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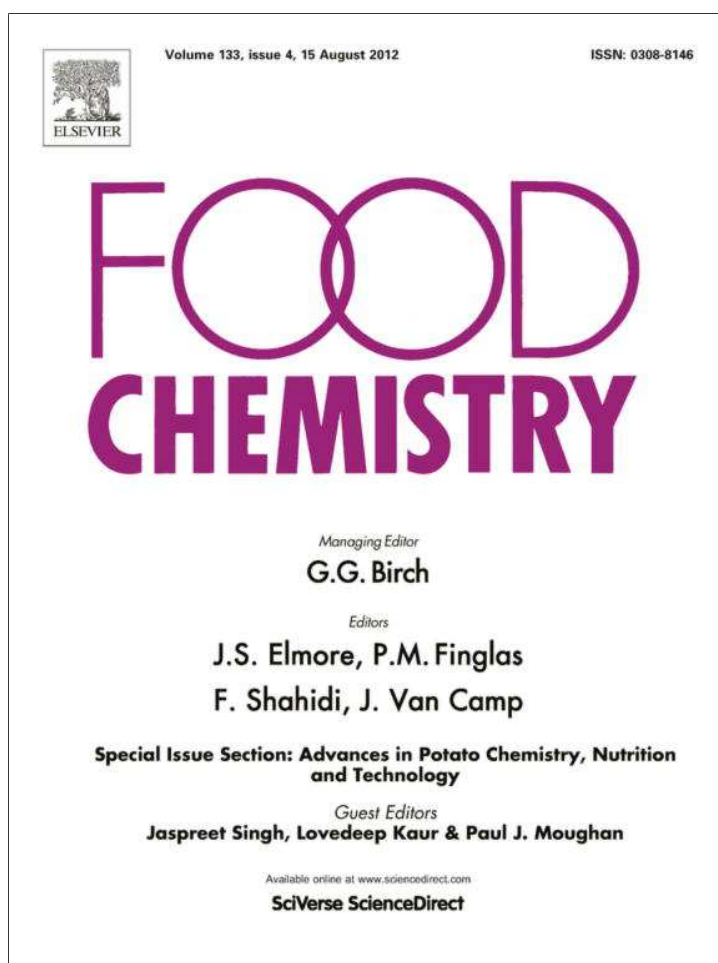
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# Food Chemistry

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## Quantitative analysis of phenolic components and glycoalkaloids from 20 potato clones and *in vitro* evaluation of antioxidant, cholesterol uptake, and neuroprotective activities

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### ABSTRACT

Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world and provides essential nutrients. With an aim to develop potato varieties for functional food or nutraceutical applications, we have conducted metabolomic profiling, total phenolics, chlorogenic acid, anthocyanins, and glycoalkaloids analyses on 20 selected potato clones within the Canadian potato breeding program of Agriculture and Agri-Food Canada. Pigmented potatoes in general contain higher levels of phenolic components, including chlorogenic acid and anthocyanins. Levels of phenolics were retained with granulation processing of pigmented potato tubers, but glycoalkaloids were significantly reduced with granulation. The pigmented potatoes also have higher antioxidant activity reaching up to 35% of that for berries, measured as their potency in scavenging DPPH radicals. Extracts of the 20 potato clones (peel, tuber, and granule) were also evaluated for *in vitro* effects on liver LDL cholesterol uptake and protection of cortical neurons from cell death caused by oxygen glucose deprivation (OGD). These potato extracts in general showed mild activity in enhancing LDL cholesterol uptake in liver HepG2 cells, and also protected cortical neurons against OGD induced cell death, with extracts from granules of six of the potato clones showing significant neuroprotective effects. The bioactive components are not dependent on pigmentation of potato clones. These novel bioactivities identified in potatoes warrant in-depth investigations in the future. Taken together, our results provide further evidence for the enhanced health beneficial components in potato.

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### 1. Introduction

The ageing population structure and increasing health care costs have placed a priority on development of new solutions for health maintenance. Advances in the understanding of the relationship between the phytochemical constituents of our plant-based food supply and health at a molecular level have increased interest in functional foods and nutraceuticals. Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world following wheat and rice to provide essential nutrients, mainly carbohydrate. As food and life style choices have been increasingly

recognised as useful approaches in prevention or delaying the onset of chronic diseases, more and more research and commercial development are focused on food phytochemicals such as polyphenolics and carotenoids (Hu, 2003; Hunter, Skinner, & Lister, 2008; Maiani et al., 2009; Minich & Bland, 2008; Rao, 2003; Spencer, 2008; Stevenson & Hurst, 2007; Thomasset et al., 2007; Tosetti, Noonan, & Albini, 2009; Voutilainen, Nurmi, Mursu, & Rissanen, 2006). Potato is a good source of polyphenolics and carotenoids, particularly the pigmented or coloured varieties (Brown, 2005; Camire, Kubow, & Donnelly, 2009; Lachman & Hamouz, 2005; Tsao, 2009). As such, the antioxidant activity of these components in potatoes has been a focus of past studies (Al-Saikh, Howard, & Miller, 1995; Han, Sekikawa et al., 2006; Lachman & Hamouz, 2005; Lachman, Hamouz, Orsak, Pivec, & Dvorak, 2008;

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Lachman et al., 2009; Leo et al., 2008; Nara, Miyoshi, Honma, & Koga, 2006; Reddivari, Hale, & Miller, 2007; Singh & Rajini, 2008). In rat studies, anthocyanins from both the red and purple pigmented potatoes showed protective effects against D-galactosamine or acetaminophen-induced liver damage (Han, Hashimoto et al., 2006; Han, Sekikawa et al., 2006; Han, Shimada, Sekikawa, & Fukushima, 2007; Ohba et al., 2007). These anthocyanins and the glycoalkaloids from potatoes have also been revealed to induce cancer cell apoptosis and may be useful in chemoprevention (Hayashi et al., 2006; Reddivari, Vanamala, Chintharlapalli, Safe, & Miller, 2007; Reddivari, Vanamala, Safe, & Miller, 2010; Yang, Paek, Kozukue, Lee, & Kim, 2006). More recently, Kaspar et al. reported their findings of giving pigmented potato (white, yellow, and purple fleshed) meals to healthy male adults for 6 weeks. Results showed that consumption of pigmented potato reduced inflammation and DNA damage (Kaspar et al., 2011).

Efforts have been made to develop new potato varieties as functional food or food material with higher levels of health beneficial phytochemicals in the past years. Antioxidant profiling of native Andean potato tubers revealed higher levels of carotenoids and polyphenolics including chlorogenic acid and the anthocyanin petanin (also known as petunidin-3-(p-coumaroylrutinoside)-5-glucoside), the latter was reported to be exceptionally high in dark-purple-fleshed cultivars (Andre et al., 2007). Genotypic variation was also found to be the predominant factor affecting the contents of phenolics and carotenoids, compared to other parameters such as location and year (Hamouz et al., 2010; Lachman et al., 2009; Reddivari, Hale, & Miller, 2007; Wegener, Jansen, Juergens, & Schuetze, 2009). Drought-stressed native cultivars were investigated for the effects of growing conditions on accumulation of polyphenolics, carotenoids and glycoalkaloids, and these components were found to be affected very cultivar-dependently for the pigmented potato cultivars (Andre et al., 2009). Beyond natural selection, genetic manipulations in transgenic potato varieties that lead to the overexpressing of key flavonoid biosynthetic enzymes was demonstrated to increase levels of phenolic acids and anthocyanins (Lukaszewicz et al., 2004).

We conducted profiling of small molecular metabolites on a selection of 20 diploid and tetraploid potato clones, and completed quantitative analyses of phenolic components and glycoalkaloids in these potato peels and tubers, as well as selected potato granules. In addition, the antioxidant capacities of these potato and potato granule extracts were assessed by using DPPH radical scavenging assay. We further investigated the role of potato bioactives in human health through the use of bioassays. *In vitro* LDL cholesterol uptake activities of these potato extracts were evaluated in HepG2 liver cells, while the neuroprotective potency was tested in primary cortical neurons. Our results, as presented in this paper, provide further evidence for the enhanced health beneficial components in both pigmented and unpigmented potato selections. Our findings support further potato breeding for functional food or nutraceutical applications.

## 2. Materials and methods

### 2.1. Reagents and chemicals

Folin–Ciocalteu's phenol reagent (2 N), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox),  $\alpha$ -chaconine,  $\alpha$ -Solanine, 2,2-dimethyl-2-silapentane-5-sulphonate sodium salt (DSS), D<sub>2</sub>O and methanol-d<sub>4</sub>, formic acid, hydrochloric acid (HCl), cyanidin chloride, malvidin chloride, pelargonidin chloride and delphinidin chloride were purchased from Sigma–Aldrich (St. Louis, MO, USA). Chlorogenic acid was from Indofine (Hillsborough, NJ, USA). Pelargonidin-3-glucopyranoside (chloride form) was purchased

from Polyphenols Laboratories AS (Sandnes, Norway) and petunidin-3-glucoside chloride, petunidin chloride and peonidin chloride were from ChromaDex (Irvine, CA, USA).

### 2.2. Potato samples

Twenty selected potato breeding clones (Fig. 1) from the Potato Research Centre, Agriculture and Agri-Food Canada (Fredericton, NB, Canada) were used in this study. These potato clones were given the sample codes of 1–20, with the selection/cultivar numbers as follows: Clone 1 (9970–02 (4×)), 2 (9970–02), 3 (F03063), 4 (F03064), 5 (F98021), 6 (12120–03), 7 (07506–01), 8 (15250–04), 9 (15255–16), 10 (11448–02), 11 (11827–09), 12 (N0634–7), 13 (Shepody), 14 (F05070), 15 (F05086), 16 (F05089), 17 (F03061), 18 (10908–06), 19 (12115–07), and 20 (11379–03). The potatoes (2.2 kg each) were cleaned with tap water before they were peeled using a hand held vegetable peeler (about 2 mm thickness) to obtain peels and tuber separately. Tubers were further sliced to small pieces, and both tuber and peel materials were then freeze-dried, milled, and stored at –20 °C prior to extraction.

Among these 20 potato selections, 16 clones were chosen for preparation of potato granules based on a commercial process for manufacturing of potato granules. In brief, 2.3 kg of potatoes were washed and peeled as described above. The peeled potatoes were sliced to ½ inch thickness. Potato slices were blanched in 3 L of 80–85 °C water with 3 g of Na<sub>2</sub>S<sub>2</sub>O<sub>7</sub> for 10 min and then sprayed with cold water for 5 min. Following blanching, the slices were steamed for about 20 min at atmospheric pressure. After steaming, cooked slices were mashed and mixed with 1.4 g of glycerides and 0.01 g of citric acid. After mixing, the mixture went through two heating and cooling cycles to reduce the moisture to around 50%. It was then conditioned at 4 °C for overnight. The cooled mixture then went through a series of drying and mixing steps in a fluid bed dryer (M501 Programmable Fluid Bed Dryer, Sherwood Scientific Ltd., Cambridge, UK) until it reached a 6–9% moisture content. Finally, the material was screened. The portion of material that passed through 60 mesh (<0.25 mm) was stored at –20 °C before extraction and analysis.

### 2.3. Sample extraction and <sup>1</sup>H NMR based metabolomic profiling

For preparation of water soluble extracts, freeze-dried potato tuber and peel samples (100 mg) were extracted with 1 mL of buffered D<sub>2</sub>O (100 mM phosphate buffer, pH 7.0; NaN<sub>3</sub> 0.4 mg/mL) with sonication for 30 min, then centrifuged at 7400g for 5 min. The supernatants were filtered through a 3 kDa Amicon® ultra centrifugal filter at 14,000g for 35 min. The filtrate solution (447 µL) was mixed with 15 mM DSS (3 µL), then 60 µL was transferred to a 1.7 mm NMR tube.

For methanol soluble metabolites extraction, the same samples (100 mg) were extracted with methanol-d<sub>4</sub> (1 mL) with sonication for 30 min and then centrifuged at 7400g for 5 min. The supernatant (447 µL) was mixed with 15 mM DSS (3 µL) and then 60 µL was transferred to 1.7 mm NMR tube. NMR spectra for these potato tuber and peel extracts were acquired on a Bruker Avance III 600 MHz NMR spectrometer operating at 600.283 MHz <sup>1</sup>H observation frequency and a temperature of 25.00 ± 0.02 °C. Automatic tuning and matching, gradient shimming, and 90° pulses calibrations were performed on each sample prior to spectral acquisition using a 1.7 mm gradient inverse probe. For all extracts, 1D NOESY spectra with pre-saturation of the water signal were acquired with 256 scans, an acquisition time of 2.73 s (64 K points), a sweep width of 20 ppm, a mixing time of 10 ms, and a relaxation delay of 4 s. Spectra were referenced using the internal DSS standard set to 0 ppm. The signals were acquired, processed and analysed using TopSpin® NMR data acquisition and processing Software



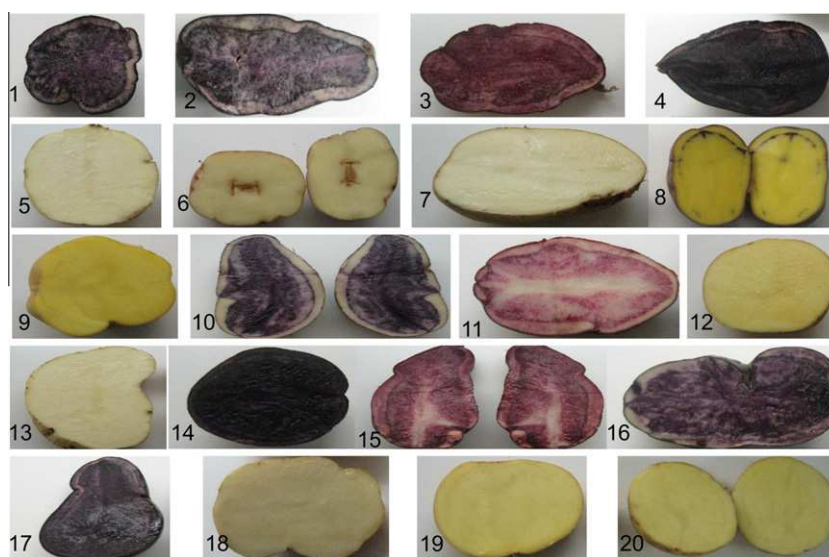


Fig. 1. Photographic images of 20 potato clones (provided by Yu and Charter).

(Bruker Biospin Ltd., East Milton, ON) integrated with the spectrometer.

Targeted profiling of tuber water extracts was conducted by processing and binning the spectra for the region of  $\delta$  0–10 ppm (water peak region  $\delta$  4.7–4.83 ppm excluded) within 0.04 ppm chemical shift window by using the NMR software package NMR Suite 5.0 (Chenomx, Edmonton, Canada). The concentration of each profiled component was the mean of two extraction replicates. Multivariate data analysis (PCA and PLS-DA) was performed by using SIMCA-P + 12.01.0 software package (Umetrics, Umea, Sweden).

#### 2.4. Total phenolics quantification

Dried potato tuber, peel, and granule powder (60 mg) were accurately weighed and added to 500  $\mu$ L 80% MeOH with 1% acetic acid in 1.5 mL microtubes. The mixture was vortexed for 30 s, followed by sonication (20 min) and then centrifugation at 16,000g (5 min). The supernatant was pipetted into a 1 mL volumetric flask. The extraction was repeated twice by using 250  $\mu$ L of the same solvent, the three extracted supernatants were combined, and adjusted using the same solvent to a final volume of 1 mL. Each sample extraction was done in triplicate.

The total phenolics content of the samples was determined according to a modified Folin–Ciocalteu (FC) colorimetric method (Zhang et al., 2006). Briefly, 20  $\mu$ L sample extract solution was loaded on 96-well plate. To each well, 40  $\mu$ L 10% FC reagent was added, mixed and incubated for 5 min in the dark at room temperature. Then 160  $\mu$ L 700 mM sodium carbonate was added, and the plate was covered with Parafilm to incubate in the dark at room temperature for 1.5 h. Finally the absorbance at 750 nm was read on a SPECTRA max M2 plate reader (Molecular Devices Corporation, CA, USA). Gallic acid was used as a standard. A five-point standard curve was made and the linear range was from 0.025 to 0.200 mg/mL. Total phenolics content of the potato samples is reported as gallic acid equivalents (GAE) in mg per gram of dry material (mg GAE/g).

#### 2.5. Quantification of chlorogenic acid and anthocyanins

The potato extract solution prepared as described above (Section 2.4) was used for quantification of chlorogenic acid and anthocyanins. Separation was conducted on an Agilent Zorbax

XDB-C18 (4.6  $\times$  150 mm, 5  $\mu$ m) column using the Agilent HPLC 1100 system, coupled with a diode array detector (DAD). For mobile phases, solvent A was water with 0.1% trifluoroacetic acid (TFA) and solvent B was acetonitrile with 0.1% TFA. The flow rate was set at 1 mL/min and the column temperature was 40  $^{\circ}$ C. The gradient scheme employed was 8% B for 10 min, then to 25% B in 15 min. The wavelength for detection of chlorogenic acid was 330 nm, whereas anthocyanins (petunidin-3-glucoside chloride and pelargonidin-3-glucoside as standards) were detected at 520 nm. Anthocyanins in potato samples containing petunidin-3-para-coumaroyl-rutinoside-5-glucoside were expressed in petunidin-3-glucoside chloride, and samples containing pelargonidin-3-para-coumaroyl-5-glucoside were expressed in pelargonidin-3-glucoside. Five-point calibration curves were used for the quantification. Linear ranges were determined to be from 0.365 to 1095  $\mu$ g/mL for chlorogenic acid, from 0.57 to 1710  $\mu$ g/mL for petunidin-3-glucoside chloride, and from 0.8 to 2400  $\mu$ g/mL for pelargonidin-3-glucopyranoside. The results were reported as mg per gram of dry sample.

#### 2.6. Quantification of anthocyanins in aglycone form (anthocyanidins)

For the tuber and peel samples, 100 mg of freeze dried powder was extracted in 5 mL of 90% methanol with 0.5% formic acid in a 20 mL scintillation vial. For 100 mg of dried granules the initial extraction was in 50% methanol with 0.5% formic acid. Samples were then sonicated for 15 min. The extract was filtered through Celite to remove bulk tuber tissue and methanol was evaporated under vacuum. The vial was washed with 1 mL of 90% methanol with 0.5% formic acid. The aqueous methanol extract was transferred to an amber septum sealed vial and adjusted to 2 mL. Two hundred microlitres of 37% LC–MS grade HCl was added to the vial. The vials were sealed and placed in a 150  $^{\circ}$ C oven for 30 min followed by immediate chilling on ice. The extract was then transferred to a pre-activated 5 g C18 Seppak column (Waters, Mississauga, Canada). Two washes of 10 mL of water were passed through the column. Anthocyanidins were eluted with 2  $\times$  5 mL of methanol with 0.1% formic acid. The anthocyanidin extract was dried using a Rotovap under vacuum. The dried anthocyanidins were redissolved in 1 mL of a solution of 85% water, 15% acetonitrile and 0.1% formic acid, and then passed through a 0.2 micron filter.

The anthocyanidin concentration of hydrolysed potato extracts was determined using a Waters nano-Acquity™ ultra-performance liquid chromatograph (UPLC) coupled to a Xevo™ quadrupole time-of-flight mass spectrometer (TOF-MS). The column was a Waters Acquity HSS T3 (1.0 × 100 mm, 1.8 μm) and the injection volume was 2 μL. Solvent A was 0.1% formic acid in water and solvent B was 0.1% formic acid in acetonitrile. The gradient elution was as follows: 0–10 min, 15–19% B; 10–14 min, 19–21% B; 14–17 min, 21% B; 17–19 min, 21–90% B. The flow rate was 50 μL/min. Mass spectrometry was carried out using a Waters Z-spray electrospray ion source in positive ionisation mode. MS parameters were as follows: capillary voltage 2.24 kV, sampling cone 45 V, extraction cone 3.7 V, source temperature 100 °C, cone gas flow 80 L/h and desolvation gas flow 800 L/h. The range of MS was 100–800 *m/z*. The microchannel plate (MCP) detector potential was set to 2100 V. Accurate mass determinations were ensured using a lockmass solution of leucine enkephalin in acetonitrile–water (50:50) introduced via a lock-spray probe at 50 μL/min. The protonated ion of leucine enkephalin at *m/z* 120.0813 and the protonated molecule at *m/z* 556.2771 were used to recalibrate the mass axis during the analyses. The anthocyanidins were dissolved in methanol, combined to form a mixed standard solution and divided equally among light-resistant glass vials. The solutions were then dried under nitrogen and stored at –20 °C. Immediately before analysis, a vial was removed from the freezer and serially dilutions were made with water/acetonitrile/formic acid (85:15:0.1). The range of calibration curves was 500–4500 pg/μL. Individual anthocyanidins were identified on the basis of their MS spectra and retention times. Each sample was injected twice and the average of the two readings is presented.

## 2.7. Quantification of glycoalkaloids

Potato peel, tuber, and granule extract solutions were prepared as described previously (in Section 2.4) and their glycoalkaloids content was quantified. Separation was conducted on an Agilent Zorbax XDB-C18 (4.6 × 150 mm, 5 μm) column using the Agilent HPLC 1100-MSD system. Solvent A was water with 0.1% formic acid and solvent B was acetonitrile with 0.1% formic acid. The gradient used was: 25% B for 5 min, then to 63% B in 10 min. The column temperature was at 30 °C, while the flow rate was set at 0.5 mL/min. Mass spectra were obtained using the Agilent MSD at the following settings: SIM 852 *m/z* for α-chaconine and 868 *m/z* for α-solanine; drying gas flow (L/min): 10; nebuliser pressure (psig): 30; drying gas temperature (°C): 350; capillary voltage (V): 4000 (positive). Standard solutions containing both α-chaconine and α-solanine were freshly prepared from stock solutions. A five-point standard curve was made. The linear ranges were from 5.12 to 5120 ng/mL for α-chaconine and from 5.54 to 5540 ng/mL for α-solanine. The results were reported as mg per gram of dry sample.

## 2.8. Determination of DPPH radical scavenging activity

Potato peel, tuber, and granule extract solutions prepared as described previously (Section 2.4) were used for determination of DPPH radical scavenging activity. The antioxidant assay was based on a 96-well plate method (Fukumoto & Mazza, 2000) with slight modification. Briefly, 10 μL of sample solution was added in each well in a 96-well plate. To each well, 100 μL of 761 μM DPPH in 80% MeOH was added. The plate was incubated at room temperature in the dark for 2 h and the absorbance at 515 nm was read on a SPECTRA max M2 plate reader (Molecular Devices Corporation, CA, USA). Trolox was used as a standard. A seven-point standard curve was made from 0.25 to 0.88 mg/mL. The results were expressed as mg of Trolox equivalents per gram of dry sample.

## 2.9. In vitro evaluation of liver LDL-cholesterol uptake activity

Potato extracts were separately prepared for evaluation of LDL-cholesterol uptake activity. Dried potato peel, tuber, or granule sample (300 mg) was extracted with 1.2 mL 80% MeOH by vortexing and then sonicating for 20 min. After centrifugation at 1300g for 5 min, the supernatant containing the aqueous MeOH extract was collected. The extraction was repeated twice using the same solvent (0.9 mL). The liquid extracts were combined, dried using a Rotavap under reduced pressure and then freeze-dried. Dried extracts were then dissolved in dimethyl sulfoxide (DMSO) at a concentration of 25 mg/mL, and stored at –20 °C prior to being assayed.

HepG2 human hepatoma cells were grown in EMEM medium (Sigma–Aldrich, Mississauga, ON) containing 10% (v/v) FBS in a humidified incubator at 37 °C with 5% CO<sub>2</sub>. Once 70–80% confluent, the cells were detached with 0.25% trypsin and seeded onto 96-well tissue culture plates (BD Biosciences, Mississauga, ON). After 24 h of incubation, the cells were treated in triplicate in EMEM medium containing 0.5% (v/v) lipid depleted foetal calf serum (Sigma–Aldrich), cholesterol (10 μg/mL), 25-hydroxylcholesterol (1 μg/mL) and the indicated concentration of potato extract. Cells treated with water, PBS and DMSO were used as negative controls and cells treated with 15 μg/mL of berberine were used as a positive control. After 16 h of treatment, the cells were washed twice with PBS and cultured in EMEM medium that contained 0.5% lipid depleted foetal calf serum and 5 μg/mL BODIPY-labelled low-density lipoprotein cholesterol (Invitrogen, CA) for 4–5 h. Afterward, cells were washed twice with PBS. The plates were read on a Varioskan fluorescent plate reader using excitation and emission wavelengths of 490 and 515 nm, respectively. LDL-cholesterol uptake was calculated as the percentage of fluorescence increment as compared with the DMSO negative control.

## 2.10. In vitro evaluation of neuroprotective activity

Potato peel, tuber and granule extracts prepared as described above (in Section 2.9) were used for evaluation of neuroprotective activity in cortical neurons.

### 2.10.1. Animal care

Experiments involving the use of animals were approved by the University Committee on Laboratory Animals (Protocol #09–030, Dalhousie University) and were conducted in accordance with the guidelines set by the Canadian Council on Animal Care. Embryonic day 15 timed pregnant CD1 out-bred mice were obtained from Charles River Laboratories (Wilmington, MA).

### 2.10.2. Preparation of primary cortical neuron cultures

Primary cortical neuron cultures were prepared from cerebral cortices of wild-type (WT) CD1 mouse embryos as described previously (Katchanov et al., 2001) with the following modifications. Pregnant CD1 females were heavily anaesthetised under isoflurane vapour (Benson Medical Industries Inc.; Markham, ON) before decapitation. Embryonic day 16 (E16) fetuses were immediately removed from sacrificed pregnant females by cesarean section and placed in a medium sized Petri dish filled with ice-cold Hank's Balanced Salt Solution (HBSS, GIBCO). Brains were cleared of meninges and cortices were isolated under a dissecting microscope. Cortices from each embryo were placed in individual wells of a 24-well plate (Corning; Lowell, MA), each submerged in 1 mL of ice-cold HBSS. Under sterile conditions, the tissue was briefly minced, transferred to 15 mL sterile conical tubes (Corning) and centrifuged at 350g for 3 min at room temperature. Dissecting solution was discarded before cortical neurons were dissociated by a 15 min incubation in 1 mL of 0.1% trypsin–EDTA solution

(Invitrogen) at 37 °C. Trypsinisation was inhibited by the addition of 1 mL of Neurobasal medium (Invitrogen) with 10% FBS and 0.01% DNase I. Tubes were mixed briefly and then cells were centrifuged again at 350g for 3 min. The supernatants were discarded and each cell pellet was suspended in 1 mL of cortical neuron medium (Neurobasal medium with 10% foetal bovine serum (FBS, GIBCO), 2% B27 supplement (Invitrogen), 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, GIBCO), 1 mM L-glutamine (Invitrogen), and 1% Gentamycin (Invitrogen)), triturated 10 times and counted using trypan blue exclusion and a hemocytometer. Cortical neurons were plated in 96-well plates (Corning) that were pre-coated with poly-D-lysine (PDL, Sigma–Aldrich) according to the procedure described by the manufacturer. Briefly, plates were coated immediately before use with 100 µg/mL PDL for 5–10 min (50 µL/well), washed three times with tissue-culture grade water and left to dry for 2 h before cells were introduced. Cortical neurons were plated at a concentration of  $1 \times 10^6$  cells/mL (100 µL/well) and medium was completely changed the day after plating to serum-free cortical neuron medium (Neurobasal medium with 2% B27 supplement, 5 mM HEPES, 1 mM L-glutamine, and 1% Gentamycin), which was replaced every 3 days in culture. Cultures were maintained in a humidified, 37 °C incubator with 5% CO<sub>2</sub>. Experiments were performed on the ninth day *in vitro* (DIV9).

#### 2.10.3. Neuroprotection assay

Lactate dehydrogenase is a stable cytosolic enzyme that is released by necrotic cells upon membrane damage. The membrane integrity of cortical neurons was assayed by measuring the release of lactate dehydrogenase (LDH) using the Cytotoxicity Detection KitPLUS (Roche Applied Science; Indianapolis, IN). This assay kit detects LDH released into culture supernates by a coupled enzymatic reaction that converts a tetrazolium salt into a red formazan product. Resulting formazan was detected using an ELx800 UV spectrophotometer (Bio-tek Instruments Inc.; Winooski, VT). Positive (100% LDH release) and negative (spontaneous LDH release) controls were prepared in triplicate according to the manufacturer's instructions. Primary cortical neuron cultures were prepared as described above. Cortical neuron cultures were exposed to vehicle (0.1% DMSO) or polyphenolic-enriched potato extract at a concentration of 1 µg/mL in serum-free cortical neuron medium for a period of 12 h on DIV8 before they were subjected to oxygen glucose deprivation (OGD). Glucose-free medium (glucose-free Dulbecco's Modified Eagle Medium (Invitrogen)) containing extract (1 µg/mL) was placed in a 96-well plate and equilibrated to 0% oxygen in a modular chamber incubator (Billups-Rothenberg; Del Mar, CA). The chamber was flushed for 4 min at 20 L/min with an anoxic gas mixture (5% CO<sub>2</sub> and Balanced N<sub>2</sub>) (PraxAIR; Dartmouth, NS) using a step-down pressure system and placed in a humidified, 37 °C incubator for 12 h. Cortical neuron medium was replaced with OGD-medium (anoxic and glucose-free) and the cultures were placed in the modular chamber incubator. The chamber was flushed again with anoxic gas and placed inside a humidified, 37 °C incubator for 12 h. Following, cell culture supernates were collected on DIV9 for determination of released LDH. Absorbance was measured at 490 nm with a reference wavelength of 620 nm. Percentage of total LDH release was calculated by following the instructions provided by the manufacturer. Background was subtracted and LDH release in each sample was expressed as a percentage of the positive control. See Fig. 5A for a diagram of the experiment timeline.

#### 2.10.4. Statistical analysis

Results are expressed as mean ± SEM (standard error of the mean) of multiple determinations performed using cultures derived from eight animals. Data were analysed using Prism 4 software for Macintosh (GraphPad Software; La Jolla, CA). Group

differences were analysed using a one-way ANOVA, and when significant, Tukey's multiple comparisons post hoc test was employed. Differences calculated between mean values of each group were considered statistically significant when  $p \leq 0.05$ .

### 3. Results and discussion

#### 3.1. <sup>1</sup>H NMR based metabolomic profiling

A visual comparison of proton NMR spectra of extracts from selected potato clones 1, 8, 9, 11, 12, and 13 is shown in Fig. 2A. The levels of phenolic components in tuber water extracts were revealed to be similar among selections, but varied more in methanol extracts. Significant differences were observed in peel methanol extracts from the four representative pigmented potato selections.

Multivariate analysis (Partial Least Square Discriminant Analysis, PLS-DA) revealed that the metabolite profiles of methanol extracts were more closely related to the colours of potatoes (Fig. 2B). Targeted profiling of small molecule metabolites (data not shown, see Supplement Fig. 1) from tuber water extracts revealed some variability among potato samples with similar colours. However, for some metabolites, such as fumarate, glucose, and fructose, the yellow or off-white potato varieties appeared to produce more than the purple or red coloured potatoes. On the other hand, the purple and red potatoes contained higher levels of asparagine, alanine, glutamate, glutamine, valine, and sucrose. These water-soluble metabolites quantified from the tuber were presented at different levels in pigmented (purple or red) and yellow or unpigmented potatoes, and contributed to the separation of these two groups. This preliminary finding in proton NMR-based metabolite profiling indicates that variation of some of the small molecule primary metabolites is associated with the pigmentation in potatoes.

#### 3.2. Total phenolics content

The levels of total phenolic components measured for these potato samples were at 3.0–12.5, 1.6–8.4, and 1.2–7.9 mg gallic acid equivalent (GAE)/g dry sample for peel, tuber, and granule, respectively (Table 1). Except for a few clones, most potato selections contained greater amount of phenolics in the peel than in the tuber, often twice as much. Granules seem to retain the total phenolic components for the majority of potato clones tested.

The total level of phenolics was highly correlated with the colour of potato clone. Purple and red pigmented potato clones contained higher levels of phenolics in both peel and tuber than observed in yellow or unpigmented clones.

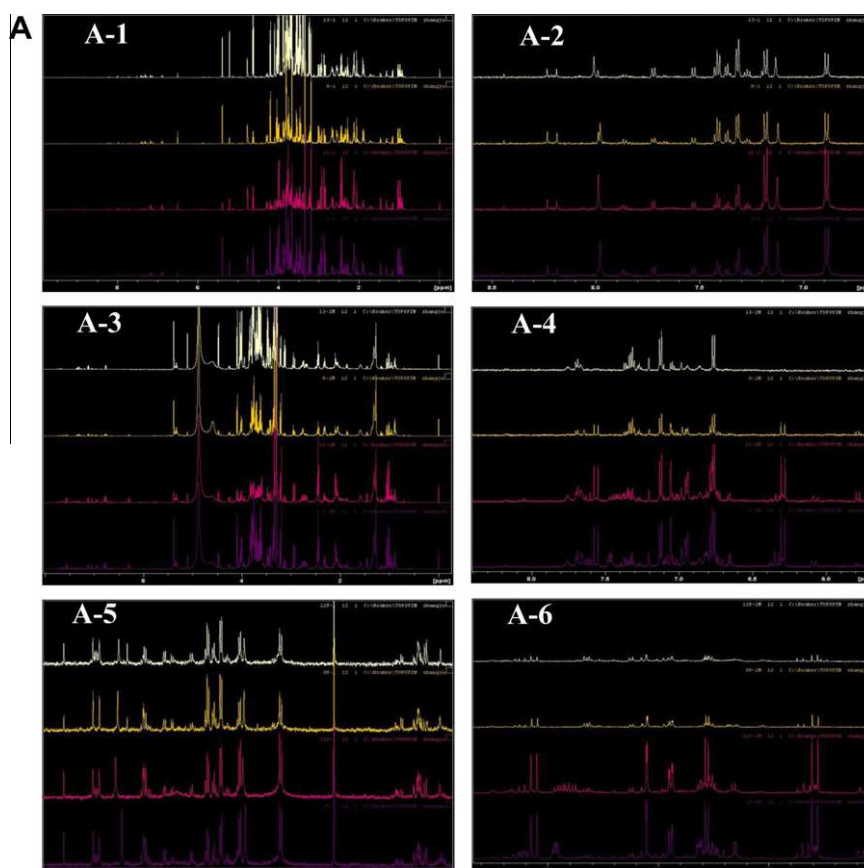
#### 3.3. Chlorogenic acid content

Chlorogenic acid has been reported to be the major phenolic components in specialty potato selections, accounting for 50–70% of the total phenolics (Reddivari, Hale, & Miller, 2007). The levels of chlorogenic acid in our 20 potato clones were found to range from 0.33 to 8.67 mg/g dry sample in peel, 0.04–4.26 mg/g in tuber, and 0.01–3.14 mg/g in granule (Table 1). Similar trends were found related to its presence in peel vs. tuber, and also the pigmented vs. yellow or unpigmented clones, as described above for the total phenolics.

#### 3.4. Anthocyanins content

Two different methods were used for anthocyanin quantification in these 20 potato clones. In the first method, an attempt was made to measure the quantity of original anthocyanins without the





**Fig. 2.**  $^1\text{H}$  NMR spectra and metabolomic profiling results. A: representative  $^1\text{H}$  NMR spectra of tuber and peel extracts (A-1 and A-2, tuber water extracts; A-3 and A-4, tuber methanol extracts; A-5, peel water extracts; A-6, peel methanol extracts). The colour of spectrum represents the colour of potato clone). B: PLS-DA plots based on global and targeted profiling of tuber extracts (B-1, water extracts; B-2, methanol extracts; B-3, targeted profiling of water extracts; B-4, loading plot of targeted profiling data. The colour of data point for B-1 to B-3 represents the colour of potato clone). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sample preparation process involving hydrolysis. Considering that red or pink potatoes (peel and tuber) contain pelargonidin-3-(p-coumaroylrutinoside)-5-glucoside as the main anthocyanin component, while the purple or blue potatoes contain petunidin-3-(p-coumaroylrutinoside)-5-glucoside as the predominant anthocyanin (Lewis, Walker, Lancaster, & Sutton, 1998), we used pelargonidin-3-glucoside and petunidin-3-glucoside as standards to quantify the total anthocyanins in these 20 potato clones (Table 1). Among the red or pink potatoes, clone 15 contained the highest amount of anthocyanins, with a total of 25.79 mg/g dry weight in peel, 14.42 mg/g in tuber, and 10.82 mg/g in granule (measured as pelargonidin-3-glucoside equivalents). In the purple or blue potato group, clone 17 had the highest level of anthocyanins (measured as petunidin-3-glucoside equivalents): 16.99 mg/g dry weight in peel, 7.33 mg/g in tuber, and 4.09 mg/g in granule. No anthocyanins were detected in the yellow or unpigmented potatoes.

In order to probe a larger range of anthocyanins, conversion to aglycones using hydrolysis and quantification using six anthocyanidin standards (malvidin, petunidin, delphinidin, peonidin, cyanidin, and pelargonidin) was done. The results are also listed in Table 1, with the total anthocyanins being the sum of the six anthocyanidins. The levels of anthocyanidins were found to be lower than anthocyanins, which is likely due to losses during hydrolysis. In the 20 potatoes, 12 clones were found to contain appreciable amount of anthocyanidins. Clone 17 had the highest levels, with 4.39 mg/g dry weight in peel, 2.99 mg/g in tuber, and 2.86 mg/g in granule. In consideration of overall levels of anthocyanidins in peel, tuber, and granule, clone 15 had the highest of the red or pink potato group,

with total anthocyanidins observed at levels of 1.42, 1.21, and 1.13 mg/g dry weight, respectively. This second method of anthocyanin quantification further confirmed that pelargonidin was the major anthocyanidin for red or pink potatoes, while petunidin was predominant in purple or blue potatoes.

There were differences in the levels of anthocyanidins between peels and tubers. For all the cultivars examined, anthocyanidin levels were higher in the peel than in the tuber. In four clones, 14, 15, 16 and 17 the level of anthocyanidins in the tuber was greater than 65% of that in the peel. The anthocyanidin levels in the clone 15 tuber were 85% of that found in the peel. These results are explained by the genetic variation in these cultivars that promotes deposition of pigment within the flesh of the tuber.

The clones 1 and 2 differed in ploidy; the former was tetraploid and the latter was diploid. These two cultivars showed similar distributions of anthocyanidins, with the diploid cultivar producing slightly higher levels.

The tubers from 16 out of 20 cultivars were used for granule production. The data in Table 1 show that anthocyanidin levels remained high after granule processing for cultivars that already had high levels of anthocyanidins. However, for cultivars with low levels of anthocyanidins, granule processing resulted in a loss of anthocyanidins, below the level of detection. There was also loss of anthocyanidins from the high producing clones, but the levels remained appreciable. For example, clone 17 had 2.86 mg/g dry weight of anthocyanidins after granule processing. In addition, the distribution of anthocyanidins was similar among granules and the freeze-dried skin and flesh samples.



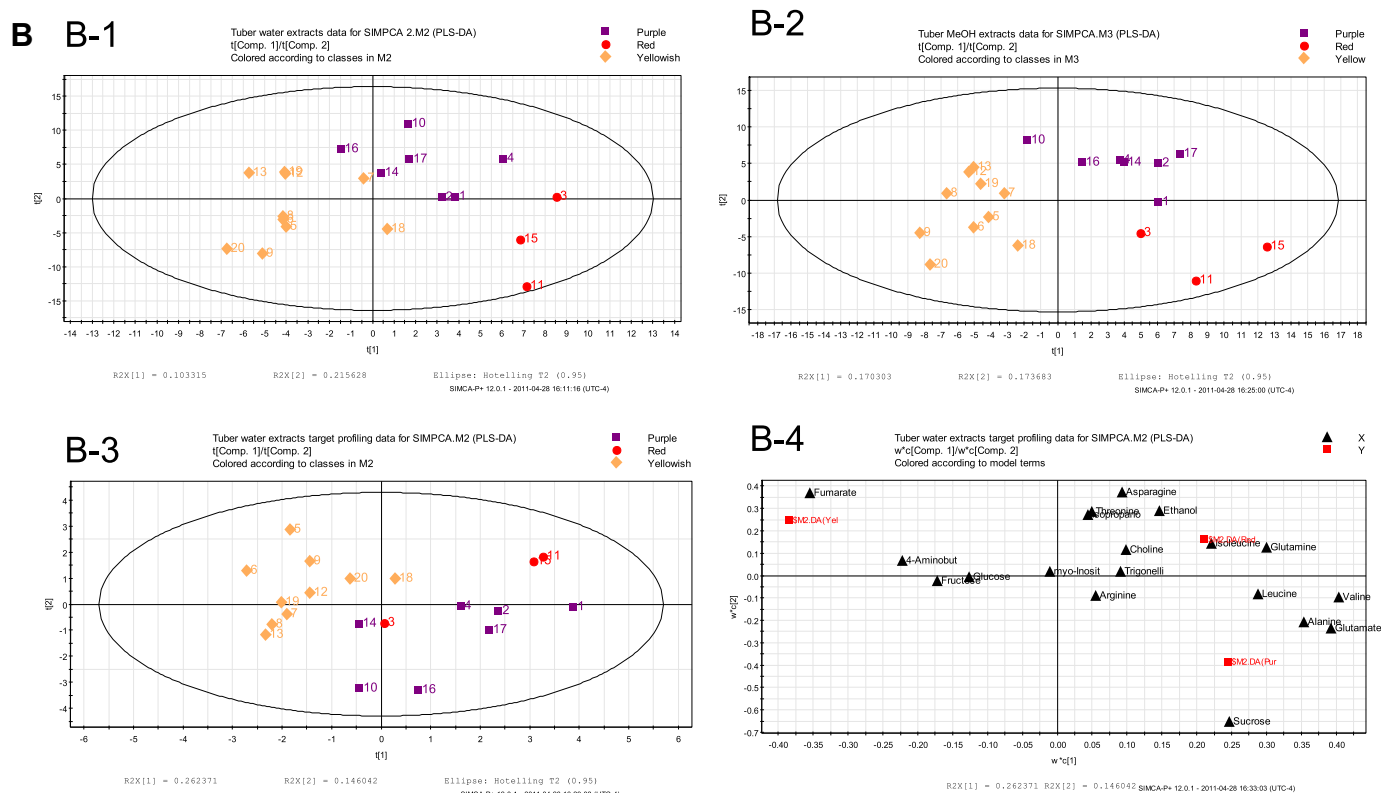


Fig. 2 (continued)

The results of this study also showed that the potatoes produced levels of anthocyanins that reached levels similar to those observed in blueberries and raspberries. Levels of anthocyanidins in berries were found to be 3.11 mg/g dry weight for fresh blueberries and 2.13 mg/g dry weight for fresh raspberries using the same quantification method as performed in the present study (Hynes & Aubin, 2008), whereas our potato clone 17 had higher levels of anthocyanidins (4.39 mg/g dry weight in the peel and 2.99 mg/g dry weight in the tuber). Unlike berries, potatoes are served as part of a main course meal and therefore incorporation of pigmented-flesh potatoes in meals can provide additional sources of anti-oxidants in the diet. Potatoes cost less to produce and result in higher yields that can be an economical source of raw material for extraction of anthocyanins. A problem associated with potato is the leaching of anthocyanins with some processing methods (e.g. boiling). Therefore finding that dried potato granules retain anthocyanins provides a product that can be used for functional food processing.

### 3.5. Glycoalkaloid content

Glycoalkaloids such as  $\alpha$ -chaconine and  $\alpha$ -solanine are minor components in potatoes with food safety concerns. In order to assess the overall health related attributes for the 20 potato clones, the glycoalkaloids were quantified by using the HPLC-MSD method (results displayed in Table 1). Similar to the other components discussed above, all peel samples contained much higher levels of glycoalkaloids than tubers. The peels of unpigmented clones (7 and 18) had the highest level of glycoalkaloids ( $\alpha$ -chaconine 4.07 mg/g dry weight for clone 7 and  $\alpha$ -solanine 3.23 mg/g for clone 18). Tubers in general contained much less glycoalkaloids, and interestingly red or purple potatoes appeared to have higher levels when compared with either the purple or blue group, or the yellow or unpigmented group. Clone 11 had the highest level of glycoalkaloids in all tuber samples (1.33 and 2.37 mg/g dry weight for  $\alpha$ -

chaconine and  $\alpha$ -solanine, respectively), and also granule samples (0.35 and 0.64 mg/g dry weight for  $\alpha$ -chaconine and  $\alpha$ -solanine, respectively).

The granulation process greatly reduced glycoalkaloid levels in the tuber, by 50–90%. As the granules still retained considerably high level of beneficial phenolics, the granulation process could be used as a step to reduce of undesirable glycoalkaloids in potato based functional food products.

### 3.6. DPPH radical scavenging activity

DPPH radical scavenging assay was used to measure the antioxidant activity of the 20 potato extracts. As shown in Fig. 3, the peels possessed the most potent antioxidant activity in all potato clones. Pigmented potato clones also outperformed the yellow or unpigmented clones, owing their higher levels of phenolic content. The tuber of clone 15 had the greatest DPPH radical scavenging activity of all the tuber samples, measured at a level of 15.17 mg Trolox equivalents (TE)/g dry weight. In comparison, the DPPH radical scavenging activities of blueberry, raspberry, strawberry, and cranberry we measured and fell in the range of 40–60 mg TE/g dry weight (data not shown). Therefore the potato clone 15 showed antioxidant activity up to 35% of that measured for berries, well recognised as a natural source of potent antioxidant.

It is also interesting to note that antioxidant activity was largely retained in several granule samples, including those from clone 3 (red), 4 (purple), 15 (red), and 17 (purple), demonstrating the potential for developing granule products with high antioxidant activity from these potato selections.

### 3.7. In vitro liver LDL cholesterol uptake activity

As shown in Fig. 4, our potato extracts from different clones enhanced LDL cholesterol uptake in liver HepG2 cells. Tuber and granule extracts had slightly higher activity than peel for most of

**Table 1**

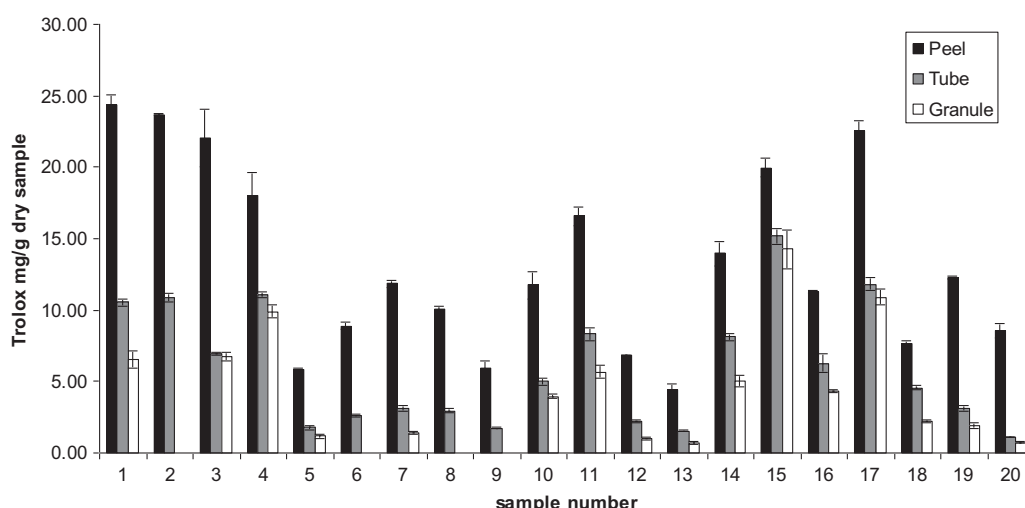
Total phenolics, chlorogenic acid, anthocyanins, and glycoalkaloids contents in peels, tubers, and granules of 20 potatoes.

Clone		TPH <sup>a</sup>	CHL <sup>b</sup>	Tpetu <sup>c</sup>	Tpelar <sup>d</sup>	Malv <sup>e</sup>	Petu <sup>f</sup>	Delp <sup>g</sup>	Peon <sup>h</sup>	Cyan <sup>i</sup>	Pelarg <sup>j</sup>	Total <sup>k</sup>	$\alpha$ -chac <sup>l</sup>	$\alpha$ -sola <sup>m</sup>
1	Peel	12.534 ± 0.609	5.752 ± 1.133	10.247 ± 1.768		0.087	2.147	0.045	0.159	0.065	0.017	2.520	0.602 ± 0.065	0.509 ± 0.059
	Tuber	5.318 ± 0.331	1.743 ± 0.261	2.923 ± 0.360		0.011	0.451	0.017	0.049	0.029	0.022	0.579	0.273 ± 0.032	0.332 ± 0.045
	Granule	3.857 ± 0.414	0.923 ± 0.187	1.627 ± 0.238		0.012	0.570	0.021	0.063	0.035	0.006	0.707	0.047 ± 0.007	0.064 ± 0.012
2	Peel	12.079 ± 0.420	6.465 ± 0.553	10.253 ± 0.502		0.132	2.663	0.069	0.223	0.083	0.015	3.184	0.811 ± 0.026	0.665 ± 0.021
	Tuber	5.300 ± 0.152	2.125 ± 0.135	3.015 ± 0.149		0.018	0.641	0.021	0.064	0.041	0.005	0.791	0.361 ± 0.011	0.446 ± 0.013
	Granule	4.073 ± 0.145	1.342 ± 0.256		24.045 ± 1.662	0.009			0.023	0.005	1.405	1.442	0.703 ± 0.046	0.808 ± 0.045
3	Peel	12.015 ± 0.686	8.660 ± 0.523		9.263 ± 0.190	0.017			0.010	0.004	0.547	0.578	0.241 ± 0.008	0.598 ± 0.014
	Tuber	4.191 ± 0.038	2.171 ± 0.062		4.458 ± 0.721				0.008	0.003	0.481	0.493	0.050 ± 0.007	0.132 ± 0.019
	Granule	4.073 ± 0.145	1.342 ± 0.256											
4	Peel	10.291 ± 0.249	3.992 ± 0.456	9.775 ± 0.908		0.091	2.631	0.060	0.187	0.084	0.011	3.063	1.036 ± 0.076	0.959 ± 0.072
	Tuber	5.425 ± 0.319	1.919 ± 0.098	5.516 ± 0.066		0.018	0.800	0.037	0.079	0.061	0.006	1.001	0.188 ± 0.008	0.343 ± 0.016
	Granule	5.234 ± 0.454	1.854 ± 0.393	3.134 ± 0.477		0.022	1.171	0.058	0.122	0.086	0.010	1.468	0.061 ± 0.008	0.121 ± 0.019
5	Peel	3.002 ± 0.152	0.728 ± 0.049				0.001					0.001	1.658 ± 0.133	0.994 ± 0.078
	Tuber	1.857 ± 0.090	0.094 ± 0.009										0.545 ± 0.052	0.420 ± 0.040
	Granule	1.521 ± 0.083	0.039 ± 0.006										0.116 ± 0.019	0.079 ± 0.016
6	Peel	4.218 ± 0.240	1.225 ± 0.075				0.001		0.008	0.002	0.001	0.012	1.595 ± 0.044	0.924 ± 0.033
	Tuber	2.188 ± 0.127	0.165 ± 0.020										0.295 ± 0.023	0.251 ± 0.020
	Granule	5.766 ± 0.223	2.783 ± 0.252										4.065 ± 0.312	2.310 ± 0.159
7	Peel	1.824 ± 0.054	0.398 ± 0.016										0.937 ± 0.021	0.628 ± 0.022
	Tuber	1.504 ± 0.139	0.126 ± 0.019										0.088 ± 0.019	0.048 ± 0.012
	Granule	1.504 ± 0.139	0.126 ± 0.019											
8	Peel	5.474 ± 0.280	3.804 ± 0.289	1.165 ± 0.104		0.010	0.149	0.002	0.033	0.008	0.133	0.335	1.127 ± 0.074	0.620 ± 0.032
	Tuber	1.920 ± 0.220	0.546 ± 0.011	0.176 ± 0.002		0.002	0.031		0.008	0.002	0.005	0.047	0.062 ± 0.002	0.060 ± 0.002
	Granule	2.942 ± 0.115	1.108 ± 0.111						0.001		0.003	0.003	1.120 ± 0.058	0.968 ± 0.267
9	Peel	2.085 ± 0.180	0.212 ± 0.007										0.207 ± 0.006	0.462 ± 0.017
	Tuber	2.085 ± 0.180	0.212 ± 0.007											
	Granule	2.085 ± 0.180	0.212 ± 0.007											
10	Peel	6.094 ± 0.501	4.266 ± 0.191	2.712 ± 0.093		0.041	0.721	0.010	0.064	0.017	0.003	0.855	2.216 ± 0.016	1.498 ± 0.005
	Tuber	2.695 ± 0.024	1.190 ± 0.122	1.814 ± 0.105		0.013	0.271	0.009	0.029	0.013	0.002	0.335	0.365 ± 0.021	0.299 ± 0.018
	Granule	2.074 ± 0.125	0.836 ± 0.073	0.883 ± 0.088		0.017	0.347	0.010	0.043	0.016	0.003	0.436	0.152 ± 0.014	0.125 ± 0.014
11	Peel	9.286 ± 0.658	5.033 ± 0.557		16.081 ± 1.842		0.018		0.037	0.015	1.143	1.214	2.752 ± 0.232	2.909 ± 0.220
	Tuber	4.234 ± 0.178	1.933 ± 0.028		5.221 ± 0.220	0.001	0.026		0.019	0.014	0.378	0.438	1.325 ± 0.063	2.369 ± 0.144
	Granule	3.455 ± 0.185	1.424 ± 0.146		3.082 ± 0.273		0.001		0.014	0.013	0.372	0.400	0.347 ± 0.040	0.639 ± 0.083
12	Peel	3.861 ± 0.118	0.867 ± 0.049									0.001	0.376 ± 0.045	0.220 ± 0.028
	Tuber	1.789 ± 0.124	0.211 ± 0.004									0.001	0.053 ± 0.001	0.034 ± 0.001
	Granule	1.244 ± 0.059	0.079 ± 0.023										0.005 ± 0.001	0.003 ± 0.001
13	Peel	3.816 ± 0.173	0.329 ± 0.004										0.703 ± 0.015	0.350 ± 0.007
	Tuber	1.635 ± 0.107	0.097 ± 0.010										0.180 ± 0.009	0.153 ± 0.008
	Granule	1.267 ± 0.124	0.026 ± 0.006										0.012 ± 0.002	0.009 ± 0.001
14	Peel	8.653 ± 0.643	3.173 ± 0.035	6.549 ± 0.070		0.103	1.973	0.024	0.003	0.002	0.011	2.115	1.313 ± 0.014	0.729 ± 0.009
	Tuber	4.824 ± 0.069	1.388 ± 0.112	4.684 ± 0.259		0.045	1.561	0.039	0.004	0.001	0.008	1.658	0.400 ± 0.030	0.361 ± 0.031
	Granule	2.929 ± 0.134	0.699 ± 0.133	2.128 ± 0.219		0.025	1.068	0.028	0.006	0.002	0.001	1.131	0.089 ± 0.010	0.064 ± 0.007
15	Peel	10.728 ± 0.497	6.195 ± 0.306		25.788 ± 0.885				0.018	0.007	1.398	1.423	1.613 ± 0.056	1.397 ± 0.057
	Tuber	8.369 ± 0.350	4.258 ± 0.173		14.421 ± 0.138				0.009	0.006	1.195	1.209	1.129 ± 0.013	1.277 ± 0.019
	Granule	7.868 ± 0.323	3.142 ± 0.089		10.823 ± 0.560				0.007	0.006	1.121	1.134	0.309 ± 0.021	0.340 ± 0.025
16	Peel	5.467 ± 0.138	2.076 ± 0.014	3.140 ± 0.032		0.040	0.643	0.007	0.007		0.003	0.699	1.721 ± 0.031	0.822 ± 0.016
	Tuber	4.030 ± 0.111	1.358 ± 0.160	1.706 ± 0.170		0.016	0.455	0.010	0.004	0.002	0.002	0.489	0.179 ± 0.010	0.134 ± 0.009
	Granule	2.679 ± 0.043	0.654 ± 0.098	0.706 ± 0.090		0.009	0.287	0.007	0.003	0.003	0.001	0.309	0.061 ± 0.011	0.045 ± 0.009
17	Peel	12.068 ± 0.449	5.071 ± 0.232	16.994 ± 0.465		0.257	3.708	0.040	0.307	0.061	0.016	4.389	0.816 ± 0.020	1.108 ± 0.033
	Tuber	5.737 ± 0.295	1.908 ± 0.195	7.331 ± 0.483		0.107	2.516	0.044	0.236	0.072	0.012	2.986	0.069 ± 0.002	0.210 ± 0.005
	Granule	5.505 ± 0.030	1.416 ± 0.114	4.093 ± 0.275		0.081	2.370	0.048	0.267	0.084	0.014	2.864	0.019 ± 0.002	0.076 ± 0.011
18	Peel	4.127 ± 0.090	1.549 ± 0.082				0.004					0.004	3.624 ± 0.303	3.229 ± 0.262
	Tuber	3.843 ± 0.347	0.761 ± 0.011										1.131 ± 0.027	1.378 ± 0.036
	Granule	2.037 ± 0.063	0.301 ± 0.076										0.283 ± 0.071	0.302 ± 0.084
19	Peel	5.758 ± 0.106	3.305 ± 0.168						0.001		0.001	0.002	0.614 ± 0.023	0.632 ± 0.026
	Tuber	2.359 ± 0.115	0.353 ± 0.030				0.001					0.001	0.040 ± 0.004	0.041 ± 0.005
	Granule	2.211 ± 0.066	0.213 ± 0.008										0.016 ± 0.001	0.017 ± 0.001
20	Peel	4.770 ± 0.304	2.392 ± 0.175										2.118 ± 0.077	2.194 ± 0.097
	Tuber	1.716 ± 0.115	0.038 ± 0.003										0.158 ± 0.006	0.185 ± 0.008
	Granule	1.807 ± 0.212	0.008 ± 0.000										0.031 ± 0.000	0.039 ± 0.001

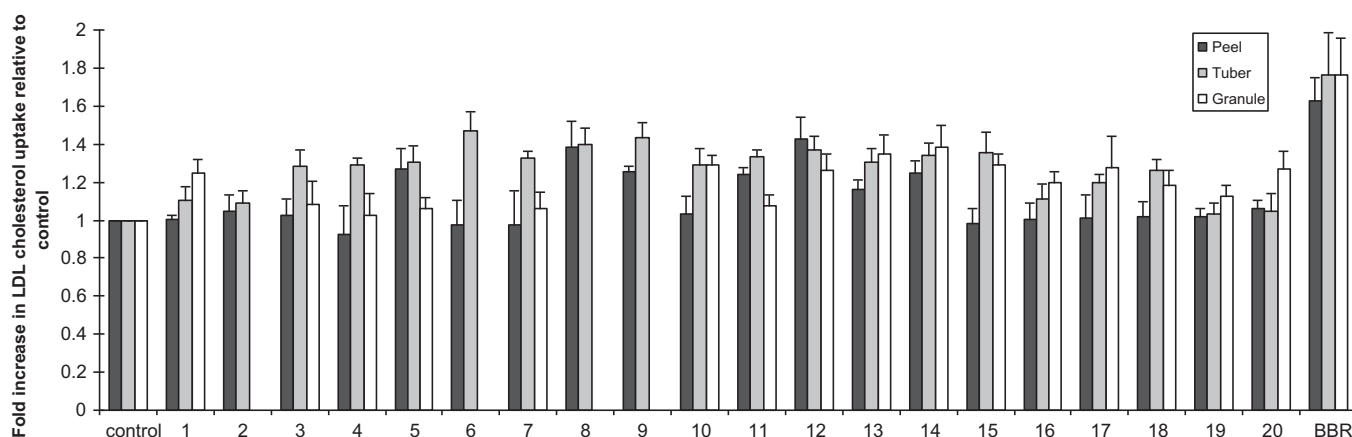
<sup>a</sup> TPH: total phenolics reported as gallic acid equivalents, mg/g dry weight.<sup>b</sup> CHL: chlorogenic acid, mg/g dry weight.<sup>c</sup> Tpetu: total anthocyanins reported as petunidin-3-glucoside chloride equivalents, mg/g dry weight.<sup>d</sup> Tpelar: total anthocyanin reported as pelargonidin-3-glucopyranoside chloride equivalents, mg/g dry weight.<sup>e</sup> Malv: malvidin chloride, mg/g dry weight.<sup>f</sup> Petu: petunidin chloride, mg/g dry weight.<sup>g</sup> Delp: delphinidin chloride, mg/g dry weight.<sup>h</sup> Peon: peonidin chloride, mg/g dry weight.<sup>i</sup> Cyan: cyanidin chloride, mg/g dry weight.<sup>j</sup> Pelarg: pelargonidin chloride, mg/g dry weight.<sup>k</sup> Total: Malv + Petu + Delp + Peon + Cyan + Pelarg, mg/g dry weight.<sup>l</sup>  $\alpha$ -Chac:  $\alpha$ -chaconine, mg/g dry weight.<sup>m</sup>  $\alpha$ -Sola:  $\alpha$ -solanine, mg/g dry weight.

the potato extracts that were evaluated. In addition, there was no correlation between liver LDL cholesterol uptake activity and the colour of the potato clone, implying that anthocyanins are not likely the bioactive components responsible for this effect. Further

investigation is needed for chemical purification and *in vitro* efficacy verification using immortalised human liver cells, and importantly to determine the cholesterol-lowering effect in pre-clinical and clinical experiments.



**Fig. 3.** DPPH radical scavenging activity of potato extracts. Data are presented as Trolox equivalents in mg per gram of dry sample, mean  $\pm$  SD calculated from measurements of three replicated extracts.



**Fig. 4.** *In vitro* effect of potato extracts on LDL cholesterol uptake in the liver cell HepG2 in the presence of cholesterol and hydroxycholesterol. Cells were treated with potato extracted at a concentration of 25  $\mu$ g/mL. LDL cholesterol uptake activity was measured using LDL-BODIPY. The negative and positive control cells were treated with vehicle and berberine (BBR, a natural product), respectively.

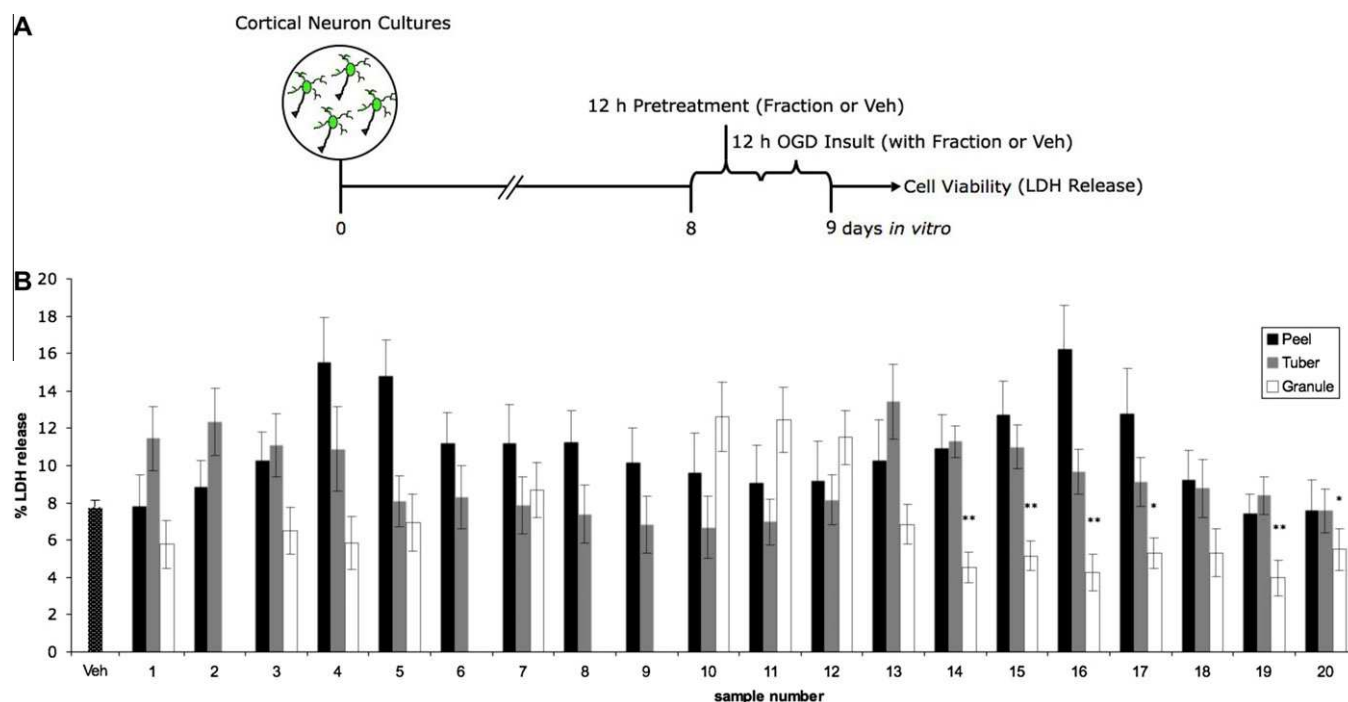
Blood cholesterol level is regulated by multiple mechanisms, including cholesterol absorption, synthesis, liver clearance, catabolism, secretion, and faecal excretion. To date, there are no natural products or drugs that can be used to lower cholesterol through increasing LDL cholesterol clearance in the liver. Therefore it is of great importance to discover and develop a novel product from a natural source, especially of food origin, that lowers blood cholesterol by increasing LDL receptor-mediated cholesterol clearance in the liver. Several potato extracts showed promising activity to increase LDL cholesterol uptake in HepG2 cells, potentially to lower blood cholesterol through a distinct mechanism of enhancing liver LDL cholesterol clearance.

### 3.8. *In vitro* neuroprotective activity

Primary cortical neuron cultures were prepared from E16 CD1 mice as described above and exposed to polyphenolic-enriched potato extracts (1  $\mu$ g/mL) on DIV8. Neuronal death was quantified immediately following a 12 h period of OGD in the presence of extract on DIV9 using the Cytotoxicity Detection KitPLUS. Resultant data were expressed as percentages of total LDH release, as compared with the positive control. Potato granule extracts of clone

14 ( $4.53 \pm 0.82\%$ ;  $n = 8$ ), 15 ( $5.15 \pm 0.79\%$ ;  $n = 8$ ), 16 ( $4.25 \pm 0.98\%$ ;  $n = 8$ ), 17 ( $5.29 \pm 0.81\%$ ;  $n = 8$ ), 19 ( $3.97 \pm 0.96\%$ ;  $n = 8$ ) and 20 ( $5.50 \pm 1.11\%$ ;  $n = 8$ ) significantly protected cortical neurons from cell death caused by OGD, as compared with vehicle (0.1% DMSO) replicates ( $7.73 \pm 0.42\%$ ;  $n = 8$ ) (Fig. 5B). The fact that only the granule extracts were neuroprotective may be due to the changing of components after the granulation process; as indicated above, granules contained less glycoalkaloids and yet retained phenolics. Therefore it is likely that the neurons better tolerated the granule, as compared with tuber and peel extracts, due to the lower level of glycoalkaloids.

In conclusion, we have conducted metabolomic profiling, total phenolics, chlorogenic acid, anthocyanins, and glycoalkaloids analyses on the 20 selected potato clones within our BioPotato research program. Pigmented potatoes in general contain higher levels of phenolic components, including chlorogenic acid and anthocyanins. The granulation process appeared to have no significant effect on these phenolics, as their levels were found to be largely retained in granules prepared from pigmented potato tubers. The pigmented potatoes also have higher antioxidant activity reaching up to 35% of that for berries, measured as the potency in scavenging DPPH radicals. Extracts of the 20 potato clones (peel, tuber, and



**Fig. 5.** *In vitro* protective effect of potato extracts in cortical neurons. (A) experiment timeline depicting fraction and OGD treatments on cortical neuron cultures. (B) Neuroprotective activity of potato extracts on cortical neuron cultures subjected to 12 h OGD. Cortical neuron cultures were exposed to extracts at a concentration of 1  $\mu\text{g/mL}$  for 12 h in serum-free conditions. Following, cells were subjected to OGD for 12 h in the presence of extract (1  $\mu\text{g/mL}$ ) and then culture supernates were collected for determination of LDH release. The values represent means  $\pm$  SEM of cells derived from eight embryos ( $n = 8$ ) ( $p < 0.05$ ,  $**p < 0.01$ ).

granule) were also evaluated for *in vitro* effect on liver LDL cholesterol uptake and the protection of cortical neurons from cell death caused by oxygen glucose deprivation. These potato extracts in general have mild activity in enhancing LDL cholesterol uptake in liver hepG2 cells, and can protect cortical neurons against OGD induced cell death, with six potato granule extracts showing significant neuroprotective effects. The bioactive components are yet to be further characterised; however our results show that the activities are not dependent on pigmentation of the potato clone. Therefore, the novel bioactivities identified from potatoes in this study warrant in-depth investigations in the future.

Taken together, our results provide further evidence in support of the enhanced health beneficial components in pigmented potato selections, as well as the importance of potato breeding for functional food or nutraceutical traits.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.foodchem.2011.08.065](https://doi.org/10.1016/j.foodchem.2011.08.065).

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