1 **Anthocyanin-rich extract from purple potatoes decreases postprandial glycemic response**
2 **and affects inflammation markers in healthy men**

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23 Abbreviated running title: Anthocyanin-rich purple potato extract decreases glycemia

24 **Highlights**

25 - Purple potato extract contained acylated anthocyanins and hydroxycinnamic acids
26 - The potato extract reduced postprandial blood glucose and insulin peaks and iAUC
27 - The hypoglycemic effect was seen in 17 healthy men after a high carbohydrate meal
28 - Acute effects were seen on some of the 90 inflammation markers studied in plasma
29 - Purple potato phenolics increased FGF-19 levels after a high carbohydrate meal

30

31 **Abstract**

32 Our recent clinical study suggested that polyphenol-rich purple potatoes lowered postprandial 33 glycemia and insulinemia compared to yellow potatoes. Here, 17 healthy male volunteers 34 consumed yellow potatoes with or without purple potato extract (PPE, extracted with 35 water/ethanol/acetic acid) rich in acylated anthocyanins (152 mg) and other phenolics (140 mg) 36 in a randomized cross-over trial. Ethanol-free PPE decreased the incremental area under the 37 curve for glucose ($p = 0.019$) and insulin ($p = 0.015$) until 120 min after the meal, glucose at 38 20 min ($p = 0.015$) and 40 min ($p = 0.004$), and insulin at 20 min ($p = 0.003$), 40 min ($p =$ 39 0.004) and 60 min ($p = 0.005$) after the meal. PPE affected some of the studied 90 inflammation 40 markers after meal; for example insulin-like hormone FGF-19 levels were elevated at 240 min 41 ($p=0.001$). These results indicate that PPE alleviates postprandial glycemia and insulinemia, 42 and affects postprandial inflammation.

43

44 **Keywords:** acylated anthocyanins; phenolics; purple-fleshed potatoes; postprandial state; 45 clinical intervention; glycemia; insulinemia; inflammation markers

46

47 **1 Introduction**

48 High blood glucose level is a risk factor for several metabolic disorders. Especially repetitive,
49 oscillating blood glucose peaks lead to oxidative stress preceding these disorders and are shown
50 to be even more deleterious than high average blood glucose both in healthy and diabetic
51 volunteers (Ceriello et al., 2008). As most of a day is spent in a postprandial state, controlling
52 blood glucose through everyday lifestyle is inevitable for maintaining health. The polyphenolic
53 blue and red colorants of various berries and fruits, the anthocyanins, and various anthocyanin-
54 rich foods, have been suggested to decrease postprandial glucose and/or insulin responses. This
55 has been seen both in healthy (Bell, Lamport, Butler, & Williams, 2017; Castro-Acosta et al.,
56 2016) and diabetic (Hoggard et al., 2013) volunteers after consumption of anthocyanin-rich
57 berries.

58 Red and purple potatoes provide a rich source of anthocyanins and other polyphenols easy to
59 adopt to an everyday diet. Anthocyanins in potatoes are composed of mainly glycosides of
60 cyanidin and pelargonidin (red varieties) or petunidin, peonidin and malvidin (purple varieties).
61 The glycosides are acylated to phenolic acids, such as *p*-coumaric acid, caffeic acid and ferulic
62 acid. In addition, coloured potatoes are rich in other phenolic compounds, such as chlorogenic
63 acid and hydroxycinnamic acids. (Giusti, Polit, Ayvaz, Tay, & Manrique, 2014; Ieri, Innocenti,
64 Andrenelli, Vecchio, & Mulinacci, 2011)

65 However, studies on the impact of acylated anthocyanins on postprandial state are still scarce,
66 and the findings have been somewhat controversial. Moser et al., 2018 reported a moderate
67 decrease of blood glucose in healthy subjects after consuming purple potato chips compared to
68 white potato chips, suggesting modulating effects of phenolics of purple potatoes on glycemia.
69 On the other hand, Ramdath et al., 2014 did not find a statistically significant difference in the
70 glycemic response in healthy men after one meal of purple, yellow or white potatoes, but the

glycemic index of the potatoes was seen to be negatively correlated to the polyphenolic content of the potato variety. In animal models, purple potatoes have been documented to lower blood glucose and cholesterol in diabetic rats (Choi, Park, Eom, & Kang, 2013) and to enhance glucose tolerance in obese Zucker rats when compared to white potatoes (Ayoub et al., 2017).

In our recent study (Linderborg et al., 2016) we found that a meal prepared from a purple potato variety (*Solanum tuberosum* L. 'Synkeä Sakari') rich in acylated petunidin and peonidin glycosides lowered postprandial glycemia and insulinemia compared to the control meal prepared from a yellow cultivar (*S. tuberosum* L. 'Van Gogh') in healthy men. In order to remove the effect of different potato varieties on postprandial metabolism in this follow-up study, anthocyanins of Synkeä Sakari were extracted with an aqueous 20 vol-% ethanol solution containing 7 vol-% of acetic acid and purified (Heinonen et al., 2016). A clinical trial was organized to investigate the effect of yellow-fleshed potatoes with and without the addition of the purple potato extract (PPE) rich in acylated anthocyanins on glycemia, insulinemia and inflammation markers in the postprandial state in healthy men. It was hypothesized that PPE lowers the highest blood glucose and insulin peaks and the area under the glucose and insulin concentration curves.

2 Materials and methods

2.1 Clinical nutrition study

2.1.1 Ethics

The study protocol was accepted by the Ethical Committee of the Hospital District of Southwest Finland. The intervention was conducted according to the Declaration of Helsinki, and registered at clinicaltrials.gov as NCT02940080. Each study subject provided their written informed consent.

2.1.2 Study participants

Seventeen healthy men aged between 18 and 45 years from the area of Turku, Finland, participated in the study. At the screening visit, a health interview was conducted, and the body mass index (BMI, 18.5–27 kg/m^2) and blood pressure (<140/80 mmHg) were measured. The volunteers were asked to participate in a fasting-state blood test in the laboratory of the Hospital District of Southwest Finland. The participants were included to the study if the test results were within the following reference values: glucose 4–6 mmol/L, alanine aminotransferase <60 U/L, creatinine <118 µmol/L, thyrotropin 0.4–4.5 mU/L, cholesterol <5.5 mmol/L, triglycerides <2.6 mmol/L and hemoglobin 130–155 g/L. The study participants were non-smokers without regular medication, and they had not participated in other clinical trials or donated blood within two months before the first intervention visit.

2.1.3 Study design

A single-blinded, cross-over study with two potato meals and a wash-out time of at least two weeks was organized. The study participants were asked to refrain from exercise and to consume only foods and drinks low in flavonoids and dietary fiber 48 hours before and 24 hours after the study meal to decrease the effect of baseline diet on their metabolism and digestion. Details on the allowed diet is provided in the Supplementary material (S1). After 12 hours of overnight fasting, the study participants consumed mashed yellow-fleshed potatoes with or without PPE and 300 mL of drinking water as breakfast. Venous blood was collected into lithium-heparin tubes at fasting state, and 20, 40, 60, 90, 120, 180 and 240 minutes after the study meal. Plasma was separated from the blood by centrifugation at 1,500 × g for 15 minutes.

2.1.4 Preparation of the meals

Floury yellow-fleshed potatoes (*Solanum tuberosum* L. 'Afra') were cultivated by Veljekset Kitola Oy, Nousiainen, Finland, and obtained simultaneously from a local grocery store. The purple-fleshed potatoes (*S. tuberosum* L. 'Synkeä Sakari') used for the anthocyanin extraction were cultivated in Kokemäki and Muhos, Finland. The anthocyanins were extracted in the LUT university from 19 kg of purple potatoes using aqueous 20 vol-% ethanol solution containing 7 vol-% of acetic acid and then further purified resulting in 1.2 L of PPE as described by Heinonen et al., 2016.

For the yellow potato portions, the yellow potatoes were washed carefully, cut in half and steam-cooked with peels for 25 minutes (0.7 mL/g of cooking water to fresh weight of potatoes). The cooked potatoes were mashed with a hand-held electric mixer, carefully homogenized and divided into portions. In total, each meal contained 350 g of cooked potatoes with peels and all remaining cooking water (110.9 g). The meals were stored at –18 °C.

In the yellow potato portion, two meal additives were used: 30 mL of PPE (corresponding to extract from 0.48 kg of fresh purple potatoes) was added to produce the study meal, and 30 mL of water was added to prepare the control meal. As PPE originally contained acetic acid (Heinonen et al., 2016) and the sensory properties of the extract needed enhancement, the pH of the two additives was adjusted to 4 by adding 9.1 mmol of acetic acid in the form of synthetic vinegar (Maustaja, Pyhäntä, Finland) to the control meal additive, and by adding 9.5 mmol and 1.7 mmol of food-grade sodium hydroxide (J.T.Baker, Deventer, Holland) to the study meal additive and the control meal additive, respectively. The amount of sodium was standardized between the meals by adding 0.4 g of sodium chloride into the control meal additive. After these additions, the total volume of the study meal and the control meal additives was 40 mL

145 per meal. The meal additives were stored at –18 °C. Prior to the clinical intervention, a yellow
146 potato portion and a meal additive were taken to a refrigerator to melt overnight. In the
147 morning, the yellow potato portion was heated using a microwave and left to cool down to
148 room temperature. Then, either the study or the control meal additive was added to the yellow
149 potato portion with 10 mL of additional water used to transfer all the residue meal additive
150 from the falcon tube to the meal.

151

152 2.2 Blood biomarkers

153 The plasma glucose and insulin concentrations were analysed in the laboratory of the Hospital
154 District of Southwest Finland as previously described (Linderborg et al., 2016). Using the
155 trapezoidal rule, the incremental areas under the glucose and insulin concentration curves
156 (abbreviated as iAUC) after each meal were calculated until the glucose and insulin levels
157 reached the fasting level. Furthermore, a total of 92 inflammation markers, listed in Table 3,
158 were analysed using cDNA multiplex immunoassay and qPCR giving semi-quantitative results
159 on a log2 scale (the Inflammation panel, Olink Proteomics, Uppsala, Sweden) from the plasma
160 samples collected at the fasting state and 240 min postprandially. Data for two inflammation
161 markers (brain-derived neurotrophic factor and interleukin 1α) were excluded due to technical
162 issues.

163 2.3 Statistical analyses

164 Power calculations for required sample size were based on the results obtained in our previous
165 study (Linderborg et al., 2016). Statistical power and effect size were calculated for significant
166 effect of added PPE extract (smaller postprandial plasma glucose in comparison to yellow
167 potato meal; t-test, $p<0.05$) using the G*power software (version 3.1.9). The obtained values

168 were utilized to calculate the number of volunteers needed for this postprandial test, which
169 turned out to be 15.

170 Statistical analyses were performed using the IBM SPSS Statistics 23.0 software (SPSS Inc,
171 Chicago, IL) for the glucose and insulin, and RStudio 1.1.456 (RStudio Team, 2016) with
172 Effsize package 0.7.4 (Torchiano, 2018) for the inflammation markers. The significance level
173 was set at 0.05, and the normality of the data was tested using the Shapiro–Wilk test. For
174 normally distributed data, the paired-samples T-test was conducted, and otherwise its non-
175 parametric counterpart, the Wilcoxon signed rank test, was used.

176 As the inflammation marker data required multiple comparisons, the false discovery rate (type
177 I error) was managed by calculating the effect size measures of Cohen's *d* and *r* score for the
178 parametric and non-parametric tests, respectively. The *r* score was calculated using the
179 equation $r = Z / \sqrt{N}$, in which *Z* is the test measure of the Wilcoxon signed rank test and *N* is
180 the total number of observations. The data was interpreted using the following reference values:
181 ≤ 0.2 equals to a small effect size; ≤ 0.5 to a medium effect size, and ≤ 0.8 to a large effect size.
182 The adjusted *p*-values (here, the *q*-values) were calculated using the Benjamini–Hochberg
183 method.

184

185 **2.4 Characterization of the meals**

186 **2.4.1 Materials**

187 For quantification of anthocyanins, flavonol glycosides and hydroxycinnamic acid derivatives,
188 HPLC-grade methanol and formic acid (VWR Chemicals, Radnor, PA) and hydrochloric acid
189 (J.T.Baker, Deventer, Holland) were used. For identification with LC-MS, MS-grade formic
190 acid (Honeywell, Morris Plains, NJ) and acetonitrile (VWR International, Fonteney-sous-Bois,

191 France) were used. For all analyses, MilliQ-grade water was used, except for the accurate mass
192 analyses in which LC-MS grade water (Merck, Darmstadt, Germany) was used.
193

194 **2.4.2 Nutrient and starch content of the potato portion**

195 The nutrient and starch content were analysed from the yellow-fleshed potato portion (350g of
196 cooked yellow-fleshed potatoes and 110.9 g of cooking water) without the meal additives.
197 Starch content was analysed in Eurofins Food Testing Netherlands in Heerenveen using
198 spectrophotometric analyses, and the nutrients (fat, digestible carbohydrates, protein, moisture
199 and ash) and energy were characterized as previously described (Linderborg et al., 2016).
200

201 **2.4.3 Analysis of ethanol and acetic acid in the purple potato extract**

202 As ethanol and acetic acid were used in the anthocyanin extraction and purification process
203 (Heinonen et al., 2016), their contents in PPE were analysed using gas chromatography. Three
204 replicate samples were taken from PPE and filtrated (0.45 µm, PTFE; VWR, Radnor, PA). The
205 analysis was carried out with a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-
206 Packard Co, Palo Alto, CA), a Hewlett Packard 7673 autosampler and a flame ionization
207 detector. The column was EC-WAX (30 m × 0.53 mm, 1.2 µm, Alltech, Nicholasville, KY).
208 Helium was used as a carrier gas with a total flow rate of 118.0 mL/min in split mode, of which
209 3.7 mL/min was directed to the column. The injection volume was 0.2 µL. The temperature of
210 the column oven was set at 80 °C, hold for 5 minutes, then increased 10 °C/min until 240 °C
211 and hold for 10 minutes. Quantification was performed using external standard curves prepared
212 from ethanol (Altia Plc, Rajamäki, Finland) and acetic acid (J.T.Baker, Deventer, Holland),
213 respectively.
214

215 **2.4.4 Analysis of free sugars and organic acids**

216 A representative share of the mashed yellow potato portion (350g of cooked yellow-fleshed
217 potatoes and 110.9 g of cooking water without the meal additives) was first freeze-dried for 48
218 hours. Three consecutive samples, 2 g each, of the freeze-dried mashed yellow-fleshed potato
219 portion were extracted using MQ-grade water and then derivatized using Tri-Sil reagent
220 (Pierce, Rockford, IL) as described by Linderborg et al., 2016 in detail.

221 For the gas chromatographic analyses, a GC-2010 Plus and AOC-20s autosampler (Shimadzu,
222 Kioto, Japan) were used. The samples were injected using AOC-20i autoinjector at 210 °C, and
223 the TMS derivatives were separated with the non-polar poly(dimethyl siloxane) GC column
224 SPB-1 (30 m × 0.25 mm, df 0.25 µm, Supelco, Bellefonte, PA), and detected using a flame
225 ionization detector at 290 °C. The carrier gas was helium (1.90 mL/min). The temperature of
226 the column oven was first 150 °C for 2 min, increased to 210 °C at 4 °C/min, and finally
227 increased at 40 °C/min until 275 °C, which was held for 5 minutes. The peaks of the TMS
228 derivatives were identified using the following external standard compounds: citric acid, malic
229 acid, sucrose (J.T.Baker, Deventer, Holland), ascorbic acid (VWR International, Fontenay-
230 sois-Bois, France), quinic acid (Aldrich, Steinheim, Germany), glucose, and fructose (Merck,
231 Darmstadt, Germany). Quantification was performed by comparing the analyte peak areas with
232 those of the internal standards, which were sorbitol (Sigma–Aldrich, St. Louis, MO) for sugars
233 and tartaric acid (Sigma–Aldrich Chemie Gmbh, Steinheim, Germany) for organic acids.
234 Correction factors were obtained by analysing mixtures of the reference compounds and
235 applied in quantification of each compound.

236

237 **2.4.5 Identification and quantification of anthocyanins**

238 Five consecutive samples of PPE were diluted with MeOH/HCl (99/1, v/v). Anthocyanins of
239 the yellow-fleshed potato portions (350g of cooked yellow-fleshed potatoes and 110.9 g of
240 cooking water without the meal additives) were extracted with MeOH/HCl 99/1 four times

241 from five samples of 1 g of freeze-dried mashed potatoes (Linderborg et al., 2016). The samples
242 were analysed using a high-performance liquid chromatograph LC-10AVP (Shimadzu, Kyoto,
243 Japan) equipped with LC-10AT pumps. 10 μL of a sample was injected with a SIL-10A
244 autosampler and detected at 520 nm with a SPD-M10AVP diode array detector connected with
245 a SCL-M10AVP data handling station. Anthocyanins were separated using a Kinetex Polar
246 C18 column (2.6 μm, 150 × 4.60 mm, Phenomenex, Torrance, CA) at 35°C. The elution
247 solvents consisted of formic acid, acetonitrile and water 5/3/92 (v/v, A) and 5/55/40 (v/v, B),
248 and elution gradient was as follows: 0–5min, 4–20% B; 5–30min, 20–22% B; 30–38min, 22–
249 28% B; 38–42min, 28–32% B; 42–50min, 32–35% B; 50–55min, 35–90% B; 55–58min, 90–
250 35% B; 58–62min, 4% B at flow rate 0.5 ml/min. The anthocyanins were quantified as
251 cyanidin-3-*O*-glucoside equivalents (Extrasynthese, Genay, France) using the external
252 standard method.

253 For identification, the anthocyanins were first separated with a Waters Acquity Ultra
254 Performance LC system linked to a Waters 2996 DAD detector using the chromatographic
255 method described above, after which the ions were detected with a mass spectrometer (Waters
256 Quattro Premier mass spectrometer with electrospray ionization) operating in the positive ion
257 mode. Full spectra between the mass range of *m/z* 100–1,400 were recorded using the capillary
258 voltage 0.8 kV, the cone voltage 15 V, the extractor voltage 2 V and the RF lens voltage 0.1 V.
259 The ion source temperature was 120 °C, the desolvation temperature 500 °C, the cone gas flow
260 100 L/h and the desolvation gas flow 650 L/h. Then, the product ions were followed by
261 colliding the selected precursor ions in the second quadrupole at the collision energy of 20 eV
262 and using an argon flow at 0.35 mL/min for further identification purposes. The MS data was
263 handled with the MassLynx 4.1 software (Waters, Milford, MA).

264 Furthermore, exact masses were measured using the high-resolution Bruker Impact
265 II™ UHR-QqTOF (Ultra-High Resolution Qq-Time-Of-Flight) mass spectrometry in positive

266 auto-MS/MS mode using electrospray ionization. The compounds were first separated using a
267 Bruker Elute UHPLC equipped with a HPG1300 pump and a diode array detector with the
268 same conditions stated above. The diode array detector response was collected in a range of
269 190–800 nm. The mass spectrometer parameters were set as follows: the capillary voltage 4.5
270 kV, the end plate offset 500 V, the nebulizer gas (N_2) pressure 2.0 bar, the drying gas (N_2) flow
271 8.0 L/min, and the drying gas temperature was 200 °C. The mass range was *m/z* 20 to 1,000.
272 Calibration was carried out by injecting 10 mM sodium formate with 180 µL/min flow rate
273 from a direct infusion syringe pump to the six-port valve for high-accuracy mass experiments
274 in the HPC mode. The mass measurement errors were calculated as the difference between the
275 individually measured accurate mass and the calculated exact mass, given in parts per million.
276 The instrument was controlled and the data was handled with the Compass DataAnalysis
277 software 4.4 (Bruker Daltonik GmbH, Bremen, Germany). In addition, literature was used to
278 aid in the identification (Andersen, Opheim, Aksnes, & Frøystein, 1991; Giusti et al., 2014;
279 Hillebrand, Naumann, Kitzinski, Köhler, & Winterhalter, 2009; Ieri et al., 2011).

280

281 **2.4.6 Flavonol glycosides and hydroxycinnamic acid derivatives**

282 Flavonol glycosides and hydroxycinnamic acid derivatives were extracted with a modified
283 method (Määttä, Kamal-Eldin, & Törrönen, 2001; Sandell et al., 2009). The samples were
284 prepared in triplicate by first diluting 1 mL of PPE and 1 g of the freeze-dried yellow potato
285 portion (350g of cooked yellow-fleshed potatoes and 110.9 g of cooking water without the
286 meal additives) into a total volume of 5 mL of MQ water. Then, the samples were extracted
287 using 10 mL of ethyl acetate, mixed vigorously for 1.5 min and centrifuged 1,000 × *g* for 5
288 min. The ethyl acetate supernatant was collected, and the pellet was extracted three times as
289 described. The ethyl acetate was evaporated using a rotary evaporator at 35 °C. The analytes
290 were diluted in methanol and filtered through 0.45 µm PTFE syringe filters.

291 The compounds were determined using an HPLC-DAD method described in detail by
292 Linderborg et al., 2016. A wavelength range of 190–600 nm was scanned. Absorption
293 maximum of 320 nm was used for hydroxycinnamic acids and caffeoylquinic acids, and 354
294 nm was used for flavonols and flavonol glycosides. Caffeoylquinic acid derivatives were
295 calculated as 3-caffeoylquinic acid equivalents, and other hydroxycinnamic acids were
296 calculated as caffeic acid equivalents (Sigma Aldrich, St Louis, MO). Flavonol glycosides were
297 calculated as quercetin-3-*O*-rutinoside equivalents (Extrasynthese, Genay, France).

298 Flavonol glycosides and hydroxycinnamic acid derivatives were identified by first separating
299 them using a Waters Acquity Ultra Performance LC system linked to a Waters 2996 DAD
300 detector using the chromatographic method described above, and then directing 0.4 mL of the
301 flow to the mass spectrometer (Waters Quattro Premier mass spectrometer with electrospray
302 ionization) operating both in the positive and negative ion modes. The capillary voltage was
303 3.5 kV (positive) or 3.6 kV (negative), the cone voltage 15 or 22 V, extractor voltage 2 or 4 V,
304 respectively, and RF lens voltage 0.0 V. Source temperature was 120 °C, desolvation
305 temperature 300 °C, cone gas flow 97 L/h and desolvation gas flow 600 L/h. The mass data
306 was collected between the mass range of *m/z* 130–800, and handled with the MassLynx 4.1
307 software (Waters, Milford, MA).

308 Identification was confirmed with the high-resolution UHPLC-Q-ToF-MS instrument
309 described in detail in the chapter 2.3.5. The HPLC conditions were as above, and the eluent
310 flow rate from the HPLC to the mass spectrometer was 0.2 mL/min. The flow was ionized
311 using negative electrospray ionization. The capillary voltage was 3.5 kV, the end plate offset
312 500 V, the nebulizer gas (N_2) pressure 1.4 bar, the drying gas (N_2) flow 9 L/min, the drying
313 gas temperature was 250 °C and collected mass range was *m/z* 20–1,000. The instrument was
314 controlled and the data was processed with the Compass DataAnalysis software 4.4.

315

316 ## 3 Results and discussion

317 ### 3.1 Characterization of the potato portion and meal additives

318 #### 3.1.1 Composition of the meals

319 The content of nutrients (Table 1) in the yellow potato portion (350g of cooked yellow-fleshed
320 potatoes and 110.9 g of cooking water without the meal additives) was similar as in our
321 previous study (Linderborg et al., 2016). The main sugar in the yellow potato portion without
322 the meal additives was glucose (1.4 g) and the main organic acid was citric acid (0.9 g). The
323 study meals contained additional glucose (4.4 mg) and citric acid (9.1 mg) per meal deriving
324 from the supplemented 30 mL of PPE. Both meals contained 0.7 mg of flavonol glycosides and
325 4.5 mg of hydroxycinnamic acid derivatives from the yellow-fleshed potato portion, and the
326 study meal contained an additional 152.4 mg of anthocyanins and 140.1 mg of
327 hydroxycinnamic acid derivatives from PPE. Furthermore, the study meal contained 0.8 mmol
328 of ethanol and 52.8 mmol of acetic acid derived from PPE.

329

330 #### 3.1.2 Identification of anthocyanins

331 Anthocyanins of 'Synkeä Sakari' were tentatively identified in our previous study (Linderborg
332 et al 2016). For the present study, the chromatographic separation was further improved leading
333 to an increased number of separated anthocyanin peaks (Figure 1A), of which 16 were
334 identified here based on the UV, MS and MS/MS data (Figure 1A, Table 2).

335 After detecting the molecular ions with mass spectrometry, the product ions from the selected
336 precursor ions were scanned using tandem mass spectrometry. Certain fragmentation patterns
337 were seen. Loss of 162 amu was regarded as a hexose (glucose or galactose), and 454 amu, 470
338 amu and 484 amu referred to a loss of a rutinose and an acyl group (coumaric acid, caffeic acid
339 and ferulic acid, respectively) from the precursor ions. However, mass spectrometric analyses
340 do not distinguish the structural isomerism without good liquid chromatographic separation

341 and corresponding reference compounds. Therefore, the coumaric acid was considered to be in
342 the *para* form, the hexose unit was considered to be a glucose, and the glucose was considered
343 to be bonded to the carbon 5 in the A-ring and the rutinose to the carbon 3' in the C-ring as
344 reported in the previous studies utilizing nuclear magnetic resonance spectroscopy for
345 identification of purple potato anthocyanins (Andersen et al., 1991; Hillebrand et al., 2009).

346 Six anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin)
347 were detected. The two major anthocyanins were identified as petunidin-coumaroyl-rutinoside-
348 glucoside and peonidin-coumaroyl-rutinoside-glucoside. Interestingly, the main anthocyanins
349 occurred also in acetylated forms which has not been reported in purple potatoes in literature
350 before. This may have been due to the high concentration of acetic acid in PPE. Furthermore,
351 the peak number 18 remained unidentified due to its low concentration and weak ionization.
352 As its UV spectrum showed a band I absorption maximum at 520 nm, it was tentatively
353 identified and quantified as an anthocyanin.

354

355 **3.1.3 Identification of flavonol glycosides and hydroxycinnamic acid derivatives**

356 Identification of the detected flavonol glycosides and hydroxycinnamic acid derivatives began
357 by determining the flavonoid class based on the band I absorption maxima in the UV-spectra,
358 and continued with more detailed identification using the retention times, mass spectra,
359 reference compounds when available, and literature. The compounds identified are listed in the
360 Table 2, and the peak numbering refers to the HPLC chromatograms in Figure 1B and 1C. The
361 main hydroxycinnamic acid derivatives in the yellow potato portion and PPE were 3-, 4- and
362 5-caffeoyl quinic acid isomers (chlorogenic acid, cryptochlorogenic acid and neochlorogenic
363 acid, respectively, $[M\text{-}H]^-$ at *m/z* 354) and hydroxycinnamic acids such as caffeic acid and *p*-
364 coumaric acid ($[M\text{-}H]^-$ at *m/z* 179 and 163, respectively). From the yellow potato portion,
365 quercetin-3-*O*-rutinoside ($[M\text{-}H]^-$ at *m/z* 610), a flavonol glycoside, was found. PPE did not

366 contain flavonol glycosides which may be due to the purification process of PPE after the
367 extraction.

368 PPE contained a caffeoyl quinic acid isomer ($[M-H]^-$ at *m/z* 353), of which the position of the
369 caffeoyl was not defined due to the lack of a reference compound. Furthermore, two isomers
370 of coumaroyl-rhamnosyl-hexoside ($[M-H]^-$ at *m/z* 472) and a coumaroyl-rhamnosyl-acetyl-
371 hexoside ($[M-H]^-$ at *m/z* 514) were identified with the aid of mass fragmentation and tandem
372 mass spectrometry. The structural isomerism of the two coumaroyl-rhamnosyl-hexosides may
373 be in the position of the hydroxyl group of the coumaric acid, and the hexose may be a glucose
374 or a galactose. As coumaroyl-rhamnosyl-hexosides have not been earlier detected in potatoes,
375 they may be breakdown-products of the acylated anthocyanins. Furthermore, two
376 hydroxycinnamic acid amides were found (King & Calhoun, 2005). Feruloyloctodopamine
377 ($[M-H]^-$ at *m/z* 329) was identified both from PPE and the yellow potato portion, and
378 feruloyltyramine ($[M-H]^-$ at *m/z* 313) was found only from the yellow potato portion.
379

380 **3.2 Glycemia and insulinemia**

381 Figure 2 presents the concentrations of plasma glucose (Figure 2A) and insulin (Figure 2B) at
382 the fasting and the postprandial states until 240 minutes after the study meal and the control
383 meal. The incremental area under the glucose curve until the time point of 120 minutes was
384 significantly lower compared to that of the control meal (p=0.019). Additionally, the study
385 meal caused a statistically significantly lower glucose response at 20 min and 40 min after the
386 meal compared with the control meal (p=0.015 and 0.004, respectively). At 240 min, the
387 glucose response was higher than the response at the corresponding time point after the control
388 meal (p=0.023). The iAUC120 min of insulin was significantly lower (p=0.015) after the study
389 meal (Figure 2, Supplementary material S2). The study meal caused lower plasma insulin

390 responses at 20, 40 and 60 minutes after the meal (p=0.003, 0.004, 0.005, respectively), and
391 increased it at 180 and 240 minutes (p=0.004 and 0.006, respectively).

392 Overall, the study meal modified the postprandial glycemic and insulinemic responses after the
393 meal compared to the control meal by ameliorating the steep increase in the levels of both
394 plasma glucose and insulin at 20–60 minutes. Thereafter, the decrease of both plasma glucose
395 and insulin were slowed down by the study meal.

396 Several possible pathways may have been involved in the biochemical mechanisms underlying
397 the glycemia modifying effects. Polyphenol-rich extracts from both purple and red cultivars
398 have been shown *in vitro* to decrease the activity of α-glucosidase, which breaks starch down
399 into glucose and maltose during digestion (Ramdath et al., 2014). Moser et al., 2018 reported
400 that purple potato polyphenols inhibit glucose transportation to Caco-2 intestine model cells *in*
401 *vitro*. In the comprehensive reviews by Hanhineva et al., 2010 and Williamson, 2013, it is
402 stated that polyphenols may modulate intracellular signaling pathways and gene expression
403 related to carbohydrate metabolism. Furthermore, anthocyanin metabolites and degradation
404 products resulting from gut microbiota metabolism may contribute to the health effects of these
405 compounds.

406 Acetic acid was used to lower the pH of the extraction medium in order to stabilize the potato
407 anthocyanins (Heinonen et al., 2016). The study meal additive contained 52.8 mmol of acetic
408 acid and to adjust the pH to the same value between the study meal and control meal additives,
409 9.5 mmol of sodium hydroxide was added to the study meal additive, and 9.1 mmol of acetic
410 acid and 1.7 mmol of sodium hydroxide were added to the control meal additive. Amount of
411 sodium was adjusted between the meals by adding 0.4 g of sodium chloride to the control meal
412 additive. Even though the pH of the meal additives were the same, the study meals contained
413 more acetic acid than the control meal due to the high content of acetic acid in PPE caused by

414 buffering effect of PPE. One dose of vinegar has been shown to lower postprandial glycemia
415 and insulinemia in healthy subjects in a dose-dependent manner (18, 23 and 28 mmol of acetic
416 acid) (Östman, Granfeldt, Persson, & Björck, 2005). Therefore, acetic acid may have partially
417 contributed to the postprandial effects seen in this study.
418
419 Despite the indication of hypoglycemic effect of acetic acid, the mechanism involved is not
420 clear. The effect may be connected to the inhibition of α-amylase, enhanced glucose uptake
421 and transcription factors as recently reviewed by Santos, de Moraes, da Silva, Prestes, &
422 Schoenfeld, 2019. Possibly low pH affects the enzyme activities resulting in reduced glycemic
423 response. It is worth to notice that the study designs between our study and the cited research
424 were different: in the cited research pH values were not adjusted between the meals, whereas
425 in our current study the pH of the meals was carefully adjusted to the same value to minimize
426 the potential effect of different pH on enzyme activities. Furthermore, in our previous study
427 (Linderborg et al., 2016), where no acetic acid was used, a meal of purple potatoes of the same
428 variety showed beneficial effects on postprandial glycemia and insulinemia compared to a
429 yellow potato meal. Finally, this type of anthocyanin-rich purple potato extract could not have
430 been prepared without acidic conditions as anthocyanins are not stable in neutral solutions.
431 Acetic acid was chosen as it is a soft acid generally accepted and used in a variety of food
432 products.
433
434 In addition, PPE contained high levels of chlorogenic acid which may also have a glycemic
435 index lowering effect (Bassoli et al., 2008). Consequently, our results may be affected not only
436 by the potato anthocyanins, but also by the difference in the contents of acetic acid and
437 hydroxycinnamic acid derivatives between the study and control meals.
438

439 **3.3 Inflammation markers**

440 Inflammation marker levels were compared between the two meal types (the study and the
441 control meals) at 240 minutes, and also between the fasting state and the 240 min time point
442 within both meal types. The fasting levels did not differ between the study and the control
443 meals (Table 3). Between the meal types at 240 minutes, the levels of C-C motif chemokine 20
444 (CCL20, $p < 0.001$) and fibroblast growth factor 19 (FGF-19, $p < 0.001$) were increased by the
445 study meal with a statistically significant difference with large effect sizes. Other markers,
446 which were also increased statistically significantly, but with only small or medium effect size,
447 were eukaryotic translation initiation factor (4E-BP1, $p = 0.045$), C-C motif chemokine
448 ligand 25 (CCL25, $p = 0.045$), interleukine 8 (IL-8, $p = 0.011$), oncostatin-M (OSM, $p = 0.005$)
449 and transforming growth factor alpha (TGF-alpha, $p = 0.045$) after the study meal compared to
450 the control meal at 240 minutes.

451 Furthermore, the levels of Fms-related tyrosine kinase (Fit3L, $p < 0.001$ and $p = 0.003$),
452 monocyte chemotactic protein 1 (MCP-1, $p < 0.001$ and $p = 0.004$), matrix metalloproteinase
453 10 (MMP-10, $p < 0.001$ and $p = 0.031$), TNF receptor superfamily member 9 (TNFRSF9,
454 $p < 0.001$ and $p = 0.013$) and TNF-related activation-induced cytokine (TRANCE, $p < 0.001$
455 and $p < 0.001$) were decreased at 240 min after control meal and study meal, respectively,
456 compared with the fasting state and at 240 minutes. FIt3L, MMP-10, MCP-1 and TRANCE
457 had a large effect size for both meals, and MMP-10 and TNFRSF9 had large effect sizes only
458 in the case of the control meal. The level of interleukin-6 (IL-6), however, was increased at
459 240 minutes compared with the fasting state, both after the control meal ($p < 0.001$) and the
460 study meal ($p < 0.001$). However, the increase had a large size effect only in the case of the
461 study meal.

462 Several markers were reduced only after the control meal at 240 min compared with the fasting
463 state. Those with large effect sizes were C-C motif chemokine 20 (CCL20, p = 0.002), T cell
464 surface glycoprotein CD5 (CD5, p = 0.001), T cell surface glycoprotein CD6 isoform (CD6,
465 p = 0.001), C-X-C motif chemokine 10 (CXCL10, $p < 0.001$), interleukin-7 (IL-7, p = 0.004),
466 interleukin-10 receptor subunit beta (IL-10RB, p = 0.003), urokinase-type plasminogen
467 activator (uPA, p = 0.001) and vascular endothelial growth factor A (VEGF-A, $p < 0.001$).

468 Interestingly, the proinflammatory cytokine IL-6 increased after both meals, as was previously
469 seen after a carbohydrate-rich meal in healthy volunteers (Steinberg, Stentz, & Shankar, 2018).
470 The study meal caused a smaller increase in IL-6 compared to the control meal; however, the
471 difference was not statistically significant between the meals. Furthermore, FGF-19 increased
472 slightly after the study meal without statistical significance but decreased statistically
473 significantly after the control meal. The FGF-19 levels were statistically different between the
474 two meals at 240 min postprandially. FGF-19 is an insulin-like ileum-derived postprandial
475 enterokine regulating bile acid homeostasis (Inagaki et al., 2005) reported to possess anti-
476 diabetic properties as it decreases glucose levels in rodents independently from insulin possibly
477 by converting glucose to lactate (Morton et al., 2013). FGF-19 also increases metabolic rate in
478 high-fat fed mice (Fu et al., 2004), regulates hepatic glucose homeostasis by suppressing
479 gluconeogenesis (Potthoff et al., 2011) and induces glycogen synthesis (Kir et al., 2011).
480 Hence, FGF-19 has been suggested to ameliorate obesity, type 1 and 2 diabetes, bile acid
481 overproduction and hepatocellular carcinoma as recently reviewed (Somm & Jornayvaz, 2018).

482 Recent studies display evidence of potato phenolics acting as anti-inflammatory agents. Kaspar
483 et al., 2011 studied blood plasma inflammatory marker levels of 12 healthy men before and
484 after a six-week daily consumption of 150 g of white, yellow and purple potatoes. They
485 reported a reduction in IL-6 and CRP levels in men who consumed purple potatoes compared
486 to those consuming white potatoes. Also Zhang et al., 2017 reported a decrease in the

487 production of IL-8 *in vitro* by adding purple potato extract rich in
488 petunidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside into TNF-alpha induced Caco-2 cells.
489 The biochemical mechanisms may involve suppression of the NF-κB pathway as activation of
490 the NF-κB leads to elevated levels of pro-inflammatory cytokines and inflammation mediators
491 (Karlsen et al., 2007). Furthermore, the phenolic metabolites and degradation products may
492 have a role in the modulation of inflammation. For example, phenolic metabolites of
493 cyanidin-3-*O*-glucoside were seen to reduce IL-6 levels in an *in vitro* cultivation of human
494 vascular endothelial cells, but the parent compound itself had no effect (Amin et al., 2015). In
495 the current study, the function and biological significance, in relation to nutrition, of
496 postprandial levels of most of the inflammatory mediators investigated are unclear, promoting
497 the need for future studies to reveal the biological relevance of these results. Furthermore, more
498 studies are needed to examine the postprandial behavior of the 90 inflammation markers as it
499 has been scarcely studied so far.

500 We studied here the postprandial inflammation response in healthy men as acute effects of
501 nutrition on postprandial inflammation response have profound relevance to human health as
502 reviewed by Muñoz & Costa, 2013. Meals have been found to cause acute postprandial
503 inflammation response even in healthy study subjects as high consumption of glucose and fatty
504 acids leads to oxidative stress inducing NFκB mediated inflammation markers. Gregersen,
505 Samocha-Bonet, Heilbronn, & Campbell, 2012 reported that an acute high-carbohydrate meal
506 excessive in calories enhances levels of IL-6 and decreases plasma total antioxidative status
507 and muscle Cu/Zn-superoxide dismutase. It was also discussed that one high-carbohydrate
508 meal may cause more severe inflammatory response than a high-fat meal. Connection of
509 dietary glucose and inflammatory response is also dose-dependent; Dickinson, Hancock,
510 Petocz, Ceriello, & Brand-Miller, 2008 reported that higher glycemic index induce higher
511 inflammatory response. Therefore, thorough investigation of postprandial inflammation status

512 after one meal in healthy study participants is essential for understanding the health effects of
513 the foods in question.

514 The statistical differences in the inflammation marker levels between the study and the control
515 meals were moderate. A single meal may not be enough to produce a large impact on the
516 inflammation status of healthy study subjects as seen in our recent publication (Nuora et al.,
517 2018), even though the meals used in our current study were rich in carbohydrates and energy.
518 Secondly, the selected time point 240 min may not have been optimal for measuring all the 90
519 selected inflammatory markers and it may have been too late for detecting the peak
520 concentration of some inflammation markers. For example, IL-6 and FGF-19 are reported to
521 peak already at 180 minutes (Steinberg et al., 2018) and 160 minutes (Morton, Kaiyala, Foster-
522 Schubert, Cummings, & Schwartz, 2014), respectively, after a high-carbohydrate meal.
523 However, we succeeded in our objective to screen a wide array of inflammation markers, but
524 for better understanding of the postprandial behavior of inflammation mediators, more
525 sampling points would have been beneficial. Lastly, one dose of PPE may have been
526 insufficient for distinguishing more significant acute effects.

527

528 **4 Conclusions**

529 In this study, we carried out a postprandial cross-over clinical study in which 17 healthy study
530 participants consumed a meal of yellow potatoes with or without the purple potato extract (PPE,
531 extracted with water/ethanol/acetic acid) rich in acylated anthocyanins and hydroxycinnamic
532 acid derivatives. The aim was to investigate whether the ethanol-free purple potato extract
533 affects glycemic, insulinemic and inflammatory responses in healthy human subjects. Our
534 results show that the purple potato extract added to a yellow potato portion (350g of cooked
535 yellow-fleshed potatoes and 110.9 g of cooking water) suppressed the postprandial plasma
536 glucose and insulin peaks and delayed the decrease in the plasma glucose and insulin levels

537 thereafter, compared to a meal of yellow potatoes. Blood glucose and insulin did not decrease
538 below the fasting levels in four hours after the study meal as they did after the control meal.
539 Therefore, our study hypothesis was supported. Besides glycemia and insulinemia, we
540 investigated the changes in the postprandial low-inflammation state by screening 90
541 inflammation markers from the plasma samples of the healthy study subjects at fasting state
542 and at 240 minutes after the meals. The energy- and carbohydrate-rich yellow potato portion
543 with or without PPE showed an inter-treatment effect on inflammation markers, such as the
544 insulin-like hormone FGF-19. As we studied here the acute effects of one meal, long-term
545 effects of purple potato phenolics should be investigated in the future.

546 In our recent study (Linderborg et al., 2016), we compared the impact of a meal of
547 purple-fleshed potatoes with that of yellow-fleshed potatoes on glycemia and insulinemia; the
548 results suggested that purple potatoes are more beneficial to human postprandial glucose
549 metabolism compared to yellow potatoes. The present study showed the findings are true also
550 of the extract of purple potatoes. Furthermore, our study confirmed extracted potato-derived
551 acylated anthocyanins and other phenolic compounds can be used as bioactive components for
552 improving the postprandial glycemic response after a high carbohydrate meal. To the best of
553 our knowledge, this is the first time such results are reported for a purple potato extract rich in
554 acylated anthocyanins and other phenolics.

555 In order to study the metabolic impact of the purple potato anthocyanins, we successfully
556 removed the possible effects of different potato varieties on biomarkers by extracting the
557 anthocyanins from the potatoes and adding them into a yellow potato portion which was also
558 used as the control meal. This excluded the effects of for example differences in the content
559 and structure of starch as well as the content of the vitamin C. Study participants acted as their
560 own control in a cross-over manner which decreased the interindividual variation related to
561 parallel studies. The baseline diet was strictly restricted concerning dietary fiber, flavonoids,

562 dietary supplements and alcohol for two days before the intervention, and one day after the
563 intervention to decrease the effect of baseline on the responses. We screened 90 inflammation
564 markers, of which a majority has not been previously reported in nutrition studies related to
565 potato phenolics. Our study is the first one to demonstrate the upregulation of the postprandial
566 level of FGF-19 after a high-carbohydrate meal by dietary anthocyanins. This is also the first
567 study in which the acute postprandial levels of 90 inflammation markers are studied after a
568 high carbohydrate meal with and without phenolic compounds extracted from purple potatoes.
569 However, our results may be partially affected by the difference in amount of acetic acid, used
570 in the extraction of PPE, between the control and study meal.

571 As a conclusion, this study shows evidence that the purple potato extract rich in acylated
572 anthocyanins decreases the postprandial glucose and insulin peaks and slows down the
573 decrease of glucose and insulin thereafter. As most of the day is spent in the postprandial state
574 and repetitive, fluctuating high blood glucose peaks are associated with oxidative stress and
575 type 2 diabetes, these findings indicate that increasing the intake of acylated anthocyanins and
576 other phenolics derived from purple potatoes as a part of a versatile and nutritious diet may
577 contribute positively to health. These health-promoting compounds may be cost-effectively
578 received from consuming purple-fleshed potatoes or similar food-grade purple potato extracts
579 used in this study. Purple potato extracts may be produced from the food industry side streams,
580 such as potato peels, and be used as a part of health-promoting functional foods.

581

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595

596 **Conflict of interest**

597 The authors declare no conflict of interest.

598

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744 **Tables**

745 **Table 1.** The nutrient composition (*n*=2), sugars and acids (*n*=3), anthocyanins (*n*=5), flavonol
746 glycosides and hydroxycinnamic acid derivatives (*n*=3) of the yellow-fleshed potato portion
747 (350g of cooked yellow-fleshed potatoes and 110.9 g of cooking water) without the meal
748 additives, and PPE (30 mL, the amount added to the study meal). Values are given as mean ±
749 standard deviation.

750

751 **Table 2.** Identification and quantification of anthocyanins, flavonol glycosides and
752 hydroxycinnamic acid derivatives in the purple potato extract (PPE) and the yellow-fleshed
753 potato portion (YP, 350g of cooked yellow-fleshed potatoes and 110.9 g of cooking water
754 without the meal additives) based on the UV, MS, MS/MS, and Q-ToF-MS data. Shown
755 positive ions are M^+ for anthocyanins and $[M+H]^+$ for hydroxycinnamic acid derivatives.
756 Amounts are given as mg per meal (referring to the anthocyanin-rich purple potato extract,
757 PPE, and the yellow potato portion) ± standard deviation.

758

759 **Table 3.** Average plasma inflammation marker levels at the fasting state and 240 min after the
760 study and control meals analysed using the cDNA-based proximity extension multiplex
761 immunoassay and qPCR. The values are means (n = 17) using an arbitrary, semi-quantitative
762 log2 scale, and variation is given as standard deviation (SD). Differences between the two
763 meals within a time point and between the two time points within each meal were statistically
764 compared using significance level of 0.05 for between-group comparisons. Furthermore, the
765 Benjamini–Hochberg corrected *p*-values (*q*-values) and effect size (Cohen's *d* or *r*, depending
766 on normality of the data), abbreviated here as ES, are listed.

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768

769 **Figures.**

770 **Figure 1.** HPLC chromatogram A) at 520 nm of purple potato extract (PPE) derived from
771 *Solanum tuberosum* L. ‘Synkeä Sakari’; B) at 320 nm of the yellow potato portion *S. tuberosum*
772 L. ‘Afra’; C) at 320 nm of the purple potato extract (PPE) from *S. tuberosum* L. ‘Synkeä
773 Sakari’. Numbering of the peaks refer to Table 2.

774

775 **Figure 2.** Plasma glucose (A) and insulin (B) concentration (*n* = 17) after the study meal (■)
776 and the control meal (▲). Values are presented as mean ± standard deviation.