The anthocyanins in black currants regulate postprandial hyperglycaemia primarily by inhibiting α-glucosidase while other phenolics modulate salivary α-amylase, glucose uptake and sugar transporters

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The anthocyanins in black currants regulate postprandial hyperglycaemia primarily by inhibiting α-glucosidase while other phenolics modulate salivary α-amylase, glucose uptake and sugar transporters.

Sisir Kumar Barik, Wendy R Russell, Kim M Moar, Morven Cruickshank, Lorraine Scobbie, Gary Duncan, Nigel Hoggard*

University of Aberdeen, Rowett Institute, School of Medicine, Medical Sciences and Nutrition, Aberdeen, AB25 2ZD, United Kingdom

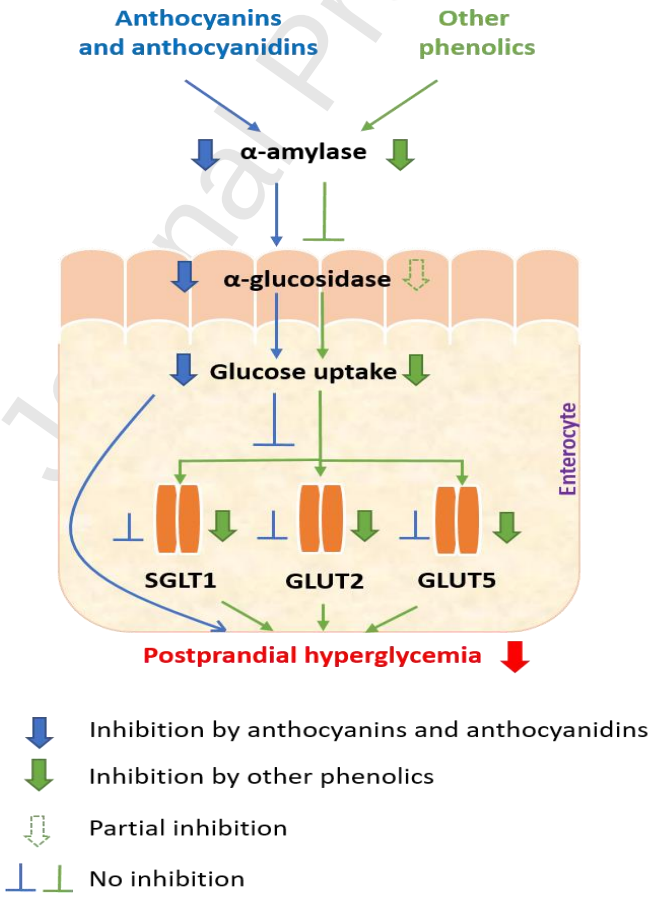
Abstract

The hypoglycaemic effects of two *Ribes* sp. i.e. anthocyanin-rich black currants (BC) were compared to green currants (GC), which are low in anthocyanins to establish which compounds are involved in the regulation of postprandial glycaemia. We determined the effect of the currants on inhibiting carbohydrate digestive enzymes (α-amylase, α-glucosidase), intestinal sugar absorption and transport across CaCo-2 cells. The digestion of these currants was modelled using in vitro gastrointestinal digestion (IVGD) to identify the metabolites present in the digested extracts by LC-MS/MS. Freeze-dried BC and IVDG extracts inhibited yeast α-glucosidase activity ($P<0.0001$) at lower concentrations than acarbose, whereas GC and IVDG GC at the same concentrations showed no inhibition. BC and GC both showed significant inhibitory effects on salivary α-amylase ($P<0.0001$), glucose uptake ($P<0.0001$) and the mRNA expression of sugar transporters ($P<0.0001$). Taken together this suggests that the anthocyanins which are high in BC have their greatest effect on postprandial hyperglycaemia by inhibiting α-glucosidase activity. Phytochemical analysis identified the phenolics in the currants and confirmed that freeze-dried BC contained higher concentrations of anthocyanins compared to GC (39.80 vs. 9.85 g/kg dry weight). Specific phenolics were also shown to inhibit salivary α-amylase, α-glucosidase, and glucose uptake. However, specific anthocyanins identified in BC which were low in GC were shown to inhibit α-glucosidase. In conclusion the anthocyanins in BC appear to regulate postprandial hyperglycaemia primarily but not solely by inhibiting α-glucosidase while other phenolics modulate salivary α-amylase, glucose uptake and sugar transporters which together could lower the associated risk of developing type-2 diabetes.

Keywords (max 6): Black currants; anthocyanins; LC-MS/MS; alpha-glucosidase; glucose uptake; glucose transporter

Abbreviations: T2DM: Type-2 Diabetes mellitus, EA: Enzyme activity, MC: 95% methanol control, AC: Acarbose, BC: Freeze-dried Black currants, GC: Freeze-dried Green currants, IVGD: In vitro gastrointestinal digestion, SSF: Simulated Salivary Fluid, SGF: Simulated Gastric Fluid, SIF: Simulated Intestinal Fluid, DBC: In vitro gastrointestinal digested Black currants, DGC: In vitro gastrointestinal digested Green currants, LC-MS/MS: Liquid chromatography-mass spectrometry, PCA: Principal component analysis, w/w: wet weight, CYA: Cyanidin, DEL: Delphinidin, MAL: Malvidin, MGL: Malvidin-3-glucoside, CGL: Cyanidin-3-glucoside, CAR: Cyanidin-3-arabinoside, PA: Protocatechuic acid, VA: Vanillic acid, PAL: Protocatechuic aldehyde, FA: Ferulic acid, HBA: Hydroxybenzaldehyde, CA: Chlorogenic acid, GA: Gallic acid, SA: Syringic acid, RV: Resveratrol.

Graphical abstract



1. Introduction

Type-2 Diabetes Mellitus (T2DM) is a prevalent endocrine-metabolic disorder with approximately 500 million cases worldwide in 2018 [1]. Prior to medication, T2DM can be managed, at least initially, through an appropriate diet and by sustaining a healthy lifestyle [2]. One of the major independent risk factors for developing T2DM is prolonged postprandial blood glucose levels referred to as hyperglycaemia [3]. Postprandial hyperglycaemia arises following a high carbohydrate meal due to the excess hydrolysis/breakdown of starch by the digestive enzymes (e.g. α-amylase and α-glucosidase) and sugar absorption across the small intestine. Recently, the consumption of black currants (BC) has recently been shown to reduce postprandial glycaemia and insulinemia in human subjects [4,5]. The most likely mechanisms involved relate to a reduction of the breakdown of carbohydrates in the mouth by α-amylase [6], in the intestine by α-glucosidase [7] and the reduced transport of glucose across the small intestine [8]. After the ingestion of a meal, the first stage of the breakdown of carbohydrate occurs in the mouth where salivary α-amylase hydrolyses α (1→4) bonds of large polysaccharides such as starch and glycogen to disaccharides like maltose [9,10]. The non-absorbed carbohydrates in the partially digested food bolus exiting the stomach [11] are broken down further to monosaccharides by the α-glucosidase enzyme located in the brush-border of the enterocytes in the small intestine and absorbed in the upper jejunum [12]. This postprandial state then activates families of glucose transporters or carriers in the small intestine, predominantly by the sodium-dependent glucose cotransporter 1 (SGLT1) and glucose transporter 2 (GLUT2), which allows the diet-derived monosaccharides (glucose) to be transported into small intestinal epithelial cells [13-15]. After a high carbohydrate diet, GLUT2 is translocated to the apical side from the basolateral side of the membrane from the intracellular vesicles, thus, allowing the absorption of the bulk amount of glucose into the small intestine [16,17]. In addition to this, fructose absorption into the cytosol is performed by another facilitative transporter called glucose transporter 5 (GLUT5), which is a fructose transporter expressed in the apical border of the epithelial cells of the small intestine [18,19].

Currently, biguanides (Metformin) are the first-line of treatment along with α-glucosidase inhibitors like Acarbose, Miglitol and Voglibose for people with high blood glucose levels or diagnosed with T2DM [20,21]**.** However, there are several complications associated with the use of these drugs, which includes liver ailments, abdominal pain, renal tumours, hepatic injury, acute hepatitis and gastrointestinal side effects such as flatulence and diarrhoea [22-25]. Diet-induced treatments from natural extracts may offer an alternative to avoid such complications. Several in vitro [7,26] and in vivo [27,28] studies have reported that diets rich in phenolic compounds, especially anthocyanins are capable of inhibiting α-glucosidase in the intestine, thus suppressing postprandial glycaemia. Previous in vitro and human intervention studies have also suggested that anthocyanins are the active component of berries in the regulation of postprandial glucose [4,5,8,29].

The black currant (Ribes nigrum) is a woody shrub in the family *Grossulariaceae* grown for its berries. It is native to temperate parts of central and northern Europe and northern Asia. In

this study, the hypoglycaemic effects of two *Ribes* sp. i.e. anthocyanin-rich black currants (BC; *Ribes nigrum L.*), were compared to green currants (GC; *Ribes nigrum*),which are low in anthocyanins. To establish which compounds are involved in the regulation of postprandial glycaemia, the phenolic profile of these currants and the metabolites produced following in vitro gastrointestinal digestion of the extracts were identified by targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS). The effect of BC, GC and selected metabolites of these currants in vitro on salivary α-amylase activity, α-glucosidase activity, and glucose uptake/transport in the intestine were determined.

2. Materials and Methods

2.1 Currants and sample preparation

GC (*Ribes nigrum*) Vertii and BC (*Ribes nigrum L.*) Ojebyn varieties were kindly provided by the James Hutton Institute, Dundee, UK. Currants which are non-toxic to humans were vacuum-freeze dried (Freezone vacuum Freeze dryer, Labconco, USA) followed by freeze-milling (Freezer mill Spex CertiPrep 6800, UK) and were vacuum-sealed and stored in -70 °C until further use.

2.2 Enzymes, reagents, and standards

Human salivary α-amylase (A0521), lyophilised α-glucosidase from *Saccharomyces cerevisiae*, EC 232-604-7 (G5003), 4-nitrophenyl α-D-glucopyranoside (*p*NPG- N1377) and acarbose (A8980) were obtained from Sigma-Aldrich, UK. Cyanidin (79457), delphinidin (43725), delphinidin-3-galactoside (04301), protocatechuic acid (03930590), vanillic acid (94770), protocatechuic aldehyde (D108405), ferulic acid (PHR1791), 4-hydroxybenzaldehyde (144088), chlorogenic acid (C3878), gallic acid (G7384), synergic acid (S6881), resveratrol (R5010) were purchased from Sigma-Aldrich, UK. Malvidin (80083), malvidin-3-galactoside (80600), cyanidin-3-galactoside (89463), cyanidin-3-arabinoside (89614), petunidin-3-glucoside (89755), pelargonidin-3-glucoside (89753), pelargonidin (80084) and peonidin (80085) were purchased from PhytoLab, Germany. Cyanidin-3-glucoside (0915S), delphinidin-3-glucoside (0938S) and malvidin-3-glucoside (0911S), were from Extrasynthese, France.

2.3 Human salivary α-amylase inhibition assay

The assay was performed following the protocol of EnzChek® Ultra Amylase Assay Kit (E33651), Invitrogen, UK. All the samples and reagents were prepared in 1X reaction buffer provided in the assay kit. Briefly 50 µL of samples were added to 50 µL (200 µg/mL) of dye-quenched substrate- DQ starch from corn, BODIPYL conjugate. Acarbose (1 mg/mL) was used as a positive inhibitor. The samples were then incubated at room temperature for 15 minutes. Ex/Em maxima of digestion product were read using a fluorescence microplate

reader (Spectramax Gemini XS- Molecular Devices, USA) at 502/512 nm. Background fluorescence was corrected by subtracting the no-enzyme control.

2.4 Yeast α-glucosidase inhibition assay

Samples were prepared by dissolving the freeze-dried currant powders in 95% methanol (20847, analR VWR chemicals, UK), placed at 37 °C in a shaking water bath for 15 min, vortexed and then filtered through a 0.22 μm syringe filter (Millex PES, Merck Millipore, UK). Samples were run along with no substrate and no enzyme controls. All the enzyme, inhibitor and substrate solutions were prepared in phosphate buffer (PBS, 0.1M; pH 6.9) and the inhibition assay was analysed using an end-point 96-well plate format. A reaction mixture containing 20 μL of PBS, 20 μL of α-glucosidase (0.5 U/mL) and 20 μL of extract were prepared and pre-incubated for 15 min at 37 °C. 40 μL *p*NPG (3mM) was then added to the mixture. After further incubation at 37 °C for 15 min, the reaction was stopped by adding 150 μL of sodium carbonate (0.2 M). Absorbance was read at 405 nm by a microplate reader (μQuant™ Microplate Spectrophotometer, BioTek Instruments Inc. USA). The α-glucosidase inhibitory activity was expressed as inhibition percentage (%) and was calculated as; %Inhibition = (ΔA Control - ΔA Sample/ΔA Control) 100, where ΔA = Individual absorbance recorded at 405 nm after subtracting from the blank.

2.5 Cell Culture

Human CaCo-2 cells are used as a model to explore intestinal epithelium absorption [30]. CaCo-2 cells were obtained from American Tissue Culture Collection (ATCC®, HTB-37TM) and were used between passages 35-48. Cells were maintained in Eagle's Minimum Essential Medium (M2279, Sigma-Aldrich, UK) containing 10% heat-inactivated fetal bovine serum (S181H, Biowest, France), 1% Penicillin-streptomycin (P4333, Sigma-Aldrich, UK), 1% (0.2 mM) L-glutamine (G7513, Sigma-Aldrich, UK), 1% MEM-non-essential amino acids (M7145, Merck, UK), 1% sodium pyruvate (S8636, Sigma-Aldrich, UK) and were cultured in a 5% CO_2 – 95% air (v/v; O_2 partial pressure of 150 Torr) 37 ^{0}C incubator (Panasonic-MCO-170AICUVH-PE, Panasonic Healthcare Ltd. Japan). Cells were cultured in 12-well plates with media change every two days and maintained for 21 days post-confluency. By this time, the CaCo-2 cells formed a confluent monolayer resembling the brush borders of the small intestine and show high levels of the expression of genes related to digestive enzymes, nutrient transporters and vitamin/mineral metabolism [31].

2.5.1 Cell viability assay (CellTitre-Blue)

The cytotoxicity of the freeze-dried currant extracts and their identified individual compounds was determined in a range of physiologically relevant concentrations [32,33] in CaCo-2 cells using the CellTiter-Blue (CTB) cell viability assay kit (Promega-G8081, UK). Cells were seeded in 96-well plates and allowed to get to confluence prior to testing. Concentrations used in our study did not show any significant effect on the metabolic capacity of the cells (Supplemental Fig. 1A & 1B).

2.6 Glucose uptake (2-deoxy-D-glucose (2DG) uptake)

The effects on glucose uptake was measured using a non-radioactive, homogeneous bioluminescent 2DG Uptake-Glo™ kit (Promega- J1341, UK) as per manufacturer's instructions. CaCo-2 cells were maintained in 12-well plates for 21 days post-confluence. Media was changed to serum-free medium 16 h before treatment. The culture medium was removed, and cells were washed twice with PBS and then exposed to different concentrations of test samples. All treatments were made in glucose-free and serum free-medium. Freshly prepared 2DG (10 mM, 50 µL) was added to each well and was incubated for 20 minutes. Then 250 µL of stop buffer (acid detergent solution) was added to each well. 75 µL of lysate was transferred to a 96-well white-walled plate followed by 25 µL of neutralization buffer and 100 µL of 2DG6P detection reagent in each well and incubated for 2 h. The luminescent signal was measured using Tecan Spark™ 10M multimode microplate reader, Switzerland. Phloretin (1 mM; P7912, Sigma) was used as an active glucose transporter positive inhibitor.

2.7 qRT-PCR (Quantitative real-time reverse transcription-PCR)

2.7.1 Total RNA isolation/cDNA synthesis

Total cellular RNA was isolated from cultured cells using TRI Reagent® (Sigma T9424) according to the manufacturer's instructions [34]. The isolated total RNA (1 µg) was used to synthesise cDNA using SuperScript™ II reverse transcriptase (Invitrogen, 18064014) as per manufactures instructions.

2.7.2 TaqMan gene expression assay

Following first strand cDNA synthesis, mRNA expression levels of sodium-independent facilitated glucose transporter-2 (GLUT2), fructose transporter-5 (GLUT5), sodium-dependent total glucose transporter (SGLT1) and B2M (β-2-microglobulin, reference housekeeping gene) were analysed using the Applied Biosystems 7500 v2.3 Fast real-time quantitative PCR instrument and TaqMan gene expression assay (TaqMan Fast Universal PCR Master Mix- no AmpErase™ UNG, Catalogue no. 4364103, Applied Biosystems™, Cheshire, UK). TaqMan® FAM-MGB hydrolysis human gene probes GLUT2/SLC2A2 (Hs01096908_m1), GLUT5/SLC2A5 (Hs01086390_m1), SGLT1/SLC5A1 (Hs01573793_m1), B2M/IMD43 (Hs00187842_m1) were purchased from Life technologies (Cat no. 4331182), UK. Primers span exon-intron borders as a control for DNA contamination. Reactions were performed in triplicates including non-template and no-reverse transcription controls. Quantitative measurements of target genes of interest relative to B2M gene expression were derived using the standard curve method. Data have been normalised to the untreated control group in each experiment and are presented as mean percentage inhibition ± standard error or means.

2.8 Phytochemical Analysis

Phytochemical extractions were performed by three methods specifically optimized for anthocyanidins (aglycones), anthocyanins (glycosides) and 'other phenolics'.

2.8.1 Anthocyanin and anthocyanidin extraction and their quantification

Internal Standard (IS); 2-amino-3,4,7,8-tetramethyl-3H-imidazo [4,5,f] quinoxaline (4,7,8-TriMeIQx, product no. A630000, Toronto Research Chemicals, Canada) in 10 mM methanolic acid and added to give a final concentration of 0.5 μg/mL.

Aglycones (anthocyanidins) extraction: Methanol/water/37% HCl, (50/33/17 (v/v/v)) was used as the solvent. Samples (0.1 g dry weight) were suspended in 3 mL of extraction solvent vortexed rigorously and placed in an ultrasound bath (DAWE Ultrasonics limited, UK) for 20 min.

Glycosides (anthocyanin) extraction: 10 mM acetic acid (A/0400/PB17 Honeywell-Fluka, USA) in methanol (34966, analR Honeywell Fluka, USA) is used as the solvent. Samples (0.1 g dry weight) were suspended in 4 mL of extraction solvent vortexed rigorously and placed in the ultrasound bath for 10 min.

The supernatants from both methods were separated by centrifugation at 3220g; 4 ℃ for 5 min (5810R, Eppendorf, Germany). The pellets were re-extracted thrice (2 mL) using their respective extraction solvents and the supernatants combined. Samples for aglycone extraction only were hydrolysed along with the pellets for 60 min at 100 °C in a thermoblock (BT3, Grant, UK). Hydrolysed samples were then allowed to cool at room temperature and an aliquot (1 mL) taken and centrifuged at 13975g (Jouan MR1822, Thermo Fisher-Scientific, USA) at 4 ℃ for 5 min. Internal standard (50 μL) was added to the samples (n=3; 450 μL), prepared in different dilutions (1:1000, 1:5000, 1:10000 and 1:50000) and these were directly analysed by LC-MS/MS.

2.8.2 LC-MS/MS analysis for anthocyanin and anthocyanidins

Quantitative determination of the anthocyanin aglycones and glycosides were performed based on calibration plots and retention times for the following compounds;

Aglycones: delphinidin, cyanidin, peonidin, pelargonidin, petunidin, and malvidin. **Glycosides**: cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, delphinidin-3-*O*-galactoside, delphinidin-3-*O*-glucoside, malvidin-3-*O*-galactoside, malvidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, and petunidin-3-*O*-glucoside.

LC-MS/MS was performed using an Agilent 1290 Infinity LC Binary pump system using an Agilent Poroshell 2.7 μM, 3 X 50 mm column from Agilent Technologies (Wokingham, UK). The mobile-phase solvents were 2% formic acid (A) and 85% acetonitrile + 15% methanol (B). Several calibration levels were prepared by diluting the stock solutions with methanol-formic acid (1%) having concentrations of 2, 10, 20, 40, 100, 200, 500 and 1000 pg/mL. Samples (4 μL injection) were run according the following gradient flow: 2.5−25% B (10.5 min), 25% B (1 min), 2.5% B (0.1 min), 2.5% B (1 min) with a starting pressure of 120 bar and constant flow rate. In all the cases, the flow rate was (500 μL/min) with an injection volume of 4 μL. The LC eluent was directed in split less mode into a 6490 triple-quadrupole mass spectrometer (Applied Biosystems, Warrington, UK) fitted with AJS-ESI (Agilent Jet Stream-Electrospray ionization source). The mass spectrometer was run with the following source settings: ion spray voltage, −4000 V; sheath gas temperature 350 °C and with a gas

flow of 13 L/min. All the metabolites were quantified using multiple reaction monitoring. Standard solutions (10 ng/μL) for all analytes were prepared and pumped directly via a syringe pump. The ion transitions for each of the analytes were determined based on their molecular (precursor) ion and a strong fragment (product) ion. The retention time, precursor ion, collision energy etc. were optimized individually for each analyte (Supplemental Table 1). The peaks obtained for aglycones and glycosides along with the internal standard were shown in the chromatogram (Supplemental Fig. 1).

2.8.3 Extraction and quantification of 'other phenolics'

All the amounts are in dry weights unless otherwise indicated. The method is followed as described by Multari et.al. [35] with some modifications. Briefly, samples, n=3 (0.1 g) were suspended in 3 mL HCl 0.2M, extracted with 5 mL ethyl acetate (EtOAc) and the layers were then separated by centrifugation (5 min; 1800g; 4 °C). The extraction was repeated twice, and the EtOAc extracts were combined. The organic layer was left to stand over sodium sulfate (anhydrous) and then filtered, and the solvent was removed under reduced pressure using rotavapor (R-114, Büchi Labortechnik AG. Switzerland) at a temperature not exceeding 40 °C. The remaining aqueous fraction obtained after EtOAc extraction was neutralized and freeze-dried. The freeze-dried pellets were suspended in NaOH (3 mL; 1 mol dm^{-3}) and stirred at room temperature for 4 h under nitrogen. The pH was adjusted to 2, and the samples were extracted with EtOAc (5 mL). This was repeated twice and processed as described above. The pH of the aqueous fraction was then brought to 7, and the aqueous fraction was freeze-dried. The freeze-dried aqueous fractions were suspended in 3 mL HCl ; 2M and incubated at 95 °C for 30 min with intermittent mixing. The samples were cooled and extracted with EtOAc (5 mL × 3) and processed as described above. End results were expressed as mean mg/kg of the samples after the LC-MS/MS analysis.

2.8.4 Preparation of extracts for LC-MS/MS analysis

The solvent used was methanol/water 4:1 containing 0.02% acetic acid. Negative-mode internal standard 1 (IS1) for mass spectrometry used was [^{13}C] benzoic acid (TJ2363, Sigma-Aldrich, Dorset, UK) 2 ng/mL in 20 μL solvent. It was prepared to give a final concentration in a sample of at 400 pg/μL. Positive-mode internal standard 2 (IS2) for mass spectrometry was (2-amino-3,4,7,8 tetramethylimidazo [4,5-f] quinoxaline (; 0.5 ng/mL in 20 μL solvent. It was prepared to give a final concentration in a sample of 100 pg/μL). The samples were then centrifuged (12500g; 5 min; 4 °C) and the supernatants were analysed by LC-MS/MS as detailed below.

2.8.4.1 LC-MS/MS for phytophenol analysis

Six gradient methods were used to separate the different categories of metabolites, which were performed using Agilent 1100 (Method 1,2 and 3) and Agilent 1200 (Method 4,5 and 6) binary pump LC-MS system fitted with a Zorbax Eclipse C18 column (5 μm, 150 mm × 4.6 mm) from Agilent Technologies (Wokingham, UK) and Aquasil C18, 5μm, 250 mm X 2.1 mm for Polyamine analysis from Thermo Fisher-Scientific (Paisley, UK) respectively. The mobile-phase solvents were 0.1% acetic acid (A) and acetonitrile containing 0.1% acetic acid

(B), except in polyamine analysis where solvents were 0.1% perfluoropentanoic acid (A) and acetonitrile containing 0.1% perfluoropentanoic acid (B).

Method 1: 10−55% B (45 min), 80% B (5 min), 80% B (2 min), 10% B (1 min), 10% B (5 min). Method 2: 15−95% B (40 min), 95% B (3 min), 15% B (1 min), 15% B (6 min). Method 3: 25−60% B (15 min), 60% B (3 min), 25% B (1 min), 25% B (5 min). Method 4: 40−90% B (13 min), 90% B (1 min), 40% B (1 min), 40% B (9 min). Method 5: 25−95% B (15 min), 95% B (3 min), 25% B (0.2 min), 25% B (3.8 min). Method 6-: 30−90% B (8 min), 90% B (2 min), 30% B (1 min), 30% B (4 min).

The flow rate were 400 μL/min and 500 μL/min for method 1 and 2 respectively, and 300 μL/min for all the other analyses. The injection volume was 20 μL The LC eluent was directed, without splitting, into an API 3200 and 4000 triple-quadrupole mass spectrometers (Applied Biosystems, Warrington, UK) fitted with a turbo ion spray source. For LC methods 1, 2, 4 and 6 the mass spectrometer was run in negative ion mode with the following source settings: ion spray voltage, −4500 V; source temperature, 400 °C; gases 1 and 2 set at 15 and 40 (units), respectively; and the curtain gas set to 10 (units). For LC method 3 and 5, the mass spectrometer was run in positive ion mode with the following source settings: ion spray voltage, 5500 V; source temperature, 400 °C; gases 1 and 2 set at 14 and 40 (units), respectively; and the curtain gas set at 10 (units). All the metabolites were quantified using multiple reaction monitoring. Standard solutions (10 ng/μL) for all analytes were prepared and pumped directly via a syringe pump. The ion transitions for each of the analytes were determined based on their molecular ion and a strong fragment ion. For several categories of compounds, it was inevitable that their molecular ion and fragment ion would be the same, but this was overcome by their different elution times [35,36]. The voltage parameters, declustering potential, collision energy, and cell entrance/exit potentials were optimized individually for each analyte.

2.9 In vitro gastrointestinal digestion

All materials were standard analytical grade. The following reagents/chemicals were purchased from Sigma-Aldrich, UK; Potassium chloride (Product no. P5405), monobasic potassium phosphate (P9791), sodium bicarbonate (S5761), magnesium chloride hexahydrate (M2393), ammonium carbonate (379999), calcium chloride dihydrate (C7902), amylase from porcine - Type VI-B (A3176), pepsin from porcine (P7000), pancreatin from porcine pancreas (P1750), Bile Extract Porcine (B8631). The chemicals/reagents purchased from Fisher Scientific, UK were Sodium Chloride (S/3160/60), Hydrochloric acid (H/1000/PB17) and Sodium hydroxide (S/4920/60).

2.9.1 In vitro gastrointestinal digestion (IVGD) model

The IVGD was a slight modification of the process described by Minekus et. al. [37]. Briefly, the IVGD consists of three important phases, which mimics the human alimentary canal starting with (A) Oral phase that contains Simulated Salivary Fluid (SSF, pH 7) followed by (B) Gastric phase- Simulated Gastric Fluid (SGF, pH 3) and (C) Intestinal phase- Simulated Intestinal Fluid (SIF, pH 7). The simulated digestion stock fluids were prepared to contain

electrolytes, enzymes, and water, filter sterilised using 0.22 μM filter, pH adjusted with 1 M NaOH or 1 M HCl and stored at 4 °C. $CaCl_2$ was added to the mixture on the day of use and not to the simulated digestion stock fluids. The working solutions were diluted 4:1 (in distilled water) and the enzyme activities were in units per mL of final digestion mixture rather than secretion activity unless stated otherwise. A control sample was also included with the same composition of electrolyte mixture, enzymes, and conditions but without any berry extracts.

All the experimental conditions were performed in a pre-set 37 °C shaking water bath (Grant OLS-200, UK) unless otherwise stated. Freeze-milled (5 g) berry extracts were mixed with 11.6 mL of water to create a 'food-like' paste. 50% (w/v) SSF including $CaCl_2$ (150 mM) and amylase (150 U/mL) was added to the paste and was allowed to mix for 2 min. The simulated food bolus was exposed to SGF at 50% (v/v) that contains porcine pepsin (16 mg/mL) and $CaCl_2$ (0.15 mM) and was incubated in the shaking water bath for 2 h. Then the simulated digestion mixture was exposed to SIF at 50% (v/v) that contains pancreatitin (20 mg/mL) and $CaCl_2$ (0.6 mM) and incubated for further 3 h whilst mixing. Samples were then centrifuged to remove the semi-digested mush at 3220g for 5 min. The supernatants were removed and set aside in falcon tubes whereas PBS is added to the pellets equal to the volume of the sample prepared and re-suspended. The samples were again centrifuged at 3220g for 5 min, supernatant removed and set aside with the previous supernatant. The collected fractions were stored at -70 °C and subsequently freeze-dried. Wet weight of the supernatants and residues were weighed separately, calculated their recovery difference and measured in equivalent weights of the freeze-dried berries for performing the assay.

2.10 Statistical Analysis

All the samples were run in triplicate for each experiment, and the results were presented as a mean ± standard error. Statistical analysis was performed using GraphPad Prism 7.0 for Windows. The data were analyzed using One-Way-Analysis of Variance (ANOVA) of Tukey's multiple comparisons test to compare the groups and Dunnet's multiple comparison test to compare a against single control as appropriate for he experiment. Data on phytochemicals were analyzed by principal component analysis (PCA), unit-variance-scaled using SIMCA-P+ 12 software (Umetrics, Cambridge, UK). The heat map was produced using Ms. Excel-2016 for Windows 10.

3. Results

3.1 Freeze-dried black and green currant extracts inhibit human salivary α-amylase

Both BC and GC extracts showed a dose-dependent inhibition of human salivary α-amylase (ANOVA $P<0.0001$) (Fig. 1A). Freeze-dried BC showed 75.7% ($P<0.0001$), 54.6% ($P=0.0013$) and 56.1% ($P=0.0010$) inhibition of human salivary α-amylase at 66, 6.6 and 0.66 μg/mL respectively, while freeze-dried GC showed 83.9% ($P<0.0001$), 68.7% ($P=0.0001$) and 66.2% ($P=0.0002$) inhibition at the same respective concentrations. The commercial

inhibitor Acarbose showed no significant inhibition (1 mg/mL; 31.4%, P=0.1024). No α-amylase activity was observed with the enzyme and substrate controls.

3.2 Freeze-dried black currant extracts inhibit yeast α-glucosidase but not the green currant extracts

Freeze-dried BC significantly inhibited yeast α-glucosidase activity (IC50 0.7 mg/mL, ANOVA P<0.0001). The inhibition of yeast α-glucosidase activity by the freeze-dried BC was dose-dependent (72.5% inhibition at 66 μg/mL; (P<0.0001); 70.5% inhibition at 13.2 μg/mL; (P<0.0001) and 24.2% inhibition at 6.6 μg/mL; (P<0.0001). At the same concentrations, freeze-dried GC showed no effect on yeast α-glucosidase activity (P=0.9974) (Fig. 1B). Acarbose, the known inhibitor of yeast α-glucosidase activity inhibited the enzyme activity by 46.7% (P≤0.001) at 1 mg/mL. No activity was observed with no enzyme and no substrate controls.

3.3. Freeze-dried black and green currant extracts inhibit 2DG uptake

Both GC and BC extracts showed a dose-dependent inhibition of 2DG uptake (ANOVA P<0.0001) in CaCo-2 cells. The IC50 for 2DG uptake inhibition was found to be 56.3 μg/mL for BC and 51.1 μg/mL for GC. BC extracts showed 55.5% and 27.1% (P<0.0001) inhibition, while GC extracts showed 59.3% and 27.7% (P<0.0001) inhibition at 6.60 and 0.66 ug mL^{-1} respectively (Fig. 1C). Both BC and GC did not show any significant inhibition at 0.06 μg/mL (P=0.9835 and P=0.1917). As a control, the glucose transporter inhibitor phloretin (1 mM) showed a 62.8% inhibition of 2DG uptake in the CaCo-2 cells (P<0.0001).

3.4 In vitro gastro-intestinal digested (IVGD) black currant extracts inhibit yeast α-glucosidase

IVGD of both BC and GC extracts were implemented to determine if the digested extracts had a similar effect on yeast α-glucosidase compared with the undigested extracts. The IVGD black currant (DBC) and green currant (DGC) extracts from the fraction modeled to represent the metabolites absorbed by the small intestine were compared. There was a significant inhibition of yeast α-glucosidase activity by the DBC extracts (ANOVA P<0.0001) (Fig. 2). The inhibition of yeast α-glucosidase activity by DBC was dose-dependent with 42.9% inhibition at 66 μg/mL (P<0.0001), 37% inhibition at 13.2 μg/mL (P<0.0001) and 17.3% inhibition at 6.6 μg/mL (P=0.0115) respectively. No inhibition of α-glucosidase activity was observed by DGC at any of these concentrations. Acarbose was found to inhibit the yeast α-glucosidase activity by 59.3% at 1 mg/mL (P<0.0001). The in vitro digested control which was composed of all the components of the IVGD model without the BC or GC extracts had no effect on the yeast α-glucosidase, which shows that the effects on the yeast α-glucosidase by the BC extracts were due to the currants and not a component of the digestion model.

3.5 Phytochemical composition of freeze-dried and IVGD extracts of black and green currants

Freeze-dried (BC, GC) and IVGD (DBC, DGC) extracts were analysed by LC-MS/MS to determine the differences in phenolic composition between these two currants and their IVGD products.

3.5.1 Quantification of anthocyanin and anthocyanidins in black and green currants by targeted liquid chromatography-mass spectrometry (LC-MS/MS)

Targeted analysis of the commonly distributed [38,39] anthocyanidins (aglycones) and anthocyanins (glycosides) was performed on both the freeze-dried and IVGD currant extracts. The quantified anthocyanidins and their glycosides significantly varied between the currants before and after the IVGD, where, BC were found to have higher levels of total anthocyanins than GC (Table 1). BC were found to be composed of 39800 mg/kg of aglycones (total anthocyanins and anthocyanidins), which decreased to 14500 mg/kg after the IVGD, suggesting a 63.4% reduction of total aglycones. GC were shown to be composed of lower levels of total aglycones, (9850 mg/kg), than BC which decreased to 970 mg/kg after IVGD suggesting a decrease of ~90% of total aglycones. BC was found to have high levels of delphinidin (28440 ± 880 mg/kg) and cyanidin (10060 ± 4330 mg/kg) compared to GC, which showed high levels of cyanidin (4980 ± 690 mg/kg) than delphinidin (3420 ± 570 mg/kg). Low levels of pelargonidin (280 ± 20 mg/kg), peonidin (360 ± 20 mg/kg), but no malvidin was detected in BC. In contrast, GC showed moderate levels of malvidin (840 ± 100 mg/kg) but no detectable amounts of pelargonidin or peonidin. Both BC and GC were found to have almost similar levels of petunidin (620 ± 30 vs 750 ± 120 mg/kg) respectively. Only peonidin (140 ± 50 mg/kg) and petunidin (210 ± 70 mg/kg) were detected in DBC, whereas no amount was detected in DGC. No amount of pelargonidin was detected in any of the IVGD currants. Among the glycosides, only glucosides of cyanidin and delphinidin were detected in the extracts of BC and DBC. DBC showed higher levels of delphinidin-3-glucoside (990 ± 120 vs. 390 ± 30 mg/kg) and cyanidin-3-glucoside (440 ± 30 vs. 230 ± 30 mg/kg) when compared to the freeze-dried extracts. Only cyanidin-3-arabinoside (230 ± 220 mg/kg) was detected in GC, whereas no amount of any of the other targeted glycoside was detected in either the freeze-dried or IVGD extracts of GC.

3.5.2 Total phenolic composition in freeze-dried and IVGD extracts of black and green currants by targeted liquid chromatography-mass spectrometry (LC-MS/MS)

The total phenolic content was estimated by sumation of all detected phenolics. This was found to be 4830 mg/kg and 4280 mg/kg (dry weight) in BC and GC respectively. The phenolics were measured both in their free and bound forms. Phenolic compounds not attached to other cell wall components (free) was found to be less in BC (180 mg/kg) compared with GC (860 mg/kg). Out of a total of 164 phenolic compounds measured in both currants in free and bound forms, 93 compounds were detected. Thirty phenolic compounds were found at a concentration above 5 mg/kg in the and these were expressed in a heat map (Table 2). BC was found to be high in most of the flavonoids, some benzoic acids, aldehydes, and acetophenones. Chlorogenic acid was found at high levels in both BC (2230 ± 550 mg/kg) and GC (600 ± 30 mg/kg). In addition, a number of ‘other phenolics’ were also detected in considerable amounts in both the currants e.g. gallic acid, protocatechuic acid,

syringic acid, vanillic acid, protocatechualdehyde, ferulic acid. The percentage of phenolic metabolites detected after the reduction due to IVGD was found to be 40.7% in BC (1960 mg/kg) and only 4.3% in GC (190 mg/kg). After the IVGD, chlorogenic acid was not detected in DGC, whereas a very small amount was detected in DBC (0.29 ± 0.03 mg/kg).

3.6 Dietary anthocyanidins and anthocyanins inhibit yeast α-glucosidase

To determine if specific anthocyanins or 'other phenolics' are responsible for the decrease in α-glucosidase activity by DBC as shown in fig. 3, fifteen target compounds were selected based on the phytochemical analysis of the digested black and green currants. Three major anthocyanin aglycones, their identified glycosides along with nine other major phenolics were selected. For these compounds, the effect on yeast α-glucosidase activity was determined. The mean percentage of inhibition of these compounds in vitro against yeast α-glucosidase activity is shown in Table 3. The anthocyanin aglycones; cyanidin (CYA, 19.1%; $P<0.001$) and malvidin (MAL, 12.5%; $P<0.001$), as well as the anthocyanin glycoside; cyanidin-3-glucoside (CGL, 15.8%; $P<0.01$) all showed significant inhibition of yeast α-glucosidase activity at the concentration of 66 μg/mL. Delphindin-3-glucoside (DGL, 10.4 %; $P<0.01$) showed significant inhibition at 33 μg/mL. In contrast, the selected individual 'other phenolics' did not show any significant inhibition of yeast α-glucosidase activity, except for the positive control resveratrol (RV), which showed a strong dose-dependent inhibition ($P<0.001$) of 87.5% to 62.3% at 66 to 0.66 μg/mL, which was greater than that seen with acarbose (16.3%, $P<0.01$; 1 mg/mL). The results suggest that the anthocyanins from BC are responsible for the inhibition of α-glucosidase and not the 'other phenolics'.

3.7 Dietary anthocyanins, anthocyanidins, and 'other phenolics' inhibit human salivary α-amylase

The individual compounds selected showed significant inhibition ($P<0.0001$) against human salivary α-amylase. Among the tested anthocyanins and anthocyanidins, the highest inhibition was shown by cyanidin-3-glucoside, 74.5% ($P<0.0001$) at a concentration of 66 μg/mL, followed by its aglycone cyanidin (70.6%, $P<0.0001$, 6.6 μg/mL) > delphinidin (62.1%, $P<0.0001$, 0.66 μg/mL) > delphinidin-3-glucoside (60.9%, $P<0.0001$, 66 μg/mL) > malvidin (58.3%, $P<0.0001$, 6.6 μg/mL) > cyanidin-3-arabinoside (57.7%, $P<0.0001$, 66 μg/mL) (Fig. 3A). Among the 'other phenolics', 4-hydroxybenzaldehyde showed the highest inhibition of 92.6% ($P<0.0001$) at a concentration of 66 μg/mL followed by chlorogenic acid (87.4%, $P<0.0001$, 0.66 μg/mL) > resveratrol (86.5%, $P<0.0001$, 6.6 μg/mL> gallic acid (80.2%, $P<0.0001$, 6.6 μg/mL) > protocatechuic aldehyde (79%, $P<0.0001$, 66 μg/mL) > ferulic acid (77.7%, $P<0.0001$, 66 μg/mL) > protocatechuic acid (75.6%, $P<0.0001$, 66 μg/mL) > syringic acid (69.7%, $P<0.0001$, 6.6 μg/mL) > vanillic acid (54.3%, $P<0.0001$ 66 μg/mL). Acarbose (1 mg/mL) showed an inhibition of 31.4% ($P=0.0004$) (Fig. 3B). 4-hydroxybenzaldehyde (HBA) was calculated to be having the lowest IC50 (0.35 μg/mL) among the phenolics, whereas cyanidin was calculated to be having the lowest IC50 (0.47 μg/mL) among the tested anthocyanins and anthocyanidins. The inhibition shown by individual compounds independently at each concentration along with their IC50 values are reported in supplemental Table 2.

3.8 Dietary anthocyanins, anthocyanidins, and 'other phenolics' inhibit 2DG uptake independently

The individual compounds were then investigated to determine if they had an effect on 2DG uptake. All the anthocyanins and anthocyanidins and a few of 'other phenolics' showed significant inhibition of 2DG uptake on human intestinal CaCo-2 cell over a 2 h period. Among the anthocyanins and anthocyanidins, highest inhibition was shown by the aglycone cyanidin by 69.1% ($P<0.0001$) at 0.66 μg/mL followed by delphinidin (68.5%, $P<0.0001$, 6.6 μg/mL) > malvidin (59.9%, $P<0.0001$, 0.66 μg/mL) > cyanidin-3-glucoside (57.2%, $P<0.0001$) > delphinidin-3glucoside (41.9%, $P<0.0001$) at 6.6 μg/mL respectively (Fig. 4A). The highest inhibition by the 'other phenolics' was 91.7% by 4-hydroxybenzaldehyde ($P<0.0001$) followed by gallic acid, (73.7%, $P<0.0001$) > ferulic acid (72.2%, $P<0.0001$) > syringic acid (63.3%, $P<0.0001$) > chlorogenic acid (56.1%, $P<0.0001$) at 6.6 μg/mL respectively (Fig. 4B).

3.9 Black and green currant extracts and their 'other phenolics' but not the anthocyanins or anthocyanidins inhibit sodium-dependent active cotransporter (SGLT1), sodium-independent facilitated glucose transporter (GLUT2) and fructose transporter (GLUT5) independently

Both GC and BC extracts significantly down regulated the glucose and fructose transporters at physiologically relevant concentrations. The mRNA expression of SGLT1 showed significant inhibition (95%; $P<0.0001$) by GC at a concentration of 6.6 μg/mL, which is also observed by the inhibition of GLUT2 (88.7%, $P=0.0114$) and GLUT5 (91%, $P<0.0001$) in a time-dependent manner. In contrast, BC showed a simultaneous significant time and dose-dependent inhibition of the target genes at 2, 16, 20 h, and at concentrations of 6.6, 0.66, 0.066 μg/mL. SGLT1 inhibition was observed by 85.9% ($P<0.0001$) at 16 h treatment, whereas GLUT2 showed 71.1% inhibition ($P<0.0001$) at 20 h and GLUT5 showed 66.5% inhibition ($P<0.0001$) at 2 h incubation with BC extracts, (Fig. 5**)**.

The effect of individual compounds on gene transcription of intestinal sugar transporters involved in glucose and fructose uptake was determined. Interestingly, a significant downregulation of the intestinal glucose transporters by certain phenolics when compared to the anthocyanins and anthocyanidins tested at physiologically relevant concentrations was observed. GLUT2 is highly inhibited by chlorogenic acid (88%, $P<0.0001$) followed by gallic acid (83.7%, $P<0.0001$) > 4-hydroxybenzaldehyde (80.2%, $P<0.0001$) > vanillic acid (67.9%, $P=0.0012$) > ferulic acid (65.4%, $P=0.0019$) > protocatechuic acid (59%, $P=0.0062$). Protocatechuic aldehyde, syringic acid, and resveratrol did not show any significant inhibition of GLUT2. In contrast, protocatechuic aldehyde (52.3%, $P=0.0153$) showed inhibition of GLUT5 along with ferulic acid (53%, $P=0.0136$). The sodium-dependent co transporter SGLT1 was significantly inhibited by chlorogenic acid (70.4%, $P=0.0005$) > gallic acid (65%, $P=0.0028$) > syringic acid (61%, $P=0.0171$) > resveratrol (61%, $P=0.0168$) > 4-hydroxybenzaldehyde (60%, $P=0.0183$) > vanillic acid (58.2%, $P=0.0236$). Protocatechuic acid, protocatechuic aldehyde, and ferulic acid did not show any SGLT1 inhibition on the human intestinal cells, (Fig. 6**)**. In addition, we did not observe any

inhibition of the targeted intestinal sugar transporters by the selected anthocyanins and anthocyanidins at any of the tested concentrations (data not shown).

4. Discussion

The consumption of BC extracts in the form of a fruit-drink has been shown to reduce postprandial glycaemia, and insulinemia [5]. Similar previous studies have shown that other soft-fruits have a similar effect on postprandial glucose [4,27]. The most likely mechanisms for the reduction in the postprandial glucose seen with the soft-fruits are the breakdown of carbohydrates in the mouth by α-amylase [6], in the intestine by α-glucosidase [7] and the reduced transport of glucose across the small intestine [8].

In this study, the effect of BC extract was investigated on all three of these potential mechanisms in vitro to determine where the extract is acting and where it is most effective. The effect of BC with GC on these proposed mechanisms of action were compared. GC has been reported to be low in anthocyanins [40,41], which are suggested to be the active ingredient in berry extracts causing the reduction in post-prandial glycaemia. Using LC-MS/MS we found that the total anthocyanin content is higher in BC and almost negligible in GC in agreement with previous reports [40,42].

Both BC and GC extracts at physiologically relevant concentrations reduced human salivary α-amylase activity by roughly the same amount in vitro, which suggests that both anthocyanins and 'other phenolics' are involved in the inhibition of α-amylase. A similar level of inhibition of α-glucosidase activity was seen in vitro with BC but no effect of GC was observed. This suggested that the anthocyanins, which are only present at high levels in BC are most likely to be responsible for the inhibition of α-glucosidase activity.

IVGD BC showed a slightly reduced level of inhibition of α-glucosidase activity compared to the undigested extract but again there was no inhibition of GC on α-glucosidase activity. The slightly reduced level of inhibition of α-glucosidase by the IVGD BC compared to the BC is probably because some anthocyanins and some non-glycosylated phenolics like catechin and epicatechin are less stable. As a result, they are sensitive to intestinal digestion, where they undergo structural modifications e.g. conjugation, transformation. Therefore, their bioavailability in the gastrointestinal tract becomes an important determinant of their in vivo activity [43,44].

The effect of BC and GC extracts in vitro on intestinal glucose uptake using the cellular 2DG uptake assay on human intestinal CaCo-2 cells that are differentiated to form a confluent monolayer representative of the small-intestinal epithelium was investigated. BC was shown to be as effective as GC in inhibiting 2DG uptake. This suggests that anthocyanins along with 'other phenolics' of the extracts are responsible for the inhibition of 2DG uptake. As cellular glucose uptake is associated with the gene expression of intestinal glucose (SGLT1 and GLUT2) and fructose (GLUT5) transporters, we studied the effect of BC and GC extracts on the mRNA expression of these intestinal sugar transporters. Both BC and GC extracts

decreased SGT1, GLUT2, and GLUT5 gene expression i.e. reduced monosaccharides transport across the intestine.

To determine which components of the extracts are responsible for the reduced breakdown of carbohydrates, by α-amylase and α-glucosidase as well as the reduced transport of glucose across the small intestine, we measured the total phenolic and anthocyanin contents of both currants and the IVGD extracts by LC-MS/MS. Principal component analysis (PCA) (Supplemental Fig. 2) of both the extracts showed that the currants were distributed in different quadrants suggesting they had distinct metabolite profiles. BC were shown to contain high levels of anthocyanins especially the aglycones of cyanidin and delphinidin, which accounted for almost ~97% of the total anthocyanins and anthocyanidins (as aglycones) present in the extract. In contrast, GC were low in anthocyanins with cyanidin-3-arabinoside being the only anthocyanin glycoside measured, in keeping with the previous reports of Maatta et.al [40]. Analysis of total phenolic components of both the currants suggested that both BC and GC in differing concentrations contain other phenolic acids such as chlorogenic acid, p-coumaric acid, gallic acid, syringic acid, caffeic acid, vanillic acid, 4-hydroxybenzaldehyde etc.

Fifteen compounds (anthocyanins, anthocyanidins, and 'other phenolics'), which were present at high levels in either BC or GC were selected for further analysis. These were analysed for their effect in vitro on the inhibition of carbohydrate digestion, uptake of glucose and gene expression of sugar transporters. The anthocyanin aglycones and glycosides of cyanidin present in high concentrations in BC showed the greatest inhibition of α-glucosidase activity [32,33]. The aglycones and the glycosides detected from BC were the most effective at inhibiting α-glucosidase activity compared to selected 'other phenolics'. Interestingly, the arabinoside of cyanidin (detected only in GC) did not inhibit the α-glucosidase activity, however, the same glycoside did inhibit human salivary α-amylase. This suggests that some anthocyanins and anthocyanidins are more effective at inhibiting specific enzymes of carbohydrate breakdown than others.

It was demonstrated that the anthocyanin aglycones and their glycosides were the most effective metabolites at inhibiting α-glucosidase activity when compared with the 'other phenolics'. Therefore, the effect of a broader selection of different anthocyanins aglycones and their glycosides on cellular glucose uptake along with the 'other phenolics' was explored. The anthocyanin aglycones and glycosides present at high levels in the BC extracts were shown to inhibit 2DG uptake in the human small-intestinal cells. Moreover, it was observed that anthocyanin aglycones were found to have greater activity than their glycoside counterparts. This may be because glycosides are partially hydrolysed by β-glycosidase in the small intestine, which explains the reported higher levels of aglycones in the jejunum [45,46]. In addition, the 'other phenolics' (e.g. protocatechuic aldehyde, chlorogenic acid, ferulic acid, gallic acid, 4-hydroxybenzaldehyde, syringic acid), detected in both BC and GC inhibited 2DG uptake, which demonstrates that BC and GC contain 'other phenolics' that may be contributing to the 2DG inhibition shown by their whole extracts.

When we looked at the effect of these metabolites on the mRNA expression of glucose (SGLT1 and GLUT2) and fructose (GLUT5) transporters, the anthocyanins or anthocyanidins showed no inhibition of expression compared to some of the 'other phenolics'. The 'other phenolics'; chlorogenic acid, gallic acid, and 4-hydroxybenzaldehyde significantly downregulated the gene expression of glucose transporters SGLT1, GLUT2 in keeping with previous studies [47,48]. This suggested these compounds are more effective than the anthocyanidins or anthocyanins in regulating the mRNA expression of these glucose transporters. This may be because these metabolites are better at crossing the basolateral side of the epithelium cells from the apical side [29]. However, this may not be the same with the fructose transporter, as the above-mentioned metabolites did not show any inhibition of GLUT5 expression, rather ferulic acid and protocatechuic aldehyde were the only two compounds among those selected having significant inhibition. Protocatechuic aldehyde was only able to inhibit the fructose transporter GLUT5 and not the glucose transporters SGLT1 and GLUT2. Therefore, this suggests that the 'other phenolics' we studied here are more important in the regulation of the gene expression of sugar transporters SGLT1, GLUT2 and GLUT5 than the studied anthocyanins or anthocyanidins [49]. This may be dependent on the concentration used or the structure of these metabolites and their ability to cross the cell membrane passively [30]. It should be noted that in this study, only the major anthocyanins and anthocyanins present in the soft-fruits [38,39] were investigated. Therefore, the possibility that other unidentified metabolites may have an effect on the glucose transporters, or that the anthocyanins we tested are more important in regulating other glucose transporters in humans cannot be excluded [50,51]. We should say that the observed effect of the phenolics at the intestine can also be enhance by the use of microencapsulation to increase the concentration of undigested phenolics reaching the intestine [52].

5. Conclusion

The consumption of black currant extract has been shown to reduce postprandial glycaemia, which is a rich source of phenolics specifically anthocyanins [5]. The in vitro data presented here showed that the extracts of both BC and GC are effective at inhibiting α-amylase in the mouth and reduced glucose transport in the intestine. However, only BC (both the extract and IVGD) are effective in inhibiting the breakdown of carbohydrates in the intestine by inhibiting α-glucosidase activity. LC-MS/MS analysis confirmed that anthocyanins are the major components compared to 'other phenolics' in BC with very low levels of anthocyanins in GC. Therefore, it is proposed that the main site of action of anthocyanins in BC is the inhibition of α-glucosidase in the intestine. Along with anthocyanins, some of the 'other phenolics', present at lower concentrations in the currants compared to the anthocyanins, e.g. chlorogenic acid, gallic acid, and 4-hydroxybenzaldehyde showed reduced carbohydrate digestion by inhibiting human salivary α-amylase and reduced sugar absorption. However, only some of the 'other phenolics' demonstrated reduced sugar transport across the small intestine by inhibiting SGLT1, GLUT2, and GLUT5. In summary, specific anthocyanins identified in black currants but low in the green currants inhibited α-glucosidase more than Acarbose, a commercial drug used for the management of T2DM. This appears to be a key

mechanism in the regulation of postprandial hyperglycaemia by anthocyanins, but not the only one, while other phenolics modulate glucose uptake and sugar transporters which together could lower the associated risk of developing T2DM.

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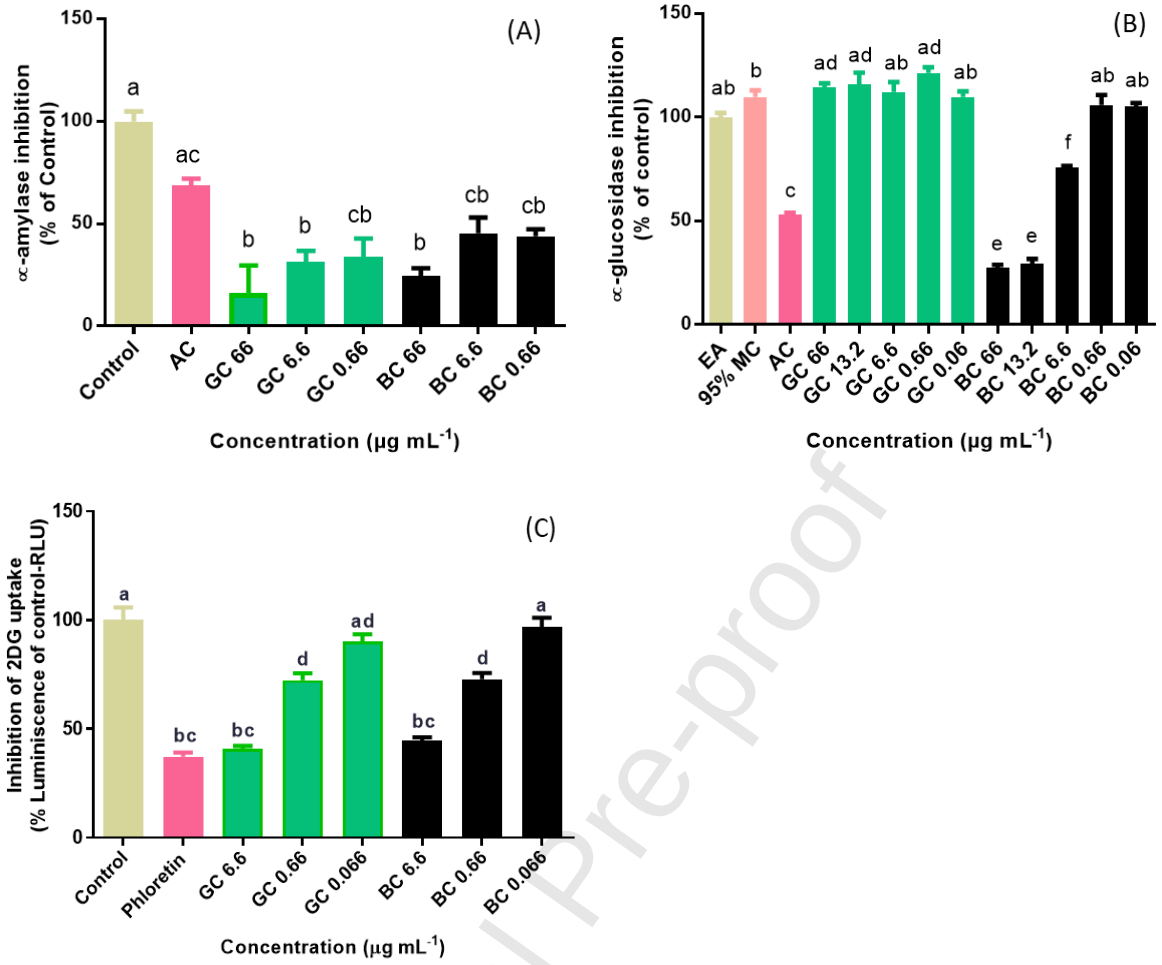


Fig. 1. Impact of freeze-dried green and black currant extracts on digestive enzymes and 2DG uptake

Activity at different fold concentrations (66 to 0.06 µg/mL) on the inhibition of **(A)** Human salivary α-amylase, (B) Yeast α-glucosidase, **(C)** 2DG uptake in human intestinal CaCo-2 cells. Enzyme activity was determined by percentage inhibition against respective controls. For the α-glucosidase assay, 95% methanol was used as the assay buffer and hence was taken as a control (without any test samples) but the percentage inhibition was determined against 100% enzyme activity. In the 2DG uptake study, the percentage inhibition of 2DG uptake was calculated against cells (control) without any treatment incubation. Phloretin was taken as a positive inhibitor. CaCo-2 cells of post-confluent 21 days were assayed after overnight serum starvation following the protocol of 2DG uptake using a Promega Glucose Uptake-Glo™ Assay kit. All the samples across the experiments were run in triplicates and are expressed as percentage inhibition ± SD of an experiment but the experiments were repeated independently at least three times on separate days with the same results. Statistical analysis was done by one-way analysis of variance of Tukey's multiple comparison tests. Values without a common letter are significantly different ($P<0.05$).

EA (Control): Enzyme activity, AC: Acarbose, MC: 95% methanol control, GC: Greencurrants, BC: Blackcurrants

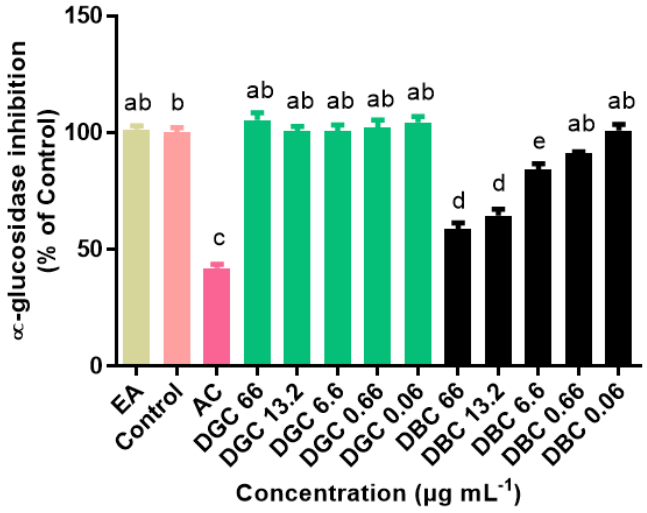


Fig. 2. Impact of in vitro **gastrointestinal digested (IVGD) green and black currant extracts on yeast α-glucosidase activity**

Percentage of enzyme inhibition was determined against enzyme activity. Samples were run in triplicates and are expressed as percentage inhibition ± SD of an experiment but the experiment was repeated independently at least three times with the same results. Statistical analysis was done by one-way analysis of variance of Tukey's multiple comparison tests. Values without a common letter are significantly different ($P<0.05$).

EA: Enzyme activity, AC: Acarbose, Control: IVGD enzyme only, DGC: IVGD Green currants, DBC: IVGD Black currants

Table 1. Anthocyanin and Anthocyanidin quantification using LC-MS/MS from freeze-fried and IVGD (in vitro **gastrointestinal digested) black and green currants.**

Contents quantified are expressed as mg/kg equivalent dry weight. nd = not detected (i.e. below the detection level).

	Black currants		***Green currants***	
	Freeze-dried	**IVGD**	**Freeze-dried**	**IVGD**
Total anthocyanins and anthocyanidins as aglycones present in the currants (mg/kg)				
Cyanidin	10063 ± 4332	6044 ± 1394	4980 ± 685	300 ± 163
Delphinidin	28442 ± 879	8079 ± 1986	3423 ± 566	569 ± 168
Malvidin	nd	81 ± 15	840 ± 100	100 ± 0
Pelargonidin	282 ± 21	nd	nd	nd
Peonidin	357 ± 19	136 ± 51	nd	nd
Petunidin	615 ± 31	211 ± 66	750 ± 120	nd
Total aglycones	**39758**	**14551**	**9853**	**969**
Detected glycosides in the currants (mg/kg)				
Cyanidin 3-O-arabinoside	nd	nd	226 ± 221	nd
Cyanidin 3-O-galactoside	nd	nd	nd	nd
Cyanidin 3-O-glucoside	232 ± 27	444 ± 31	nd	nd
Delphinidin 3-O-galactoside	nd	nd	nd	nd
Delphinidin 3-O-glucoside	392 ± 32	989 ± 124	nd	nd
Malvinidin 3-O-galactoside	nd	nd	nd	nd
Malvinidin 3-O-glucoside	nd	nd	nd	nd
Pelargonidin 3-O-glucoside	nd	nd	nd	nd
Petunidin 3-O-glucoside	nd	nd	nd	nd
Total glycosides	624	1433	226	nd
Undetected glycosides in the currants (mg/kg)				
Total	**39134**	**13118**	**9627**	**969**

Table 2. Heat map showing the 30 phenolic compounds, which were quantified above or approximately 5 mg/kg dry weight (highest to lowest) from the freeze-dried black currant (BC), green currants (GC) extracts and IVGD black currant (DBC), green currants (DGC) extracts using LC-MS/MS

Sl. no.	Compounds	Phenolics (mg/kg dry weight)								Total phenolics			
		BC		DBC		GC		DGC		BC	DBC	GC	DGC
		Free	Bound	Free	Bound	Free	Bound	Free	Bound				
1	Chlorogenic acid	nd	2229.1 ± 545.21	0.29 ± 0.03	nd	597.58 ± 29.90	nd	nd	nd				
2	Gallic acid	13.79 ± 0.64	311.96 ± 6.36	95.32 ± 1.73	2.61 ± 0.12	1.75 ± 0.08	653.41 ± 34.23	1.04 ± 0.01	6.71 ± 0.13				
3	Protocatechuic acid	18.37 ± 3.21	566.28 ± 4.92	294.78 ± 9.59	41.57 ± 1.49	3.79 ± 0.17	489.82 ± 86.11	1.03 ± 0.05	20.50 ± 0.32				
4	p-coumaric acid	4.26 ± 0.54	168.62 ± 6.18	359.57 ± 1.83	46.42 ± 0.34	1.80 ± 0.19	522.36 ± 33.25	14.92 ± 0.23	16.47 ± 0.12				
5	Syringic acid	60.71 ± 26.46	77.25 ± 0.47	366.04 ± 7.63	72.08 ± 5.83	0.88 ± 0.06	507.71 ± 39.33	0.17 ± 0.01	31.45 ± 2.11				
6	Caffeic acid	4.68 ± 0.37	192.32 ± 3.37	109.17 ± 1.85	25.09 ± 0.17	2.23 ± 0.06	305.83 ± 31.77	19.26 ± 0.82	29.27 ± 0.63				
7	Vanillic acid	7.36 ± 1.63	37.75 ± 1.02	134.35 ± 1.47	20.01 ± 0.98	nd	186.65 ± 14.16	1.00 ± 0.11	3.69 ± 0.01				
8	1,2-hydroxybenzene	nd	47.734 ± 1.06	nd	nd	nd	nd	nd	nd				
9	Benzoic acid	1.56 ± 0.60	40.38 ± 0.31	42.27 ± 0.11	4.36 ± 0.08	1.05 ± 0.22	109.46 ± 15.36	0.69 ± 0.04	3.57 ± 0.04				
10	4-hydroxyphenylacetic acid	11.26 ± 1.30	21.50 ± 1.81	4.28 ± 0.37	0.79 ± 0.09	nd	21.50 ± 2.75	nd	nd				
11	4-methoxycinnamic acid	nd	16.59 ± 0.91	27.45 ± 0.50	1.48 ± 0.04	nd	105.93 ± 17.20	nd	nd				
12	Myricetin	nd	104.66 ± 0.10	0.95 ± 0.03	0.39 ± 0.01	23.39 ± 0.50	7.32 ± 1.08	nd	1.18 ± 0.01				
13	Catechin	nd	70.79 ± 0.19	27.0 ± 0.32	nd	29.50 ± 1.63	72.45 ± 1.58	nd	nd				
14	Epicatechin	nd	90.52 ± 0.03	21.51 ± 0.47	nd	64.58 ± 3.52	37.34 ± 0.51	nd	nd				
15	p-hydroxybenzoic acid	2.52 ± 0.06	24.0 ± 1.90	11.25 ± 0.21	1.58 ± 0.01	0.07 ± 0.02	28.71 ± 3.01	3.23 ± 0.04	4.18 ± 0.02				
16	2,5-dihydroxybenzoic acid	5.25 ± 0.43	3.27 ± 0.08	nd	nd	nd	7.43 ± 0.00	nd	nd				
17	Salicylic acid	1.19 ± 0.06	67.17 ± 0.12	0.95 ± 0.02	0.15 ± 0.0	1.01 ± 0.04	6.49 ± 0.60	0.11 ± 0.00	0.43 ± 0.00				
18	Protocatachaldehyde	13.05 ± 7.46	31.87 ± 2.87	8.89 ± 0.20	3.09 ± 0.15	0.36 ± 0.03	34.02 ± 1.49	0.43 ± 0.01	1.32 ± 0.04				
19	Epigallocatechin	nd	65.64 ± 0.05	nd	nd	31.57 ± 0.97	4.88 ± 1.14	nd	nd				
20	3,4-dihydroxyphenylacetic acid	nd	2.22 ± 0.08	2.73 ± 0.23	nd	nd	58.81 ± 4.68	nd	nd				
21	Quercetin-3-Glucoside	0.05 ± 0.01	50.50 ± 0.03	147.26 ± 2.34	12.65 ± 0.03	36.08 ± 1.76	26.40 ± 0.61	1.00 ± 0.02	5.96 ± 0.25				
22	Ferulic acid	5.32 ± 0.40	44.41 ± 1.57	13.31 ± 0.17	3.42 ± 0.05	0.56 ± 0.00	58.24 ± 5.14	2.37 ± 0.05	5.17 ± 0.01				
23	Quercetin	0.04 ± 0.01	49.72 ± 0.04	2.58 ± 0.07	0.90 ± 0.04	1.76 ± 0.08	4.50 ± 1.75	0.23 ± 0.01	1.13 ± 0.02				
24	3,4,5-trihydroxybenzaldehyde	nd	41.87 ± 0.31	2.62 ± 0.07	nd	nd	57.54 ± 1.21	0.91 ± 0.03	0.27 ± 0.00				
25	Gossypin	nd	34.49 ± 0.01	13.15 ± 0.25	0.66 ± 0.02	9.59 ± 0.17	12.46 ± 0.45	nd	nd				
26	Quercitrin	nd	32.54 ± 0.00	1.59 ± 0.06	0.49 ± 0.00	19.07 ± 0.87	2.44 ± 0.10	0.04 ± 0.00	0.39 ± 0.02				

Sl. no.	Compounds	Phenolics (mg/kg dry weight)								Total phenolics			
		BC		DBC		GC		DGC		BC	DBC	GC	DGC
		Free	Bound	Free	Bound	Free	Bound	Free	Bound				
27	p-anisic acid	nd	18.19 ± 0.04	7.56 ± 0.40	0.61 ± 0.02	nd	28.78 ± 4.07	nd	0.14 ± 0.00				
28	Gallocatechin	nd	28.21 ± 0.24	nd	nd	7.28 ± 0.24	5.93 ± 0.72	nd	nd				
29	Sinapic acid	1.23 ± 0.11	9.05 ± 0.49	2.28 ± 0.20	0.59 ± 0.03	nd	9.02 ± 0.93	0.66 ± 0.04	1.14 ± 0.00				
30	p-hydroxybenzaldehyde	0.33 ± 0.09	4.07 ± 0.33	0.90 ± 0.04	0.28 ± 0.01	0.05 ± 0.01	2.0 ± 0.01	0.08 ± 0.00	0.07 ± 0.00				

Colour grade scale

Low Medium High

Contents quantified are expressed as mg/kg equivalent dry weight. nd = not detected (i.e. below the detection level).

Table 3. Impact of individual identified dietary phenolics from green and black currants on yeast α-glucosidase

Mean % inhibition against enzyme activity (control)	**Identified dietary phenolic compounds from black and green currants**														
	Positive inhibitor	Anthocyanins and anthocyanidins						Other Phenolics							
Concentration (μg mL^{-1})	**AC**	**CYA**	**DEL**	**MAL**	**CGL**	**DGL**	**CAR**	**PA**	**VA**	**PAL**	**FA**	**HBA**	**CA**	**GA**	**SA**	**RV**

66	16.3[b]	19.1[c]	5.7	12.5[c]	15.8[b]	9	5.4	3.8	6.6	0.3	11.5	9.6	1.9	6.8	2.78	87.5[c]
33	8.3	6.4	8.4[b]	6.3	10.6	10.4[b]	0	8.7	0	0.8	5.9	0	2.5	4.4	0	76.6[c]
13.2	1.2	4.7	6.8[a]	2.5	6	9.7	0	3.2	0.1	6	1.2	0	0	3.1	0	68.7[c]
6.6	0	2.7	0	5.3	4.1	6.1	0	4.7	1	6.2	0	8.5	0.8	0	0.4	69.5[c]
0.66	0	6.1	4.2	0	4.6	2.6	0	9.1	8	10.8	0	6.6	0	0	3.08	62.3[c]

The table represents mean percent inhibition by compounds at each concentration against 100% enzyme activity. Data is a representation of three independent experiments, where values with superscripts have statistically significant difference against the control. Statistical significance was analysed using one-way analysis of variance of Dunnet's multiple comparison tests compared to single control using GraphPad Prism 7.0 for Windows 10.

a= $P<0.05$, b= $P<0.01$, c= $P< 0.001$,

AC = Acarbose, CYA: Cyanidin, DEL: Delphinidin, MAL: Malvidin, CGL: Cyanidin-3-O-glucoside, DGL: Delphinidin-3-O-glucoside, CAR: Cyanidin-3-O-arabinoside, PA: Protocatechuic acid, VA: Vanillic acid, PAL: Protocatechuic aldehyde, FA: Ferulic acid, HBA: 4-hydroxybenzaldehyde, CA: Chlorogenic acid, GA: Gallic acid, SA: Syringic acid, RV: Resveratrol

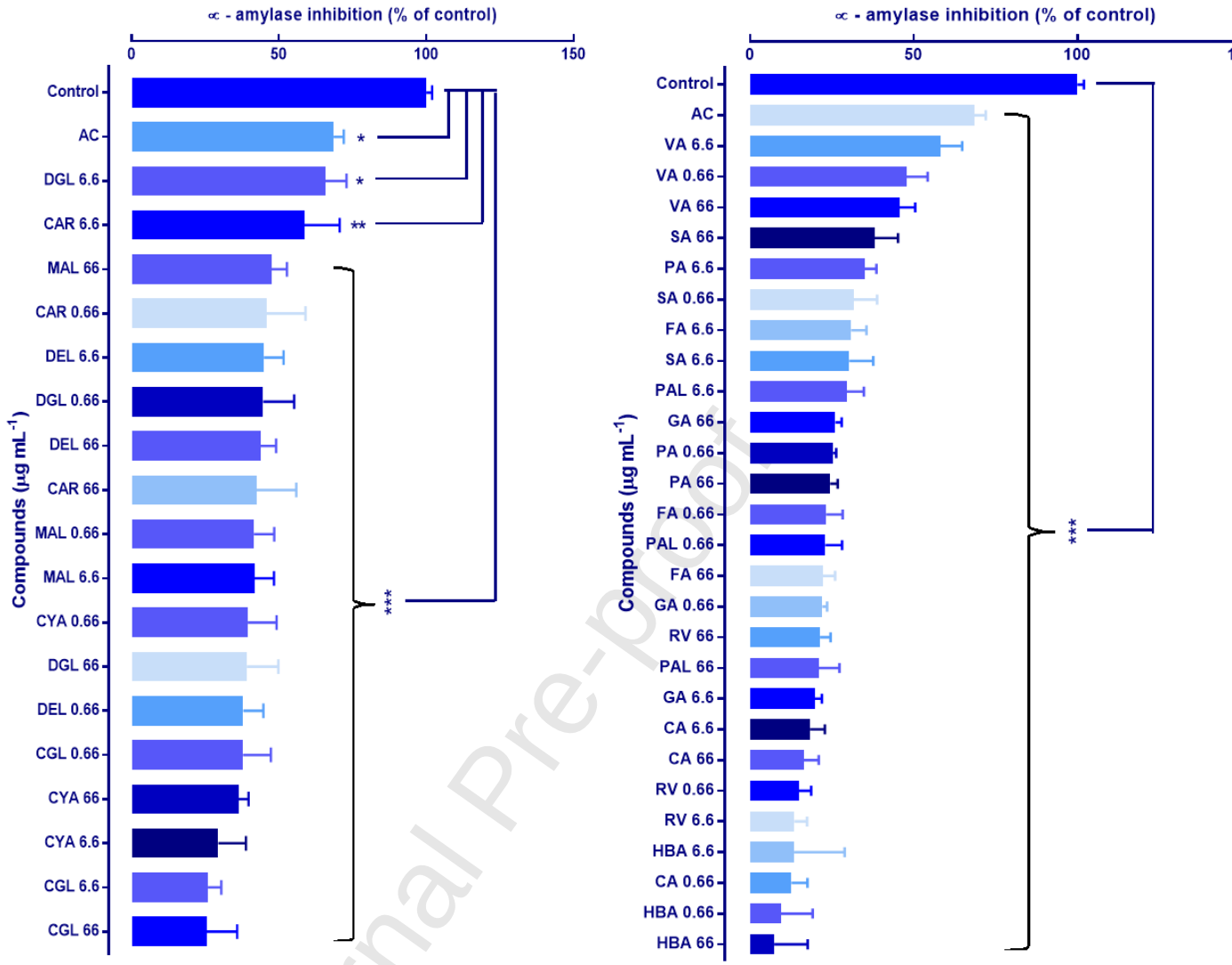
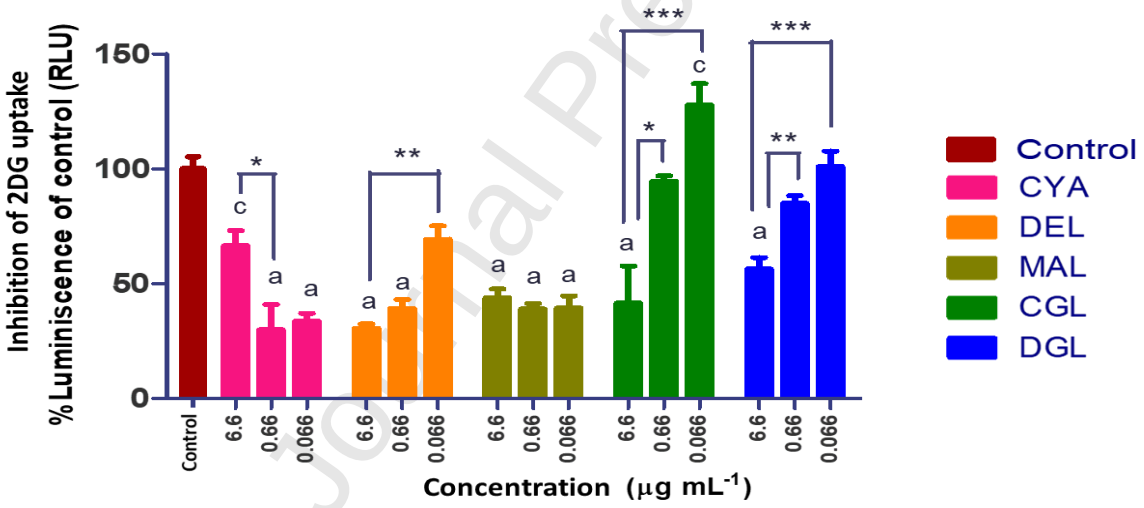


Fig. 3. Effect of selected dietary phenolics on human salivary α-amylase, (A) Anthocyanins and anthocyanidins, (B) Other phenolics

Impact of dietary phenolics on the inhibition of human salivary α-amylase at three different concentrations (66, 6.6, 0.66 µg/mL). Enzyme inhibition were determined by percentage inhibition against control (100% α-amylase activity). Samples were run in triplicates and the data is expressed as ± SEM of at least three independent experiments. Statistical analysis was done by one-way analysis of variance of Dunnet's multiple comparison tests using GraphPad prism 7.0 for Windows 10.

Control: 100% α-amylase activity, AC: Acarbose. (I) Flavonoids; CYA: Cyanidin, DEL: Delphinidin, MAL: Malvidin, CGL: Cyanidin-3-glucoside, DGL: Delphinidin-3-glucoside, CAR: Cyanidin-3-arbinoside. (II) Phenolics: PA: Protocatechuic Acid, VA: Vanillic acid, PAL: Protocatechuic aldehyde, FA: Ferulic Acid, HBA: 4 hydroxybenzaldehyde, CA: Chlorogenic Acid, GA: Gallic Acid, SA, Syringic acid, RV: Resveratrol



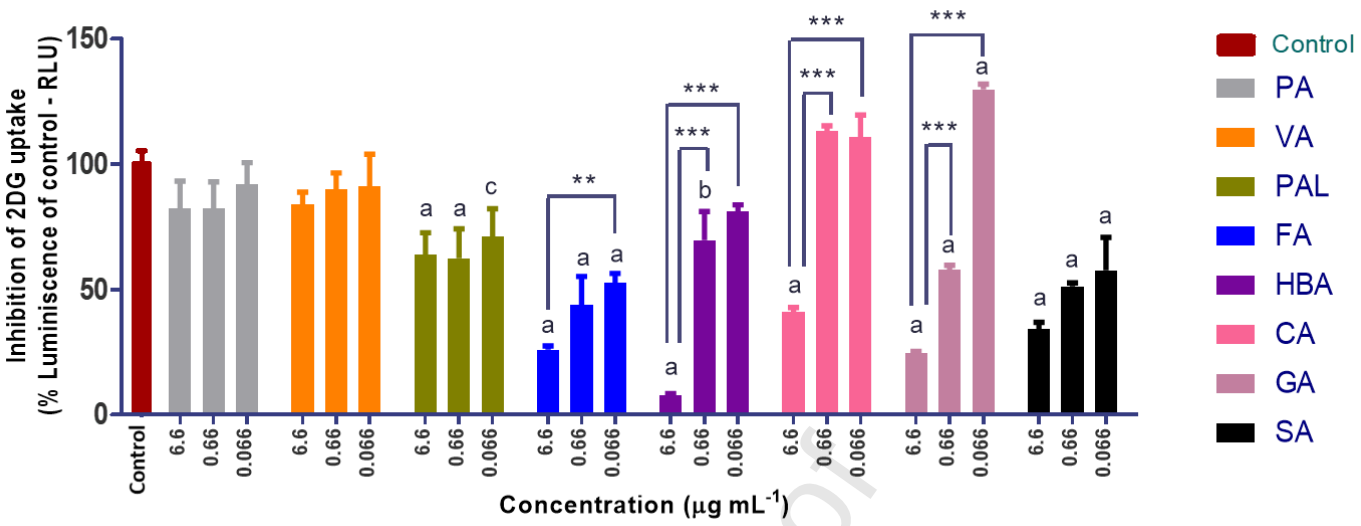
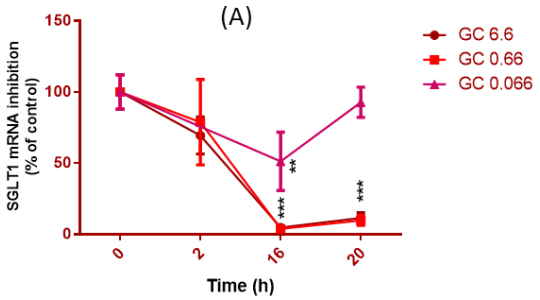
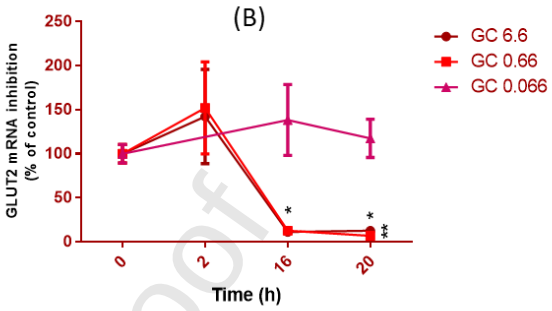


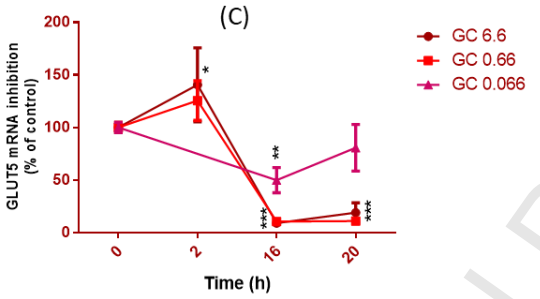
Fig. 4. Impact on 2DG uptake in CaCo-2 cells on treatment exposure with targeted individual dietary phenolics, (A) Anthocyanins and anthocyanidins, (B) Other phenolics

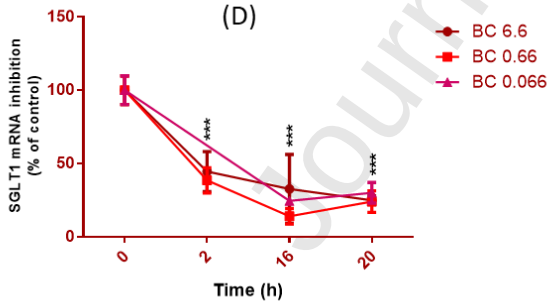
Inhibition of 2DG uptake shown by selected dietary phenolics at three different concentrations (6.6, 0.66 and 0.066 µg/mL). Percentage inhibition of 2DG uptake was calculated against cells (control) without any treatment incubation. CaCo-2 cells of post-confluent 21 days were assayed after overnight serum starvation following the protocol of 2DG uptake using a Promega Glucose Uptake-Glo™ Assay kit. Data represented are ± SEM of three independent experiments. Statistical significance was calculated by Two-way analysis of variance of Tukey's multiple comparison tests for comparison among groups and One-way analysis of variance of Dunnet's multiple comparison test for comparison of each concentration against a single control.
a=P<0.001, **b**=P<0.01, **c**=P<0.05 are statistically significant from the untreated control.
Statistical significance between the concentrations and within the same group are indicated by asterisk's, ***=P<0.001, **=P<0.01, *=P<0.05

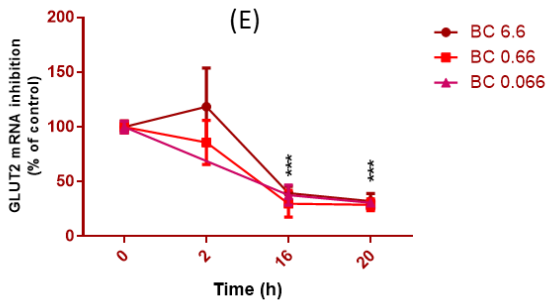
CYA (Cyanidin), DEL (Delphinidin), MAL (Malvidin), CGL (Cyanidin-3-glucoside), DGL (Delphinidin-3-glucoside), MGL (Malvidin-3-glucoside), PA (Protocatechuic acid), VA (Vanillic acid), PAL (Protocatechuic aldehyde), HVA (Homovanilic acid), FA (Ferulic acid), 4HBA (4-hydroxybenzaldehyde), HA (Hipuric acid), CA (Chlorogenic acid), GA (Gallic acid), SA (Syringic acid).











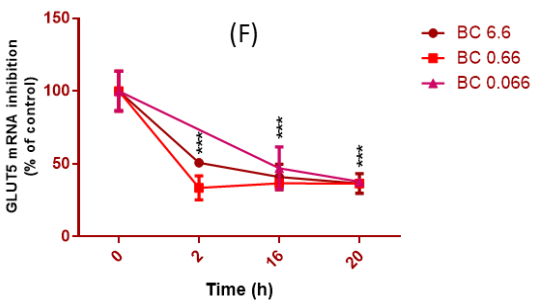


Fig. 5. Impact of freeze-dried green and black currants on the mRNA expression of glucose (SGLT1 and GLUT2) and fructose (GLUT5) transporters.

CaCo-2 cells were treated with freeze-dried currant extracts at different concentrations (6.6, 0.66 and 0.066 μg/mL) for three time-point incubations (2, 16, and 20 h). Target genes were normalised to levels of B2M as housekeeping gene. Green currants and black currants showing effects on the mRNA expressions of (**A, D**) SGLT1 (**B, E**) GLUT2 and (**C, F**) and GLUT5. Data are presented as mean percentage inhibition (relative to the control group) ± SEM (n = 9) and is analysed through One-way ANOVA's Dunnett's multiple comparison tests compared with the control group. *P<0.05, **P<0.01 and ***P<0.0001

GC: Green currants, BC: Black currants

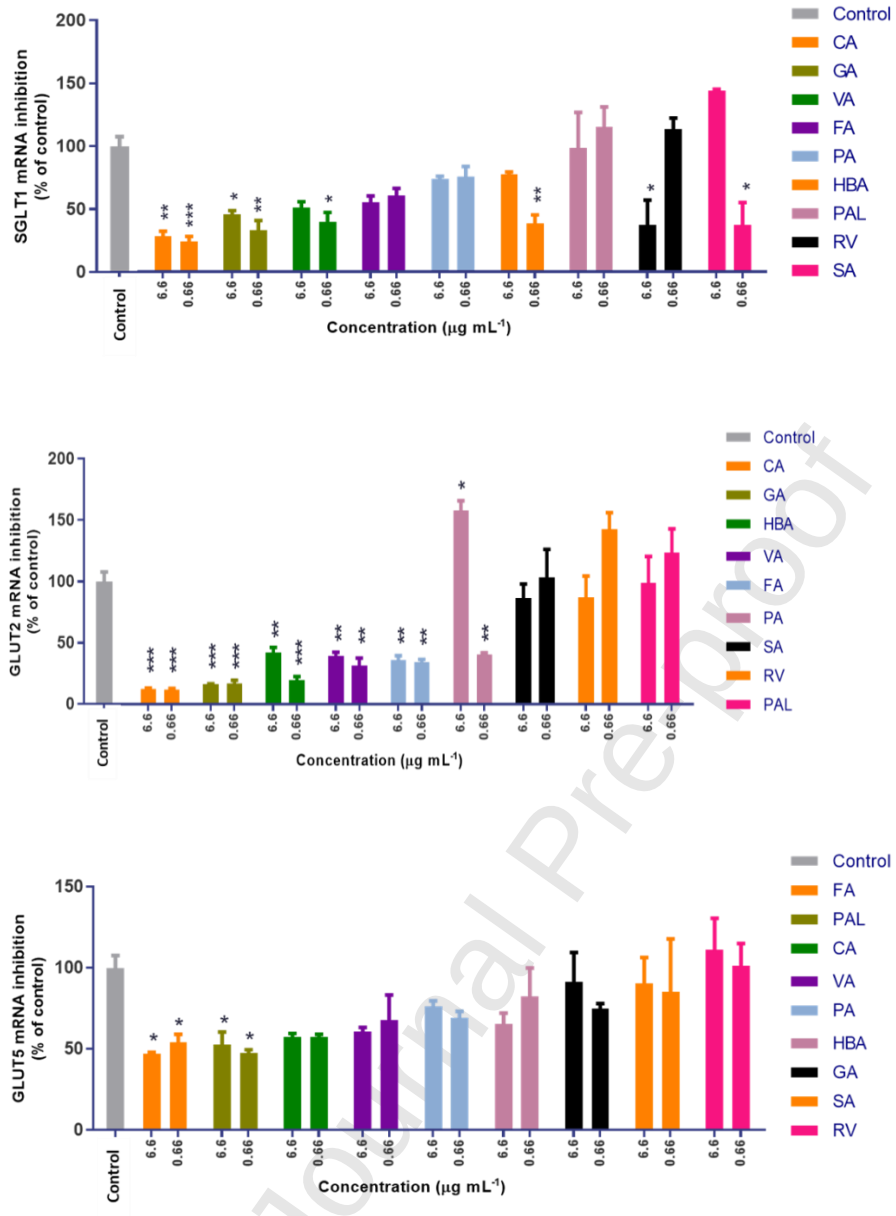


Fig. 6. Impact of individual dietary phenolics on the mRNA expression of glucose (SGLT1 and GLUT2) and fructose (GLUT5) transporters.

CaCo-2 cells were treated with dietary phenolics independently at two concentrations (6.6 and 0.66 µg/mL) for 16 h. Target genes were normalised to levels of B2M as housekeeping gene. Dietary phenolics showing effects on the mRNA expressions of (**A**) SGLT1 (**B**) GLUT2 and (**C**) and GLUT5. Compounds are shown according to their linear trend of inhibition. Data are presented as mean percentage inhibition (relative to the control group) ± SEM (n = 9) and is analysed through One-way ANOVA's Dunnett's multiple comparison tests compared with the control group. *P<0.05, **P<0.01 and ***P<0.0001.

Supplementary data:

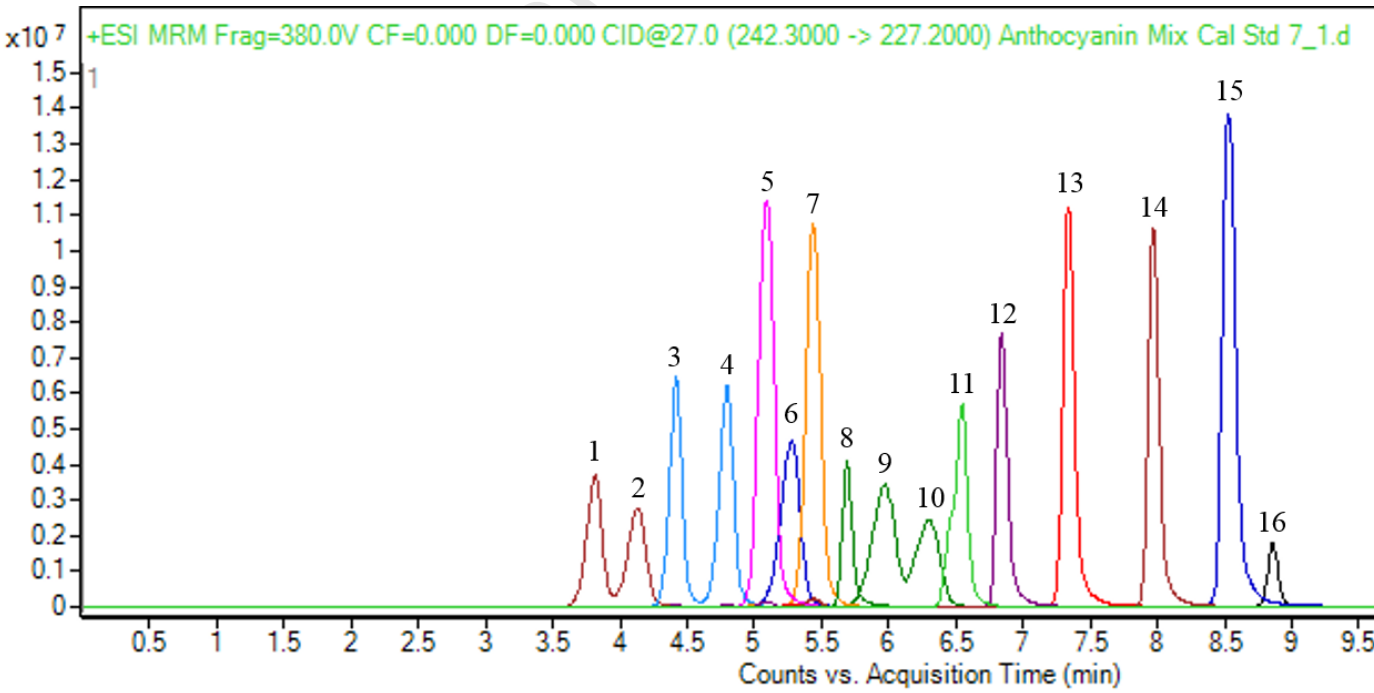
Supplemental Table 1. Transitions and analytical parameters used for the optimization of each analyte in LC-MS/MS

Compound Name	Retention Time (min)	Precursor Ion	Product Ion	Collision Energy	Polarity
Cyanidin 3-*O*-galactoside	4.39	449.1	287.2	20	Positive
Cyanidin 3-*O*-glucoside	4.75	449.1	287.2	20	Positive
Cyanidin 3-*O*-arabinoside	5.06	419.1	287.1	24	Positive
Delphinidin 3-*O*-galactoside	3.75	465.1	303.1	16	Positive
Delphinidin 3-*O*-glucoside	4.05	465.1	303.1	19	Positive
Malvidin 3-*O*-galactoside	5.93	493.1	331.1	28	Positive
Malvidin 3-*O*-glucoside	6.25	493.1	331.1	20	Positive
Petunidin 3-*O*-glucoside	5.24	479.1	317.1	24	Positive
Pelargonidin 3-*O*-glucoside	5.43	433.1	271.1	16	Positive
Cyanidin	6.85	287.1	137.1	36	Positive
Delphinidin	5.68	303.1	229.1	35	Positive
Malvidin	8.87	331.1	315	28	Positive
Petunidin	7.34	317.1	302	22	Positive
Pelargonidin	7.99	271.1	121.1	36	Positive
Peonidin	8.54	301.1	286.1	21	Positive
4,7,8-TriMeIQx (IS)	6.60	242.3	227.2	27	Positive

Supplemental Table 2. Estimated IC50 (effective inhibitory concentrations) of individual targeted dietary phenolics identified from the black and green currants and evaluated at three different concentrations (66, 6.6, 0.66 µg/mL) on the inhibition of human salivary α-amylase.

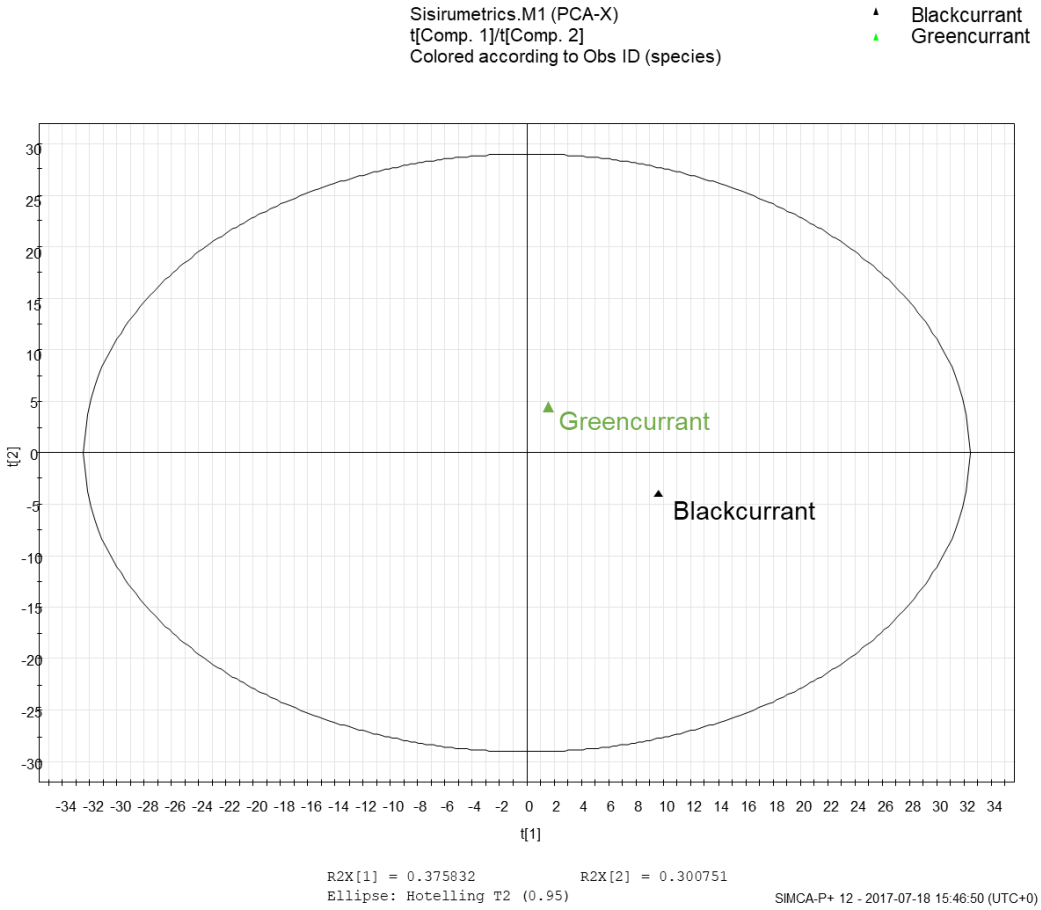
Sl. no.	Dietary phenolics from black and green currants	Estimated IC50 (µg mL^{-1})
	Anthocyanins and anthocyanidins	
1	Cyanidin	0.5
2	Delphinidin	0.5
3	Malvidin	16.4
4	Cyanidin-3-glucoside	2.6
5	Delphinidin-3-glucoside	41.8
6	Cyanidin-3-arabinoside	56.9
	Other phenolics	

1	Protocatechuic acid	29.9
2	Vanillic acid	6.0
3	Protocatechuic aldehyde	23.9
4	Ferulic acid	0.4
5	4-hydroxybenzaldehyde	0.4
6	Chlorogenic acid	0.6
7	Gallic acid	32.8
8	Syringic acid	31.3
9	Resveratrol	30.7



(1) Delphinidin galactoside: 3.75', (2) Delphinidin glucoside: 4.05', (3) Cyanidin galactoside: 4.39', (4) Cyanidin gluco
5.06', (6) Petunidin glucoside: 5.24', (7) Pelargonidin glucoside: 5.43', (8) Delphinidin: 5.68', (9) Malvidin galactoside
6.25', (11) 4,7,8-TriMelQx IS: 6.60' , (12) Cyanidin: 6.85', (13) Petunidin: 7.34', (14) Pelargonidin: 7.99', (15) Peonidi

Supplemental Fig. 1. LC-MS/MS chromatograms of internal standard, targeted anthocyanidins and anthocyanins at a concentration of 500 pg/µL.



Supplementary Fig. 2. Principal Component analysis (unit-variance-scaled) plot of the t[1] and t[2] axes, showing discrimination based on all the phytochemicals measured from green and black currants.

References:

[1] Kaiser AB, Zhang N, Der Pluijm W, V.A.N. Global Prevalence of Type 2 Diabetes over the Next Ten Years (2018-2028). Diabetes. 2018;67:202.

[2] Hu FB. Globalization of Diabetes The role of diet, lifestyle, and genes. Diabetes Care. 2011;34:1249-1257.

[3] Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: A review of current trends. Oman Med J. 2012;27:269-273.

[4] Castro-Acosta ML, Stone SG, Mok JE, Mhajan RK, Fu C-, Lenihan-Geels GN, Corpe CP, Hall WL. Apple and blackcurrant polyphenol-rich drinks decrease postprandial glucose, insulin and incretin response to a high-carbohydrate meal in healthy men and women. J Nutr Biochem. 2017;49:53-62.

[5] Castro-Acosta ML, Smith L, Miller RJ, McCarthy DI, Farrimond JA, Hall WL. Drinks containing anthocyanin-rich blackcurrant extract decrease postprandial blood glucose, insulin and incretin concentrations. J Nutr Biochem. 2016;38:154-161.

[6] Johnson MH, Lucius A, Meyer T, de Mejia EG. Cultivar Evaluation and Effect of Fermentation on Antioxidant Capacity and in Vitro Inhibition of alpha-Amylase and alpha-Glucosidase by Highbush Blueberry (Vaccinium corombosum). J Agric Food Chem. 2011;59:8923-8930.

[7] Boath AS, Stewart D, McDougall GJ. Berry components inhibit alpha-glucosidase in vitro: Synergies between acarbose and polyphenols from black currant and rowanberry. Food Chem. 2012;135:929-936.

[8] Alzaid F, Cheung H, Preedy VR, Sharp PA. Regulation of Glucose Transporter Expression in Human Intestinal Caco-2 Cells following Exposure to an Anthocyanin-Rich Berry Extract. PLoS One. 2013;8:e78932.

[9] Williamson G. Possible effects of dietary polyphenols on sugar absorption and digestion. Molecular Nutrition & Food Research. 2013;57:48-57.

[10] Hanhineva K, Torronen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkanan H, Poutanen K. Impact of Dietary Polyphenols on Carbohydrate Metabolism. International Journal of Molecular Sciences. 2010;11:1365-1402.

[11] Liao D, Zhao J, Gregersen H. Gastrointestinal tract modelling in health and disease. World journal of gastroenterology. 2009;15:169-176.

[12] Puls W, Krause HP, Muller L, Schutt H, Sitt R, Thomas G. Inhibitors of the rate of carbohydrate and lipid absorption by the intestine. Int J Obes. 1984;8:181-190.

[13] Kellett GL, Brot-Laroche E. Apical GLUT2: A major pathway of intestinal sugar absorption. Diabetes. 2005;54:3056-3062.

[14] Kellett GL, Brot-Laroche E, Mace OJ, Leturque A. Sugar absorption in the intestine: The role of GLUT2. Annu Rev Nutr. 2008;28:35-54.

[15] Satoh T, Igarashi M, Yamada S, Takahashi N, Watanabe K. Inhibitory effect of black tea and its combination with acarbose on small intestinal a-glucosidase activity. J Ethnopharmacol. 2015;161:147-155.

[16] Scow JS, Tavakkolizadeh A, Zheng Y, Sarr MG. Acute "adaptation" by the small intestinal enterocyte: A posttranscriptional mechanism involving apical translocation of nutrient transporters. Surgery (USA). 2011;149:601-605.

[17] Chaudhry RM, Scow JS, Madhavan S, Duenes JA, Sarr MG. Acute Enterocyte Adaptation to Luminal Glucose: A Posttranslational Mechanism for Rapid Apical Recruitment of the Transporter GLUT2. J Gastrointest Surg. 2012;16:312-319.

[18] Burant CF. Fructose transporter in human spermatozoa and small intestine is GLUT5. J Biol Chem. 1992;267:14523-14526.

[19] Patel C, Douard V, Yu S, Gao N, Ferraris RP. Transport, metabolism, and endosomal trafficking-dependent regulation of intestinal fructose absorption. The FASEB Journal. 2015;29:4046-4058.

[20] Etxeberria U, Laura de la Garza A, Campion J, Alfredo Martinez J, Milagro FI. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. Expert Opin Ther Targets. 2012;16:269-297.

[21] Wang H, Liu T, Huang D. Starch Hydrolase Inhibitors from Edible Plants. Adv Food Nutr Res. 2013;70:103-136.

[22] Amarowicz R, Troszynska A, Shahidi F. Antioxidant activity of almond seed extract and its fractions. Journal of Food Lipids. 2005;12:344-358.

[23] Fujisawa T, Ikegami H, Inoue K, Kawabata Y, Ogihara T. Effect of two alpha-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. Metabolism-Clinical and Experimental. 2005;54:387-390.

[24] Shobana S, Sreerama YN, Malleshi NG. Composition and enzyme inhibitory properties of finger millet (Eleusine coracana L.) seed coat phenolics: Mode of inhibition of alpha-glucosidase and pancreatic amylase. Food Chem. 2009;115:1268-1273.

[25] Hiroyuki F, Tomohide Y, Kazunori O. Efficacy and safety of Touchi Extract, an alpha-glucosidase inhibitor derived from fermented soybeans, in non-insulin-dependent diabetic mellitus. J Nutr Biochem. 2001;12:351-356.

[26] McDougall GJ, Kulkarni NN, Stewart D. Current developments on the inhibitory effects of berry polyphenols on digestive enzymes. Biofactors. 2008;34:73-80.

[27] Hoggard N, Cruickshank M, Moar K, Bestwick C, Holst JJ, Russell W, Horgan G. A single supplement of a standardised bilberry (Vaccinium myrtillus L.) extract (36 % wet weight

anthocyanins) modifies glycaemic response in individuals with type 2 diabetes controlled by diet and lifestyle. Journal of nutritional science. 2013;2:e22.

[28] Wedick NM, Pan A, Cassidy A, Rimm EB, Sampson L, Rosner B, Willett W, Hu FB, Sun Q, van Dam RM. Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. Am J Clin Nutr. 2012;95:925-933.

[29] Manzano S, Williamson G. Polyphenols and phenolic acids from strawberry and apple decrease glucose uptake and transport by human intestinal Caco-2 cells. Mol Nutr Food Res. 2010;54:1773-1780.

[30] Bornsek SM, Ziberna L, Polak T, Vanzo A, Ulrih NP, Abram V, Tramer F, Passamonti S. Bilberry and blueberry anthocyanins act as powerful intracellular antioxidants in mammalian cells. Food Chem. 2012;134:1878-1884.

[31] Fleet J, Wang L, Vitek O, Craig B, Edenberg H. Gene expression profiling of Caco-2 BBe cells suggests a role for specific signaling pathways during intestinal differentiation. Physiol Genomics. 2003;13:57-68.

[32] Cai H, Thomasset SC, Berry DP, Garcea G, Brown K, Steward WP, Gescher AJ. Determination of anthocyanins in the urine of patients with colorectal liver metastases after administration of bilberry extract. Biomed Chromatogr. 2011;25:660-663.

[33] Kaliora AC, Kanellos PT, Kalogeropoulos N. Gallic acid bioavailability in humans. In: Anonymous Handbook on Gallic Acid: Natural Occurrences, Antioxidant Properties and Health Implications. ; 2013. pp. 301-312.

[34] Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: Twenty-something years on. Nat Protoc. 2006;1:581-585.

[35] Multari S, Neacsu M, Scobbie L, Cantlay L, Duncan G, Vaughan N, Stewart D, Russell WR. Nutritional and Phytochemical Content of High-Protein Crops. J Agric Food Chem. 2016;64:7800-7811.

[36] Neacsu M, Vaughan N, Raikos V, Multari S, Duncan GJ, Duthie GG, Russell WR. Phytochemical profile of commercially available food plant powders: their potential role in healthier food reformulations. Food Chem. 2015;179:159-169.

[37] Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, Carriere F, Boutrou R, Corredig M, Dupont D, Dufour C, Egger L, Golding M, Karakaya S, Kirkhus B, Le Feunteun S, Lesmes U, Macierzanka A, Mackie A, Marze S, McClements DJ, Menard O, Recio I, Santos CN, Singh RP, Vegarud GE, Wickham MSJ, Weitschies W, Brodkorb A. A standardised static in vitro digestion method suitable for food - an international consensus. Food Funct. 2014;5:1113-1124.

[38] Ghosh D, Konishi T. Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. Asia Pac J Clin Nutr. 2007;16:200-208.

[39] Hertog MGL, Hollman PCH, Hertog MGL, Katan MB. Content of Potentially Anticarcinogenic Flavonoids of 28 Vegetables and 9 Fruits Commonly Consumed in the Netherlands. J Agric Food Chem. 1992;40:2379-2383.

[40] Maatta K, Kamal-Eldin A, Torronen R. Phenolic compounds in berries of black, red, green, and white currants (Ribes sp.). Antioxidants & Redox Signaling. 2001;3:981-993.

[41] Slimestad R, Solheim H. Anthocyanins from black currants (Ribes nigrum L.). J Agric Food Chem. 2002;50:3228-3231.

[42] Mattila PH, Hellstrom J, Karhu S, Pihlava J, Vetelainen M. High variability in flavonoid contents and composition between different North-European currant (Ribes spp.) varieties. Food Chem. 2016;204:14-20.

[43] Schantz M, Berg S, Betz M, Kulozik U, Leick S, Rehage H, Schwarz K, Baum M, Richling E. Triggered Gastrointestinal Release of Anthocyanins from Bilberries (Vaccinium myrtillus L.). X International Symposium on Vaccinium and Other Superfruits. 2014;1017:381-386.

[44] Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Med Cell Longev. 2009;2:270-278.

[45] Hassimotto NMA, Genovese MI, Lajolo FM. Absorption and metabolism of cyanidin-3-glucoside and cyanidin-3-rutinoside extracted from wild mulberry (Morus nigra L.) in rats. Nutr Res. 2008;28:198-207.

[46] Tsuda T, Horio F, Osawa T. Absorption and metabolism of cyanidin 3-O-ß-D-glucoside in rats. FEBS Lett. 1999;449:179-182.

[47] Welsch C, Lachance P, Wasserman B. Dietary Phenolic-Compounds - Inhibition of Na+-Dependent D-Glucose Uptake in Rat Intestinal Brush-Border Membrane-Vesicles. J Nutr. 1989;119:1698-1704.

[48] Hossain SJ, Kato H, Aoshima H, Yokoyama T, Yamada M, Hara Y. Polyphenol-induced inhibition of the response of Na+/glucose cotransporter expressed in Xenopus oocytes. J Agric Food Chem. 2002;50:5215-5219.

[49] Walton MC, McGhie TK, Reynolds GW, Hendriks WH. The flavonol quercetin-3-glucoside inhibits cyanidin-3-glucoside absorption in vitro. J Agric Food Chem. 2006;54:4913-4920.

[50] Luna-Vital D, Weiss M, Gonzalez dM. Anthocyanins from Purple Corn Ameliorated Tumor Necrosis Factor-α-Induced Inflammation and Insulin Resistance in 3T3-L1 Adipocytes via Activation of Insulin Signaling and Enhanced GLUT4 Translocation. Mol Nutr Food Res. 2017;61:1700362.

[51] Chen Z, Li W, Guo Q, Xu L, Santhanam RK, Gao X, Chen Y, Wang C, Panichayupakaranant P, Chen H. Anthocyanins from dietary black soybean potentiate glucose uptake in L6 rat skeletal muscle cells via up-regulating phosphorylated Akt and GLUT4. Journal of Functional Foods. 2019;52:663-669.

[52] Lavelli V, Sri Harsha PSC. Microencapsulation of grape skin phenolics for pH controlled release of antiglycation agents. Food Res Int. 2019; 119:822-828