Ex Vivo Fecal Incubation Model

Broccoli sprouts and Brussels sprouts were *in vitro* digested using an oral, gastric, and intestinal phase as previously published (1–10). Briefly, salivary amylase was added to simulate the oral phase of digestion which was followed by a gastric phase where samples were acidified to a pH of 2.5 with hydrochloric acid and pepsin was added. Then sodium hydroxide was added to neutralize the samples (pH 7) and bile salts, pancreatin, and mucin were added for the intestinal phase of digestion. For fecal bacterial cultivation a 20% fecal slurry (w/v) was made from fecal material from 10 healthy volunteers (6 female, and 4 male, age 17-51, Lee Biosolutions) and sterile PBS (0.1 M pH 7). 500 µL of fecal slurry was mixed with 10 mL of Brain Heart Infusion Broth (BHI) with hemin and vitamin K, per the manufacturer's recommendation, and either 500 µl of filter sterilized *in vitro* digested broccoli sprouts (Broc), 500 µL of filter sterilized *in vitro* digested Brussels sprouts (Brus), 500 µL of Broc and 500 µL of Brus were added (Combo) or a negative control *in vitro* digestion (NC). NC contained reverse osmosis water, equivalent in volume to the water content of broccoli sprouts and underwent the same *in vitro* digestion procedure as described above with the same enzymes, chemicals and equipment. Broc and Brus digests were scaled to be equivalent in concentration to a human consuming ½ cup of broccoli or Brussels sprouts, or in the case of the combination, ½ cup of broccoli sprouts and ½ cup of Brussels sprouts. This combination was included as Broc and Brus contain many similar but also some distinct phytochemicals and thus by combining the vegetables we increased the dose and broadened the range of phytochemicals from cruciferous vegetables which can be achieved in the kitchen as a mixed vegetable dish. Fecal cultures were incubated at 37°C for 24 h in anaerobic conditions (11). Fecal culture medium was then vortexed, sampled, centrifuged (13,000 × g, 10 min) and supernatants frozen in liquid nitrogen.

Metabolomic Analysis

Metabolites from fecal culture medium were extracted (100 µL culture/100 µL ice cold 80:20, v/v, methanol:water), mixed vigorously, and clarified by centrifugation (13,000× g for 10 min). The supernatants were further diluted 1:10 (v/v) with ice cold 80:20 methanol:water (v/v) and transferred to mass spectrometry (MS) vials. Briefly, HPLC was performed on a Shimadzu Nexera system with a phenyl-3 stationary phase column (Inertsil Phenyl-3, 5 µm, 4.6 × 150 mm, GL Sciences) coupled to a quadrupole time-of-flight MS (AB SCIEX TripleTOF 5600), as previously described (12,13). The samples were randomized, auto-calibration was performed every two samples, and a quality control sample, composed of a pooled aliquot from each sample, was analyzed every 10 samples. MS/MS information was obtained for all samples using information dependent acquisition (IDA), while sequential window acquisition of all theoretical spectra (SWATH) was performed only on quality control samples. Spectral data were processed using Progenesis QI (NonLinear Dynamics v2.4). Peak deconvolution for $[M + H]^+$, $[M + Na]^+$, and $[M + NH_4]^+$ adducts in positive ionization mode, and $[M-H]^-$, $[M + FA\text{-}H]^-$, and $[M-H_2O-H]^-$ in negative ionization mode was performed in Progenesis QI. Feature intensities were normalized in Progenesis QI across samples by total ion current of all features.

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