Soft fruit phytochemicals reduce levels of oxidative DNA damage in cell models of colorectal cancer

Coates, EM[1]* Gill, C.I.R[1], McDougall G.J[2] and Rowland, I[3]

[1]Northern Ireland Centre for Diet and Health (NICHE), Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland. BT52 1SA. [2]Scottish Crop Research Institute (SCRI), Plant Products and Food Quality, Mylnefield, Invergowrie, Dundee, Scotland, DD2 5DA. [3]Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, University of Reading, Reading, UK. RG6 6AH. *coates-e@ulster.ac.uk

Colorectal Cancer (CRC)

- Is the second most common cancer in the Western world, responsible for ~ 492 000 deaths and 945 000 new cases each year (1)
- Involves multiple sequential mutations in key genes controlling cell functions including proliferation and apoptosis (2)
- Initiation of CRC may be due to oxidative DNA damage causing permanent sequence changes (3)
- CRC risk is related to a typical Western diet - high in animal fat, sugar and meat, and low in fibre, fresh fruit and vegetables (4)
- Plant polyphenols may exert anti-cancer effects (5) and *in vitro* and *in vivo* studies have shown a protective effect of berries against CRC (reviewed in 6)
- Serum bioavailability of polyphenols is poor but colonic cells are in direct contact with these substances (7)

Aim

Use an *in vitro* model simulating the conditions of gastrointestinal digestion to produce "colon-available" extracts

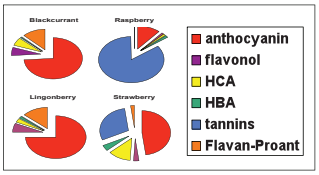
- 4 berry types: blackcurrant, lingonberry, strawberry and raspberry

Determine the anti-genotoxic effects of extract on a model of CRC representing the initiation stage of development.

Phytochemical Profile

- 4 Berries were chosen for their differences in phytochemical profile

Figure 1: Phytochemical profile of selected berries



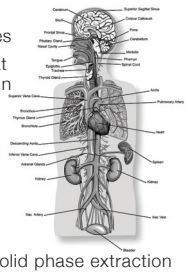
Methods

***In vitro* digestion:**
Homogenisation of berries

Gastric digestion: pH 2 at 37°C for 2 hrs with pepsin

Pancreatic digestion: Diffusion of bicarbonate to raise pH to 7.5 at 37°C over 2 hrs with pancreatin and bile salts

Stabilization by acidification to pH 2 and removal of bile salts by solid phase extraction

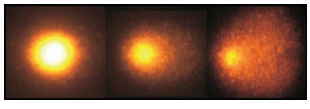


Analysis of polyphenol composition by LC-MS performed before and after "digestion"

Single Cell Gel Electrophoresis (Comet) Assay

- The anti-genotoxic potential of the colon-available berry extract was assessed using the Comet assay (% tail DNA)
- HT29 colon adenocarcinoma cells were pre-incubated with extract for 24 hr:
- 0, 1.56, 3.125, 6.25, 12.5, 25 & 50µg/ml gallic acid equivalents (GAE)
- 75µM H_2O_2 challenge for 5 min on ice

Figure 2: Comet assay - DNA migration is proportional to the number of single strand DNA breaks



Statistical Analysis

- Results expressed as mean of 3 independent experiments. One way ANOVA, Dunnett's T and T-3, and 4-way Bonferroni comparisons were performed. Significance was accepted at $p \leq 0.05$

Results

***In vitro* digestion**

- Post-digest or "colon-available" extract was reduced in tannins and anthocyanins but enriched in other polyphenols and polyphenol breakdown products (8).

Figure 3: LC-MS comparison of pre- and post-digest of extract. Decreased components ⌒, increased components ⌒ after "digestion"

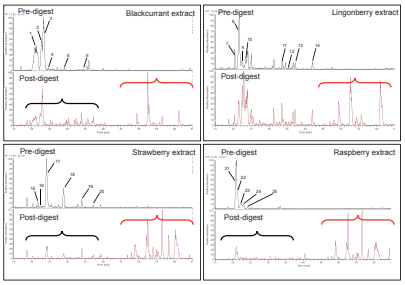


Figure 4: characterization of polyphenols in each berry type before "digestion," peak numbers correspond with peaks in figure 3

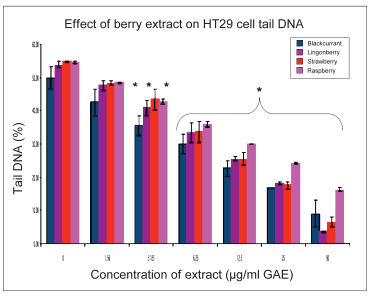
Blackcurrant			
Peak No	RT	Main m/z	Tentative ID
1	21.18	611, 303	delphinidin-3-O-rutinoside
2	22.87	449, 287	cyanidin-3-O-glucoside
3	23.87	595, 287	cyanidin-3-O-rutinoside
4	24.81	433, 595, 271	cyanidin-3-O-rutinoside
5	29.46	479, 317, 625	myricetin-3-O-glucoside
6	34.47	465, 303	quercetin-3-O-glucoside
Lingonberry			
Peak No	RT	Main m/z	Tentative ID
7	20.11	265, 461.9	hydroxycinnamic acid derivative
8	21.64	449, 287	cyanidin-3-O-galactoside
9	22.45	449, 287	cyanidin-3-O-glucoside
10	23.53	419, 287	cyanidin-3-O-arabinoside
11	33.56	303, 465	quercetin-3-O-glucoside
12	35.36	434.8, 303	quercetin arabinoside
13	37.25	449, 303	quercetin rhamnoside
14	42.05	592.8, 303	quercetin-4-HMG-rhamnoside
Strawberry			
Peak No	RT	Main m/z	Tentative ID
15	21.82	409, 579	proanthocyanidin B3
16	22.42	449, 287	cyanidin-3-O-glucoside
17	24.25	433, 271	pelargonidin-3-O-glucoside
18	28.94	519, 271	pelargonidin-3-malonylglucoside
19	34.05	479, 303	quercetin-3-glucoronide
20	36.92	449, 287	kaempferol-3-O-glucoside
Raspberry			
Peak No	RT	Main m/z	Tentative ID
21	21.02	611, 287	cyanidin-3-O-sophoroside
22	21.32	757, 287	cyanidin-3-O-glucosylrutinoside
23	22.3	449, 287	cyanidin-3-O-glucoside
24	22.94	741, 595, 287	pelargonidin-3-O-glucosylrutinoside
25	24.19	595, 271	pelargonidin-3-O-sophoroside

- Anthocyanins, tannins and hydroxycinnamic acids were reduced by *in vitro* digestion (shown in black)
- Some flavonols were relatively increased (shown in red) and polyphenol breakdown products were detected in the "colon-available" extract but not in the raw extract

Comet Assay:

- Significant dose-dependent anti-genotoxic effect observed after 24 hr pre-incubation with all berry extracts:
- 3.125 - 50µg/ml GAE (blackcurrant, lingonberry and raspberry)
- 6.25 – 50µg/ml GAE (strawberry)
- No genotoxic effect was observed at any concentration for all berry extracts (data not shown)
- Comparative differences (significant 3.125 - 50µg/ml GAE) in anti-genotoxic activity between berry types was observed with raspberry having the least potent anti-genotoxic activity

Figure 5: Anti-genotoxic effect of "colon-available" berry extract, 75µM H_2O_2 challenge. *Significance was accepted at $p \leq 0.05$, n=3



SUMMARY

- Berry polyphenols are sensitive to digestive conditions (pH, temperature, enzymes)
- "Colon-available" extract significantly reduced H_2O_2 induced oxidative DNA damage in HT29 cell model
- *In vivo* a decrease in DNA damage may lead to a decrease in initiation of the CRC process
- *In vitro* anti-genotoxic activity data for "colon-available" extract supports other studies showing the potential protective effects of berries in colon cancer

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