Whiteknights, Reading, UK

Previous work has shown that berry extracts rich in polyphenols exert anti-cancer effects including manipulation of proliferation and apoptotic pathways in vitro (1). A large proportion of dietary polyphenols reach the colon and are subject to the action of fermentative microbiota and subsequent degradation. The resulting metabolites may interact with the colonic epithelium and alter colon cancer risk. However, comparably little is known about the effects on berry supplementation on the composition of the colonic microbiota or the profile of faecal phenolics. In this study, we monitored changes in human faceal microbiota and metabolic profiles after dietary supplementation with raspberries.

MATERIALS AND METHODS

Ten male fasting subjects consumed 200 g raspberry puree for four consecutive days. Stool samples were collected from each subject, just before and just after the supplementation. Faceal water samples were extracted as described previously (2). Fresh stool samples were also frozen at -80 C before micrbiota composition analysis by DGGE (3). Derivatized faecal water samples were analyzed by GC-MS on a Thermo-Finnigan Trace DSQ following a published protocol (4). Peaks were identified using standards and their MS properties and levels quanitified against standard curves of relevant compounds.

RESULTS

Of the 116 components consistently found in all ten subjects before and after supplementation, 58 were unambigously identified, a further 25 were putatively identified and 33 could not be idenitified. The identified components were lipid and bile acid derivatives (32), phenolic components (20) and nitrogen-containing heterocylic compounds (6) (Fig. 1). The major phenolic components were similar to previous reports (4) with phenylacetic acid and phenylacetic acid derivatives present in higher concentrations than phenylpropionic derivatives with five-ten fold lower amounts of cinnamic and benzoic acid derivatives (Table 1).

Fig. 1 Major components of faceal water

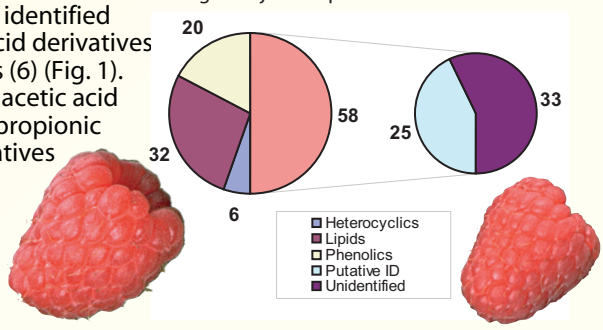
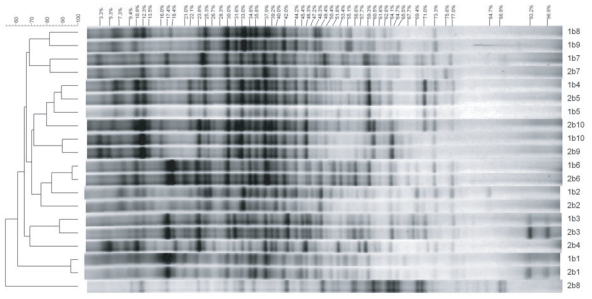


Table 1. Changes in faecal phenolics post-supplementation

Compound	Elevated *	Range (µg/ml)		Concentration (µM)	
		lowest	highest	lowest	highest
Phenylacetic acid	7	30.00	650.00	220.588	4779.412
4-hydroxyphenylacetic acid	6	2.00	26.00	13.158	171.053
3-hydroxyphenylacetic acid	5	1.50	85.00	9.868	559.211
3, 4-dihydroxyphenylacetic acid	2	0.10	75.00	0.595	446.429
3-phenylpropionic acid	6	0.10	210.00	0.667	1400.000
3(4-hydroxyphenyl)propionic acid	5	0.10	70.00	0.602	421.687
Benzoic acid	1	1.50	8.70	12.295	71.311
Salicylic acid	2	0.10	0.75	0.725	5.435
4-hydroxybenzoic acid	2	0.13	10.70	0.906	77.536
3, 4-dihydroxybenzoic acid	6	0.10	1.20	0.649	7.792
2, 5-dihydroxybenzoic acid	2	1.20	5.00	7.792	32.468
4-methoxybenzoic acid	0	0.10	11.50	0.658	75.658
Isoferulic acid	1	0.16	1.20	0.825	6.186
Caffeic acid	1	3.00	10.80	16.667	60.000
Sinapic acid	2	0.01	0.47	0.022	2.098
Naringenin	1	0.07	7.30	0.257	26.838
Hesperetin	0	0.01	8.00	0.033	26.490
Epicatechin	3	0.20	6.10	0.699	21.329
Catechol	2	0.05	3.50	0.455	31.818
Trihydroxybenzene	3	0.01	0.12	0.008	0.952

Certain phenolic metabolites, phenylacetic acid (7/10), 4-hydroxyphenylacetic acid (6/10), 3-hydroxy phenylacetic acid (5/10) and 3, 4-dihydroxy benzoic acid (6/10) were significantly increased in subjects. Subjects with increased levels of certain components did not show increases in other similar phenolics or in all phenolics.

Fig. 2 DGGE analysis of pre- and post supplementation microflora



DGGE profiling revealed considerable inter-individual variation in the composition of the colonic microbiota before raspberry supplementation (Fig. 2) However, principal component analysis confirmed that supplementation did not significantly or consistently alter the composition of the colonic microbiota. In addition, no specific microbiota alterations could be correlated to the presence (or absence) of metabolites.

CONCLUSIONS

Supplementation with 2.5 UK portions of raspberries over 4 days (a daily dose of ~ 300 mg phenolics and 40 mg anthocyanins) did not consistently alter the faceal phenolic profile in ten free-living volunteers. In fact, when increases were noted after supplementation, the levels observed were within the concentration range noted within the ten subjects. This suggests that the natural dietary variation in this study group may be greater than the extra dietary load imposed by raspberry supplementation. Perhaps understandably, a 4-day supplementation was not sufficiently long to alter the microbiota profiles of the ten volunteers.

However, increased faecal 3, 4-dihydroxybenzoic acid levels in 6 out of 10 volunteers was consistent with the observation of increased faecal levels after a single dose of 70 mg cyanidin glucoside in fasting volunteers (5). Indeed, elevated faceal levels of 3, 4-dihydroxybenzoic acid were reported following long-term supplementation with anthocyanin-rich bilberries (6), which suggests that this compound is a potential faceal biomarker for anthocyanin intake.

References
1. Coates et al. (2007). J. Carcinog. 6, 4. 2. Gill et al. Cancer Epidemiol. Biomarkers. Prevent. 13, 1199. 3. Steer et al. (2000) Nutr. Res. Review, 13, 229. 4. Jenner et al. (2005) Free Rad. Biol. Med. 38, 763. 5. Vitaglione et al. (2007) J. Nutr. 137, 2043. 6. Puuponen-Pimia et al. (2007) PSE abstract B15, VTT symposium 249. Helsinki

Acknowledgements
We acknowledge funding from NI DEL CAST awards and the Scottish Government Rural Environment and Research Analysis Directorate.