Queen Margaret University
EDINBURGH

Project 9357R4: Effect of Purple Majesty Consumption on Antioxidant Status and Markers of Cardiovascular Risk in Healthy Volunteers

Final Report

Research team: Dr Catherine Tsang
Dr Emad Al-Dujaili
Michael Clapham
Nacer Foudil Smail
Dr Suzana Al-Moosawi

Table of Contents

Abbreviations

CRP	C-reactive protein
CVD	Cardiovascular Disease
FRAP	Ferric Reducing Ability of Plasma
GAE	Gallic Acid Equivalents
HDL	High Density Lipoproteins
HOMA IR	Insulin resistance measure
LDL	Low Density Lipoproteins
PWV	Pulse wave velocity
UV	Ultra-violet
v/v	volume/volume
λ max	Wavelength of maximum absorption

Aims of the study

Potatoes (*Solanum tuberosum L*) provide a rich and varied source of vitamins and minerals. A new variety of purple potato has been introduced to Scotland. Purple Majesty contains phenolic compounds, in particular Anthocyanins (red pigments). As potent antioxidants *in vitro* the absorption, bioavailability and protective effects of Purple Majesty *in vivo* have yet to be addressed.

A study was conducted at Queen Margaret University, Edinburgh, UK to investigate the antioxidant capacity and levels of total phenolics of Purple Majesty *in vitro* and *in vivo* and to assess their absorption, bioavailability and influence on markers of CVD in healthy volunteers. Purple Majesty and Osprey were provided by Albert Bartlett Ltd. Potatoes were analysed from the same batch prior to human consumption in the clinical studies.

This study set out to achieve the following aims:

1. To Quantify the *in vitro* Antioxidant Capacity, Total Phenolic Content and Total Anthocyanin Content of Purple Majesty

2. To Determine the Absorption and Bioavailability of Purple Majesty in Healthy Volunteers

3. To Investigate the Influence of Purple Majesty on the Antioxidant status and Markers of CVD

1. Materials and Methods

1.1 Chemicals and Reagents

Gallic acid and Folin-Ciocalteau's phenol regent were purchased from Sigma (Poole, Dorset, UK). Methanol (HPLC) grade and HCl were purchased from Rathburn Chemicals (Walkerburn, Peebleshire, UK). Acetone, acetic acid (glacial) and di-sodium hydrogen phosphate (Na_2HPO_4) were obtained from Fisher Scientific (Loughborough, Leicestershire, UK). Disodium carbonate (Na_2CO_3), di-sodium hydrogen orthophosphate and sodium dihydrogen orthophosphate dehydrate were purchased from BDH Chemicals Ltd (Poole, UK). All other chemicals and reagents were obtained from Sigma (Poole, Dorset, UK) unless otherwise stated.

1.2 Determination of total phenolics

The method of Singleton and Rossi (1965) was used to determine the total phenolic content of potatoes. This method determines phenols and oxidized substances by producing a blue colour from reducing yellow-heteropoly phosphomolibdate-tungstate anions. In brief, 200 µL of a 1:5 dilution of sample was added to 10 mL of a 1:10 dilution of Folin and Ciocalteau reagent and 1.8 mL of distilled water. After 5 min 7.0 mL of a Na_2CO_3 solution (115 g L^{-1}) was added and the reaction mixture was left at room temperature for 2 h. The absorbance of the solution was read at λ 765 nm against a water blank. The optical density was compared to a standard curve prepared with 50 to 500 mg L^{-1} gallic acid and results are expressed as gallic acid equivalents (GAE).

Total phenolics in plasma were also estimated with Folin-Ciocalteau reagent using a modification of the method of Swain and Hillis (1959). This method avoids interference from proteins in biological samples (Serafini et al 1998). In brief, 500 µL of sample was added to 1 mL of 1.0 M HCl and vigorously vortexed for 60 s. Following incubation at 37°C for 60 min, 1.0 mL of a 2.0 mol/L NaOH in 75 % methanol was added and the resulting mixture vortexed for 3 min. 1.0 mL of 10 % (v/v) phosphoric acid was added and the sample was centrifuged at 1500 x g for 10 min. The supernatant was removed and kept on ice in the dark and the pellet was extracted again by adding 1.0 mL of a

solution 1:1 (v/v) acetone:water and centrifuged for 10 min at 2700 x g. The two supernatants were combined and filtered through at 0.45 µm filter (Millipore, Tyne and Wear, UK) and 200 µL of sample was assayed for total phenolics with Folin's reagent as described above and expressed as µM GAE.

1.3 Colorimetric analysis of Anthocyanins

The anthocyanin content of Purple Majesty and Osprey (for comparison) was estimated using a pH shift method adapted from Ribereau-Gayon and Stonestreet (1965). Two sets of tubes were set up each containing 140 µL of sample and 140 µL of 0.1 % concentrated HCl in 95 % ethanol. 1.5 mL of 2 % concentrated HCl (pH 0.6) was added to one set of tubes and 1.5 mL of pH 3.5 buffer (300 mL 0.2 M Na_2HPO_4) and 700 mL of 0.1 M citric acid (adjusted to pH 3.5 with 0.1 M citric acid) to the other. Absorbance was read at 700 nm to allow for correction of the haze and then at 520 nm for anthocyanin determination. Anthocyanins were quantified as cyaniding-3-glucoside, the major anthocyanin in purple potatoes, using the extinction co-efficient 29 600 L cm $^{-1}$. At pH < 1.0 anthocyanins are found entirely in their red flavylium form allowing determination of the total anthocyanins. At pH 3.5 the flavylium form of the anthocyanin is primarily in equilibrium with the colourless carbinol, therefore the absorbance is due to polymeric anthocyanins or interfering brown substances. The difference in absorbance between pH < 1.0 and pH 3.5 is due to the free anthocyanin content.

1.4 FRAP-derived Antioxidant Determination

The FRAP assay determined by Benzie and Strain (1996) was used to estimate the antioxidant capacity of potatoes and biological samples. This method measures the ability of a solution to reduce a ferric-2,4,6-tri-2-pyridyl-s-triazine (TPTZ) complex (Fe^{3+}TPTZ) to the ferrous form (Fe^{2+}), producing an intense blue-colour with absorption λ at 593 nm. The reaction is non-specific and any half-life reaction which has a less positive redox potential, under reaction conditions, than the Fe^{3+} / Fe^{2+} - TPTZ half life reaction will drive the Fe^{3+} - TPTZ reduction. In the FRAP assay excess Fe^{3+} is used and the rate limiting factor is the Fe^{2+} -TPTZ and hence colour formation, is the reducing ability of the sample. The absorbance change of the aliquot diluted 1:40 in

distilled water is due to the combined reductive activity of all the reacting antioxidants present within a sample. Optical density was compared to a standard curve prepared with 0 – 1.0 mM ferrous sulphate ($FeSO_4$) and results are expressed as the mean concentration of Fe^{2+} produced/mM.

1.5 Measurement of Fasting Plasma Lipids

Fasting plasma lipids including total cholesterol, HDL, LDL cholesterol and triacylglyercerol concentrations were determined at the Department of Biochemistry, Western General Hospital, Edinburgh, UK using a multi-channel analyser. LDL cholesterol concentrations were calculated using the Friedewald formula (Friedwald et al. 1972).

1.6 Statistics

Data are presented as mean values ± standard deviation (SD), n=3. Each sample was analysed in triplicate (unless otherwise stated) and calibrated against relevant standards where appropriate. Independent 2-sample paired t-tests were used to assess differences between groups and an unpaired t-test was used to assess differences within groups. Values at $P < 0.05$ were considered statistically significant. ns = not significant, *P<0.05, **P<0.01, ***P<0.001, as compared to relevant control. Analysis was carried out using SPSS software.

2. Analysis of Antioxidant Capacity in Purple Majesty

Purple Majesty and Osprey potatoes were supplied by Albert Bartlett Ltd (Airdrie, UK). Potatoes were stored in the dark at 4°C throughout the study period. Potatoes from the same batch were analysed *in vitro* prior to human consumption in the bioavailability and clinical study. Osprey was selected to act as a control or placebo in the clinical study.

Raw and cooked Purple Majesty and Osprey were freeze dried in vacuo overnight and a weighed amount of the dried extract was reconstituted in a mixture of 50:50 methanol:water (v/v) and 10:90 acetone:water (v/v), and analysed for their total phenolic content and total anthocyanins (Table 2.1) and antioxidant capacity (Table 2.2), respectively.

Table 2.1 Recovered weights from freeze dried potatoes for total phenolic analysis

Samples	Fresh weight before freeze drying (g)	Weight after freeze drying (g)	Dry weight used for analysis (g)	Volume (mL)	Corresponding to fresh weight (g)
Raw white(1)	20	4.10	0.1	20	0.49
Raw white (2)	20	4.01	0.1	20	0.50
Raw white (3)	20	3.81	0.1	20	0.52
Cooked white (1)	20	3.98	0.1	20	0.50
Cooked white (2)	20	4.16	0.1	20	0.48
Cooked white (3)	20	4.00	0.1	20	0.50
Raw purple (1)	20	3.68	0.1	20	0.54
Raw purple (2)	20	3.23	0.1	20	0.62
Raw purple (3)	20	3.51	0.1	20	0.57
Cooked cooked(1)	20	3.27	0.1	20	0.61
Cooked purple (2)	20	3.45	0.1	20	0.58
Cooked purple (3)	20	3.07	0.1	20	0.65

Table 2.2 Recovered weights from freeze dried potatoes for FRAP-derived antioxidant analysis

Samples	Fresh weight before freeze drying (g)	Weight after freeze drying (g)	Dry weight used for analysis (g)	Volume (mL)	Corresponding to fresh weight (g)
Raw purple	20	4.00	2	80	10.00
Raw white	20	3.88	2	80	10.31
Cooked purple	20	2.90	2	80	13.79
Cooked white	20	3.00	2	80	13.33

Purple Majesty contained significantly higher levels of antioxidants and total phenolics in their raw form compared with Osprey ($p<0.001$). Following cooking there was a slight reduction in the levels of antioxidant capacity (Figure 2.1; $p=0.01$) and total phenolics (Figure 2.2; $p=0.03$). Total anthocyanins were only detected in Purple Majesty, as

expected. There was a slight decrease in the levels of total anthocyanins following cooking, however this was not significant (Figure 2.3; p=0.353).

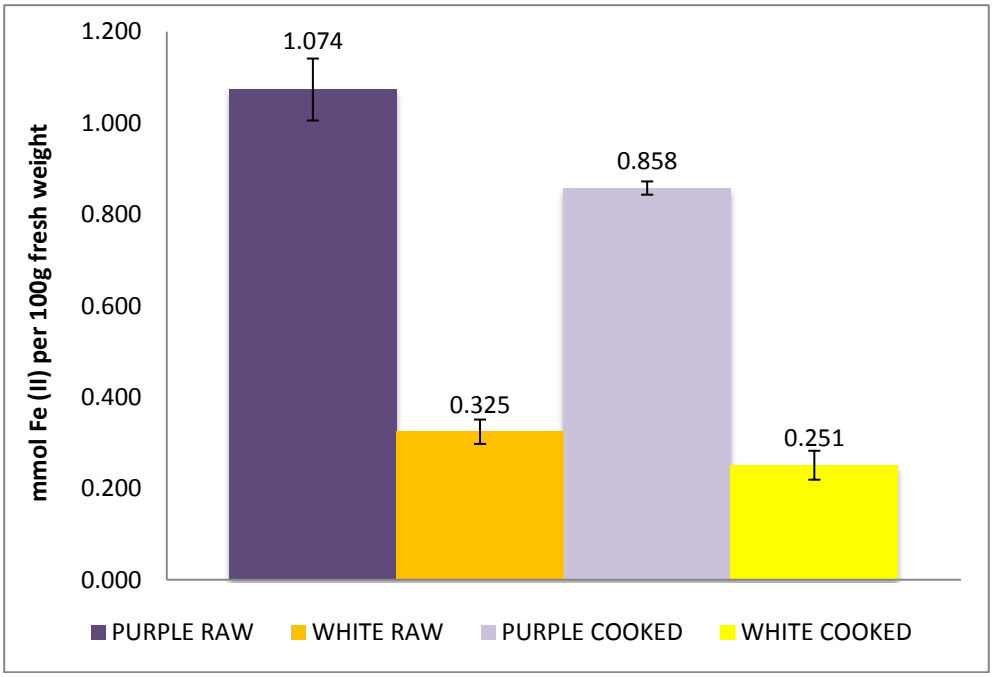

Figure 2.1 Antioxidant Capacity of Purple Majesty and Osprey white potatoes. Results expressed as mmol Fe (II) per 100 g fresh weight ± SD.

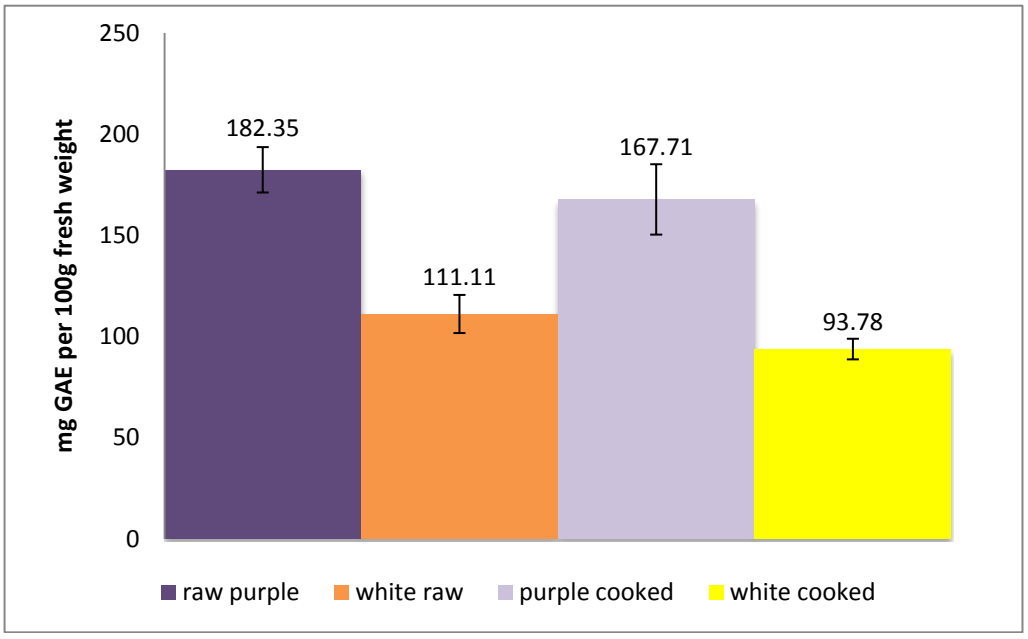

Figure 2.2 Total Phenolic levels of Purple Majesty and Osprey white potatoes. Results expressed as mg gallic acid equivalents (GAE) per 100 g fresh weight ± SD.

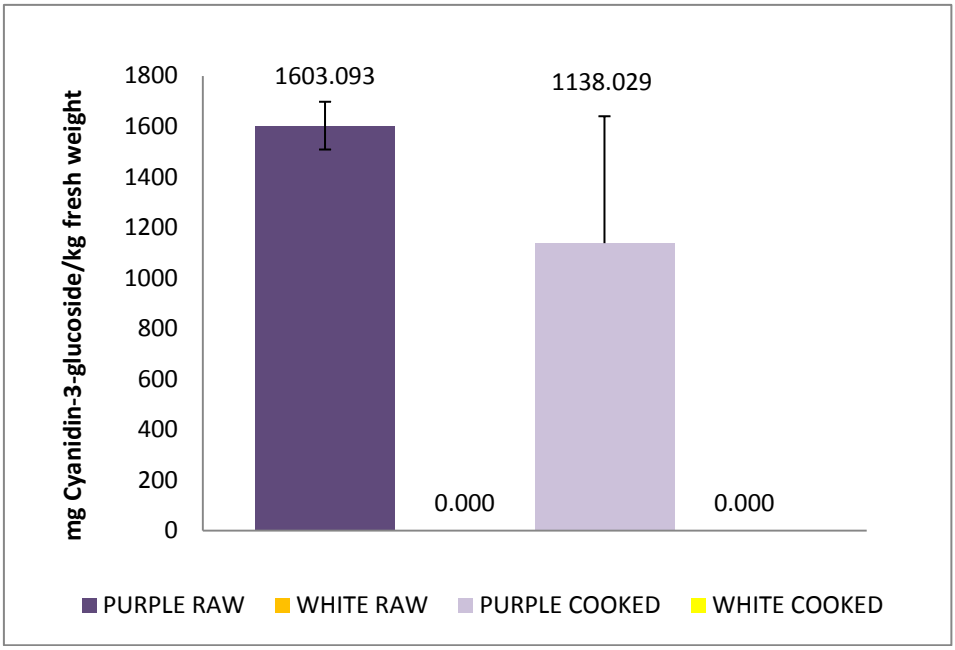

Figure 2.3 Total Anthocyanin levels of Purple Majesty and Osprey white potatoes. Results expressed as mg cyanidin-3-glucoside per kg fresh weight ± SD.

In summary, Purple Majesty was found to contain higher levels of antioxidants, total phenolics and total anthocyanins, as compared with Osprey. Domestic processing of Purple Majesty did not significantly influence the levels of total anthocyanins. Although there was a slight reduction in the levels of total phenolics, antioxidant capacity was retained. The levels of antioxidants in Purple Majesty remained higher than Osprey potatoes.

3. Absorption and Bioavailability of Purple Majesty

A bioavailability study was conducted in five healthy male volunteers (age range: 27-60 years) in order to assess the absorption and bioavailability of the antioxidants from Purple Majesty. Subject characteristics are shown in Table 3.1.

Table 3.1 Subject Characteristics

Subject	Height (m)	Weight (kg)	BMI (kg/m^2)
001	1.7	97.4	33.7
002	1.78	75.8	23.9
003	1.75	78	25.5
004	1.74	81	26.7
005	1.75	73	23.8
Mean ± SD	**1.74 ± 0.03**	**81 ± 9.6**	**26.7 ± 4.1**

Subjects attended the clinical facilities at QMU, Edinburgh following an overnight fast and baseline measurements were taken. Blood was collected at baseline and following an acute consumption of cooked Purple Majesty (400 g) at 1h, 2h, 4h and 24h post-consumption. Urine samples were also collected at 0-2 h; 2-6 and 6-24 h.

Antioxidant capacity was determined in the FRAP-derived antioxidant assay of Benzie and Strain (1996) as described in section 1.4. A peak plasma concentration was reached 1-2 hours post-consumption of Purple Majesty. Levels ranged from 1040 ± 80 µmol/L Fe^{2+} at baseline and increased to 1190 ± 355 and 1110 ± 243 µmol/L Fe^{2+} after 1 and 2 hours, respectively. The levels remained higher than baseline up to 24 h post-consumption of Purple Majesty. Figure 3.1 shows the results in mmol/L Fe^{2+}. Similar results were obtained for urinary FRAP assay. Figure 3.2 shows the individual response in urinary FRAP levels.

The levels of total phenolics were assessed in plasma using a modification of the Folin-Ciocalteau method as described by Serafini *et al.* (1998) (section 1.3). A peak plasma concentration was reached after 2 hours post-consumption of Purple Majesty. Levels ranged from 174.7 ± 28.3 mg L^{-1} GAE at baseline and increased to 178.6 ± 25 mg L^{-1} GAE after 2 hours (Figure 3.3). Urinary total phenolics concentration was also increased following Purple Majesty intake as shown in Figure 3.4.

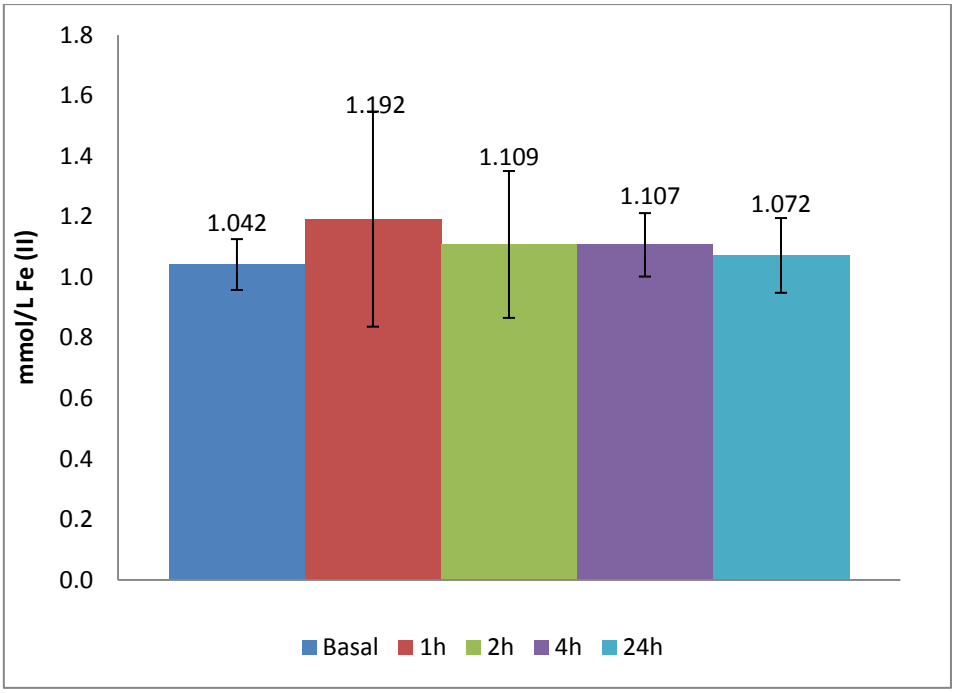

Figure 3.1 Antioxidant Capacity of Plasma from baseline-24h post-consumption of Purple Majesty. Results are expressed as mean mmol/L Fe^{2+} (n=5), ± SD.

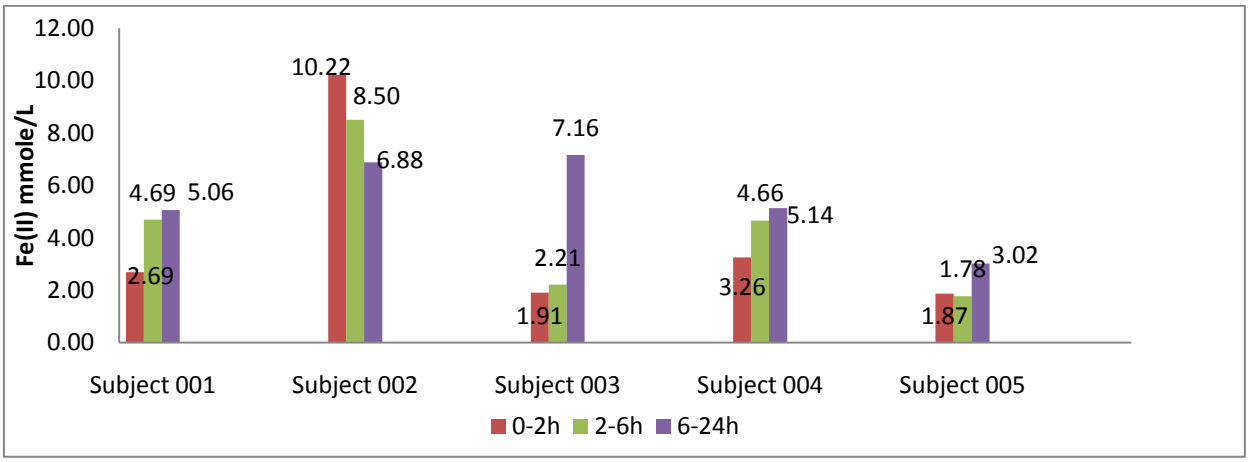

Figure 3.2 Individual Antioxidant Capacity responses in urinary FRAP levels following Purple Majesty consumption

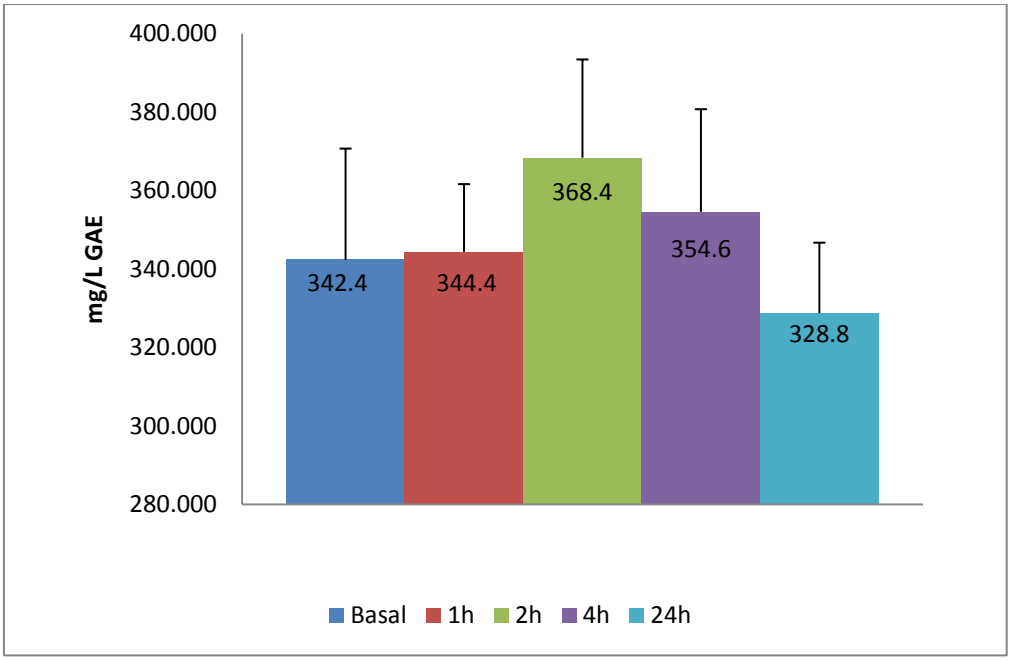

Figure 3.3 Total Phenolic concentration of Plasma from baseline-24h post-consumption of Purple Majesty. Results are expressed as mean mg/L GAE (gallic acid equivalent) (n=5), ± SD.

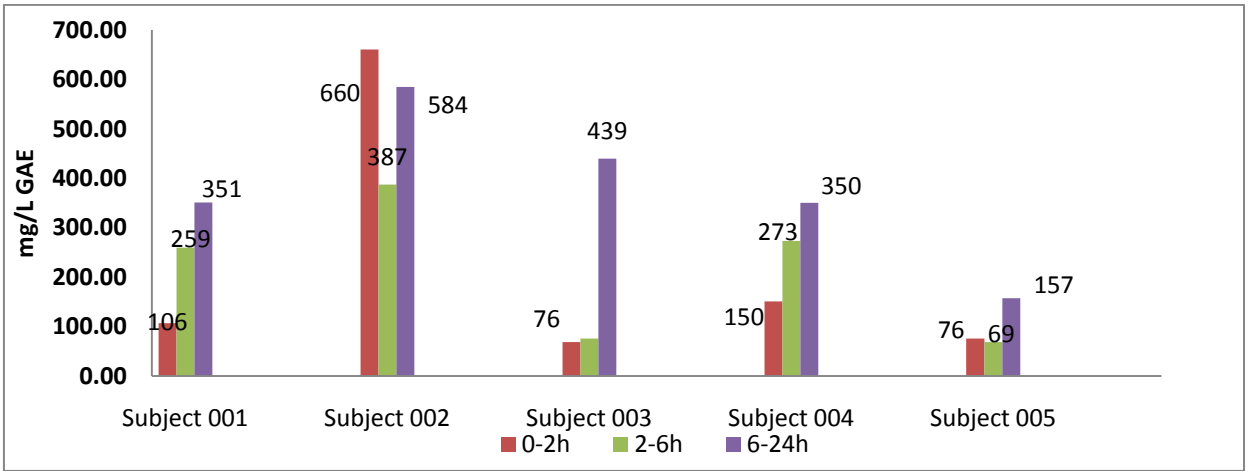

Figure 3.4 Urinary total phenolics concentration in individuals following Purple Majesty consumption.

In summary, acute consumption of 400 g of Purple Majesty showed a marked increase in the levels of total phenolics in the plasma and urine with enhanced antioxidant capacity in healthy volunteers. The results suggest that phenolic compounds from Purple Majesty are absorbed into the bloodstream and are bioavailable as evidenced

by the peak plasma antioxidant capacity 1-2 hours post-consumption. The urinary profile shows a progressive increase in antioxidant capacity and total phenolics levels up to 24 hours indicating the prolonged effect following the consumption of purple Majesty.

4. The Influence of Purple Majesty on Markers of CVD

The influence of Purple Majesty consumption on markers of cardiovascular risk was investigated. A randomised, placebo controlled cross-over study was conducted in healthy male and female volunteers (n=14). Subjects were asked to consume 200 g of Purple Majesty or Osprey (as a placebo) for 2-weeks followed by a wash-out period before commencing the second arm of the intervention (Figure 4.1).

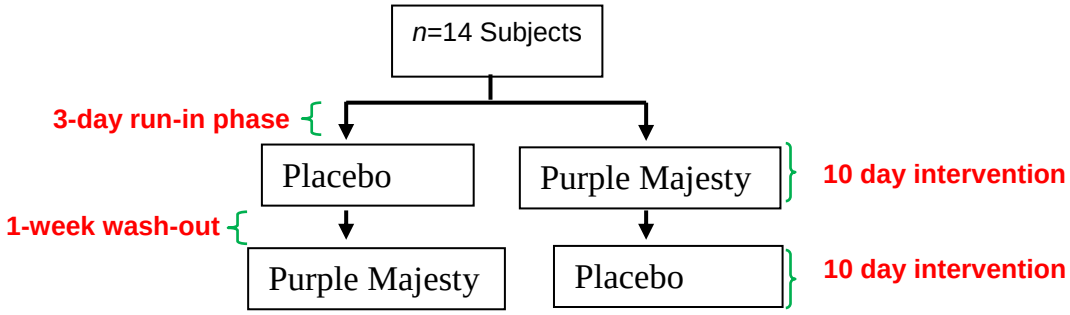

Figure 4.1 Schematic diagram of study design

Systolic and diastolic blood pressure was measured in addition to the non-invasive assessment of vascular tone (arterial stiffness) as determined by pulse-wave velocity (PWV). Plasma lipid profile (HDL, LDL and triglycerides), glucose, insulin and C-reactive protein (a measure of inflammation) were also measured. Antioxidant capacity and total phenolics were measured in plasma as previously described in section 3.

Table 4.1 shows the results from the anthropometric and blood pressure measurements as baseline and following consumption of Purple Majesty or Osprey. PWV a measure of arterial stiffness was significantly reduced from 6.5 ± 0.5 m/sec at baseline to 6.3 ± 0.5 m/sec following consumption of Purple

Majesty (p=0.049). This effect was not observed following consumption of Osprey. There were no significant changes in body weight, BMI, systolic or diastolic blood pressure following 10 days consumption of Purple Majesty. However, there was a trend towards a decrease in systolic blood pressure following Purple Majesty consumption but this did not reach significance.

Table 4.1 Anthropometric and blood pressure measurements

Variable	Baseline	After 10-days treatment with Purple Majesty	After 10-days treatment with Osprey	Significance
Weight (kg)	66.9 ± 11	67.1 ± 11.1	67 ± 10.7	ns
BMI (kg/m^2)	22.7 ± 3.2	22.8 ± 3.1	22.8 ± 3.0	ns
Systolic BP (mmHg)	115.5 ± 11.8	114.5 ± 13.9	115 ± 11.8	ns
Diastolic BP (mmHg)	70.1 ± 8.6	71.6 ± 10.6	69.3 ± 9.8	ns
PWV (m/sec)	6.5 ± 0.5	6.3 ± 0.5*	6.6 ± 0.6	p=0.049

Data expressed as mean ± SD (*n*=14); Significance based on paired t-test measurements between placebo and Purple Majesty

There were no significant changes in plasma trigylcerides, total cholesterol, HDL, LDL or the ratio of total cholesterol to HDL-cholesterol. No changes were detected in blood glucose, insulin, insulin resistance (as measured by HOMA-IR) or C-reactive protein (Table 4.2). There was also no significant difference in dietary fat, carbohydrate, protein or energy intake during the Purple Majesty and the Osprey potato consumption (data not shown).

Table 4.2 Routine biochemical measurements

Variable	Baseline	After 10-days treatment with Purple Majesty	After 10-days treatment with Osprey	Significance
Triglycerides	0.9 ± 0.5	0.8 ± 0.3	0.8 ± 0.3	ns
Total cholesterol	4.6 ± 1.3	4.2 ± 1.1	4.3 ± 0.9	ns
LDL-cholesterol	2.7 ± 0.9	2.4 ± 0.9	2.4 ± 0.8	ns
HDL-cholesterol	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	ns
Total Chol/HDL	3.2 ± 0.5	2.9 ± 0.9	2.9 ± 0.9	ns
C-reactive protein	5.0 ± 0	5.5 ± 1.5	5.0 ± 0	ns
Glucose	1.8 ± 0.3	4.6 ± 0.5	4.8 ± 0.4	ns
Insulin	6.2 ± 4.1	6.4 ± 5.5	8.4 ± 6.4	ns
HOMA IR	1.3 ± 0.9	1.3 ± 1.1	1.8 ± 1.5	ns

Data expressed as mean ± SD (*n*=14); Significance based on paired t-test measurements between placebo and Purple Majesty

Plasma total phenolic levels were higher following Purple Majesty consumption, 442.33 ± 92.2 mg/L GAE compared with levels following consumption of Osprey, 385.5 ± 46.4 mg/L GAE (Figure 4.2). However, this did not reach significance (p=0.292). Plasma antioxidant capacity was also higher following Purple Majesty consumption, 0.88 ± 0.1 mmol/L Fe $^{2+}$ compared with capacity following consumption of Osprey, 0.86 ± 0.16 mmol/L Fe $^{2+}$ (Figure 4.3). However, this did not reach significance (p=0.249).

In summary, Purple Majesty consumption over a 10 day period resulted in a statistically significant decrease in arterial stiffness suggesting improved vascular tone. No other significant changes in any other marker of CVD were observed following Purple Majesty consumption.

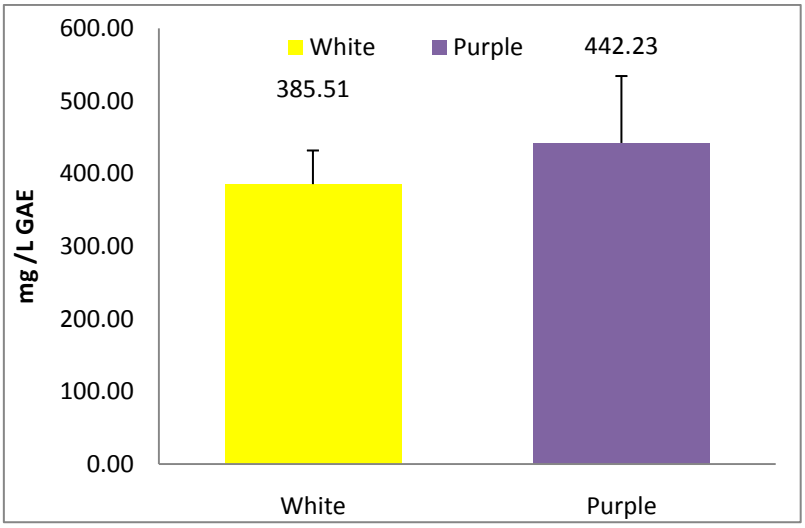

Figure 4.2 Levels of total phenolics in plasma following Purple Majesty and Osprey consumption. Results are expressed as mean mg/L GAE ± SD (n=3).

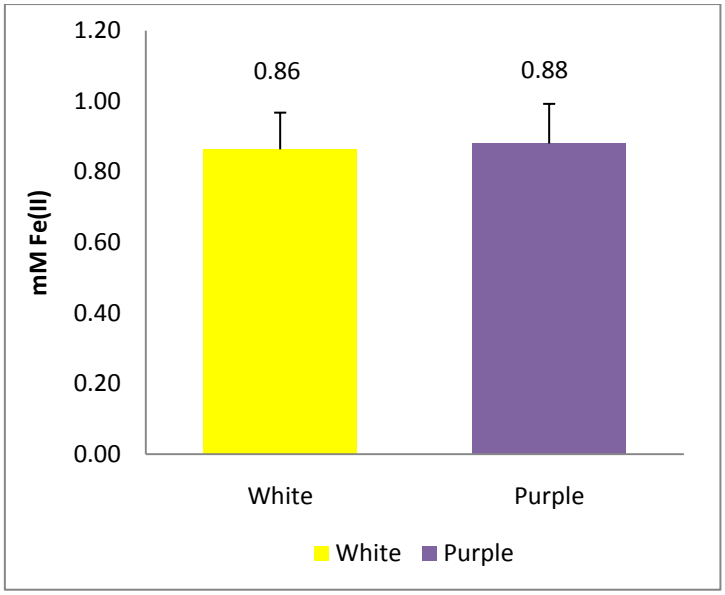

Figure 4.3 Antioxidant Capacity of Plasma. Results are expressed as mean mmol/L Fe^{2+}± SD.

5. Summary and conclusion

Purple Majesty contained high levels of total phenolics, total anthocyanins and a high antioxidant capacity. A slight reduction in the levels of total phenolics (not total anthocyanins) was observed following domestic processing, however antioxidant capacity remained high. Phenolic compounds from Purple Majesty appear to be absorbed into the blood stream and bioavailable. Metabolite profiling is underway in an attempt to identify specific metabolites associated with Purple Majesty which may be contributing to the ex vivo antioxidant capacity. Daily consumption of Purple Majesty over 10 days was well tolerated by volunteers and was associated with reduced arterial stiffness in healthy volunteers, suggesting improved vascular tone. Purple Majesty as part of a healthy balanced diet may be beneficial to health, and the findings from this report may help to support the rationale to incorporate potato consumption as part of the 5-a day of fruit and vegetable intake.