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Effect of ethnicity and a fermentable fibre on the *in vitro* colonic metabolism of polyphenols

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The anti-inflammatory and antioxidant properties of polyphenols-rich foods could reduce the risk of chronic diseases including cancer[1]. Only a small amount of polyphenols are absorbed in the duodenum and transferred to the plasma after phase I and II metabolism. The majority of polyphenols enter the colon and are metabolised by the gut microbiota. Understanding the role of gut bacteria in the metabolism of polyphenols is important to establish potential health benefits. Dietary habits and food matrix interactions should be considered as they play an important role in modulating the metabolic capacity of gut bacteria. The gut microbiota varies between different ethnic groups[2] which might be due to either dietary habits or genetics. The aim of this study is to understand the effect of ethnicity and a rapidly fermentable fibre (raftiline) on the colonic metabolism of a common dietary polyphenol (rutin).

Rutin (28 µmoles) was incubated for 24 hours with or without raftiline (1 g) in a 50 ml *in vitro* fermentation model[3] with fresh faecal samples from 14 healthy participants aged 23–43 (Indian $n = 8$, European $n = 6$). Participants followed a diet low in polyphenols and fibre for 3 days prior to providing faecal samples. The short chain fatty acids (SCFA) were measured by gas chromatography **with** flame ionization detector at $t = 0$, 2, 4, 6 and 24 h, alongside with pH. Phenolic acids were measured at $t = 0$, 6, 24 h by gas chromatography–mass spectrometry.

Three phenolic acids were identified as the main colonic metabolites of rutin 3-hydroxyphenylpropionic acid (3OH-PPA), 3,4-dihydroxyphenylaccetic acid (3, 4OH-PAA), and 3-hydroxyphenylacetic acid (3OH-PAA).

	6h fermentation								*24h fermentation*							
	Indian ($n = 8$)				European ($n = 6$)				Indian ($n = 8$)				European ($n = 6$)			
	R		R + Raf		R		R + Raf		R		R + Raf		R		R + Raf	
(µg/ml)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
3,4OH-PAA	0.1	0.1	n.d.	–	0.1	0.2	0.1	0.1	3.3	3.4	n.d.	–	1.9	2.6	0.1	0.2
3OH-PAA	0.7	1.1	n.d.	–	0.1	0.2	0.4	0,8	0.1	0.2	0.1	0.2	1.0	1.7	0.5	1.0
3OH-PPA	8.5	4.2	2.3	2.8	3.9	3.3	2.9	3.7	6.0	10.0	1.8	2.7	6.1	5.6	3.3	3.9
Sum of 3	9.4[a]	4.4	2.3	2.8	4.1b	3.5	3.3	4.3	9.4	8.5	1.9	2.9	9.0	4.2	3.8	4.8

Mean values followed by different letter are significantly different between groups, $p < 0.05$.

The fermentation of rutin alone led to higher levels of phenolic acids at 6 h in slurries from Indian compared to European participants (9.4 SD 4.4 *vs.* 4.1 SD 3.5 µg/ml, $p < 0.05$); no difference in SCFA production was observed between groups. At the same time point, when both rutin and raftiline were fermented, higher levels of SCFAs was observed in slurries from Indian compared to Europeans participants (20.1 SD 7.7 *vs.* 8.8 SD 8.3 µmoles/ml, $p < 0.05$); no difference was observed for phenolic acids production between groups. None of the differences observed at 6 h remained significant at 24 h.

Overall, addition of raftiline to the fermentation medium significantly inhibited the formation of phenolic acids at 6 h (2.7 SD 3.4 *vs.* 7.1 SD 4.7 µg/ml, $p < 0.01$) and 24 h (2.9 SD 3.6 *vs.* 9.2 SD 6.7 µg/ml, $p < 0.01$). As expected, raftiline significantly increased production of SCFA after 6 h (15.3 SD 9.6 *vs.* 0.3 SD 0.5 µmoles/ml, $p < 0.01$) and 24 h (27 SD 13.9 *vs.* 1.0 SD 1.1 µmoles/ml, $p < 0.01$) and reduced the pH from 7 to 4.5 after 24 hours of incubation (pH remained at 7 throughout the incubation when rutin was fermented alone).

The dietary fibre intake and ethnic-specific colonic microbiota should be considered in understanding the colonic metabolism of plant polyphenolics and their potential health benefits.

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