Influence of cultivar and year on phytochemical and antioxidant activity of potato (*Solanum tuberosum* L.) in Ontario

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Hu, C., Tsao, R., Liu, R., Sullivan, J. A. and McDonald, M. R. 2012. **Influence of cultivar and year on phytochemical and antioxidant activity of potato (*Solanum tuberosum* L.) in Ontario**. Can. J. Plant Sci. **92**: 485–493. Phytochemicals in coloured vegetables are responsible not only for the colour, but also for nutritional quality. In this study 11 cultivars of potato, with a wide range of skin and flesh colours, grown over two years were tested for the total phenolic content (TPC), total anthocyanin-content (TAC) and total antioxidant activity (TAA). Results showed significant variations among cultivars for both years. TPC was 1.2–3.6 mg gallic acid equivalent (GAE) g^{-1} dry weight (DW) in 2008 and 0.98–2.81 mg GAE g^{-1} DW in 2009. Total anthocyanin content was 0.70–1.92 mg cyanidin-3-glucoside equivalent (Cy3g E) in 2008 and 0.05–1.52 mg Cy3g E g^{-1} DW in 2009, respectively. The TAA also varied among different potato cultivars, with values of 12 to 64 and 6.3 to 20 μmol ascorbic acid equivalents (AAE) g^{-1} DW in 2008 and 2009, respectively for the FRAP (ferric reducing/antioxidant power) assay, and from 42 to 168 and 75 to 174 μmol trolox equivalents (TE) g^{-1} DW in 2008 and 2009 in the ORAC (oxygen radical absorption capacity) assay, respectively. The purple fleshed tubers, such as 'Mackintosh Black', demonstrated the highest antioxidant activities, indicating that anthocyanins are important antioxidants. Our results suggest that purple/red potato cultivars have a greater potential as functional foods for enhanced human health benefits.

Key words: Anthocyanins, pigmented potatoes, phenolic content

Hu, C., Tsao, R., Liu, R., Sullivan, J. A. et McDonald, M. R. 2012. **Effet du cultivar et de l'année sur la concentration de composés phytochimiques et l'activité des antioxydants chez la pomme de terre (*Solanum tuberosum* L.) en Ontario**. Can. J. Plant Sci. **92**: 485–493. Les composés phytochimiques engendrent non seulement la couleur, mais aussi la qualité nutritive des légumes pigmentés. Dans le cadre de cette étude, les auteurs ont déterminé la concentration totale de composés phénoliques (CTP), la concentration totale d'anthocyanine (CTA) et l'activité totale des antioxydants (ATA) de 11 variétés de pomme de terre à chair et à pelure de couleur très variable, cultivées pendant deux ans. Les résultats révèlent d'importantes variations entre les cultivars au cours des deux années. La CTP se situait entre 1,2 et 3,6 mg d'équivalent d'acide gallique (EAG) par gramme de poids sec (PS) en 2008, et entre 0,98 et 2,81 mg de EAG par g de PS en 2009. La concentration totale d'anthocyanine s'établissait à 0,70-1,92 mg d'équivalent de cyanidine-3-glucoside (Cy3g E) par g de PS en 2008, et à 0,05–1,52 mg de Cy3g E par g de PS en 2009. L'ATA varie aussi avec le cultivar, avec des valeurs de 12 à 64 et de 6,3 à 20 μmol d'équivalent d'acide ascorbique par g de PS en 2008 et 2009, respectivement avec le dosage du pouvoir antioxydant/de réduction des ions ferriques, et des valeurs de 42 à 168 et de 75 à 174 μmol d'équivalent de trolox par g de PS en 2008 et 2009 avec le dosage de la capacité d'absorption des radicaux oxygénés, respectivement. Les tubercules à chair mauve comme la Mackintosh Black démontrent le plus fort potentiel antioxydant, signe que les anthocyanines sont d'importants antioxydants. Les résultats laissent croire que les pommes de terre mauves/rouges présentent les plus grandes possibilités comme aliment fonctionnel, en raison d'effets plus bénéfiques sur la santé.

Mots clés: Anthocyanines, pommes de terre pigmentées, teneur en composés phénoliques

Potato is one of the most important staple foods of the human diet worldwide after wheat and rice (Woolfe and Poats 1987). Potatoes are a source of dietary starch, but are also rich in proteins, fibre, and minerals such as calcium, potassium, and phosphorus (Kolasa 1993). Plant secondary metabolites such as flavonoids and carotenoids have also been found in potato (Friedman 1997; Lewis et al. 1998), and their potential health benefits to humans have been studied in recent years. Phytochemicals such as phenolic compounds are strong antioxidants in vitro (Rice-Evans et al. 1996; Yang et al. 2004), and have been shown to improve the antioxidant

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Abbreviations: **AAE**, ascorbic acid equivalent; **Cy3gE**, cyanidin-3-glucoside equivalent; **DW**, dry weight; **FeCl3**, ferric chloride; **FRAP**, ferric reducing/antioxidant power; **GAE**, gallic acid equivalent; **ORAC**, oxygen radical absorption capacity; **TAA**, total antioxidant activity; **TAC**, total anthocyanin content; **TPC**, total phenolic content

status of plasma in humans (Carbonneau et al. 1998; Serafini et al. 1998, 2000). Phytochemical antioxidants such as chlorogenic acid and anthocyanins help prevent breast and prostate cancers (Reddivari et al. 2007), colon cancer (Camire et al. 2009), and lower the risk of cardiovascular disease (Robert et al. 2006).

Several factors, including genetics (Al Saikhan et al. 1995; Reyes et al. 2005), environmental conditions during the growing season (Reyes et al. 2005), maturity (Howard et al. 2000), and processing (Brown 2005), are known to affect the phytochemical contents and thus the antioxidant activity of vegetable crops. In particular, longer days and cooler temperatures were shown to increase the content of anthocyanins and total phenolics of purple and red fleshed potatoes by 1.4 and 2.5 times (Reyes et al. 2004). Cultivars of potato are known to contain different levels of phenolics, and potatoes with pigmented skin and flesh in particular have been shown to be a good source of antioxidants (Brown 2005; Han et al. 2006; Andre et al. 2007).

Increasing evidence shows that pigments in fruits and vegetables are always related to antioxidants (Delgado-Vargas et al. 2000; Hooper and Cassidy 2006), and antioxidants are key to the prevention of chronic diseases (McDevitt et al. 2005). This implies that fruit and vegetables with pigmented skin and/or flesh have higher levels of antioxidants. Both the skin and flesh can be pigmented in some potato cultivars (Reyes et al. 2005).

While the individual phenolic compounds may have different physiological roles, and their chemical identities and concentrations in plants have been analysed using sophisticated instrumentation, the total phenolic content (TPC), however, is frequently assessed using a simple and rapid spectrophotometric Folin-Ciocalteu method (Ronald et al. 2005). Because there are difficulties in measuring the true antioxidant status of food products in vivo, the antioxidant activities of foods or food components have been estimated using in vitro chemical models. There are two major mechanisms to explain how antioxidants deactivate the free radicals, one is hydrogen atom transfer, and the second is single electron transfer (Ronald et al. 2005). Two assays are commonly used to measure these mechanisms, the ORAC assay (oxygen radical absorption capacity) for the hydrogen atom transfer mechanisms and the FRAP assay FRAP (ferric reducing/antioxidant power) for the single electron transfer mechanisms. ORAC detects the inhibition of peroxyl radical capacity by antioxidants and it is considered to be the most biological relevant assay due to the peroxyl radical, which is the predominant free radical found in lipid oxidation in foods and biological samples (Ronald et al. 2005). The assay uses fluorescent markers and is very sensitive, and requires only a relatively low concentration of the sample. The FRAP assay measures the reducing power of the samples (Benzie and Szeto 1999), but does not measure compounds that act by radical quenching (Ou et al. 2002). Using the combination of FRAP and ORAC assays would be an effective way to detect the antioxidant capacities in most botanical samples. In addition, both the ORAC and FRAP assays are simple, reliable and used as standard methods for many chemical labs. The most abundant phenolic compounds, chlorogenic acid and anthocyanins, are distributed in the hydrophilic fraction of potato extracts (Brown 2005; Leo et al. 2008).

This study was conducted at the same site over 2 yr to determine the levels of antioxidants and phenolic compounds in a range of Ontario-grown potatoes, and to determine if variation in weather over 2 yr would have an important influence on the antioxidant levels in potatoes.

The specific objectives of the study were: (1) to determine the effect of cultivar on antioxidant activity, total anthocyanin content and total phenolic content in the hydrophilic fraction of potato tubers (2) to determine the consistency of levels of these groups of compounds over 2 yr to identify weather related effects, and (3) to determine the relationship between antioxidant activity and total phenolic content of potato.

MATERIAL AND METHODS

Field Production and Tuber Processing

Field trials were conducted at the Elora Research Station, University of Guelph (Elora, Ontario, Canada, lat. 43°41′N, long. 80°26′W) on a Conestoga silt loam soil over 2 yr in 2008 and 2009. The potatoes were planted between May 21 and May 26 in 2008 and May 19 and May 21 in 2009. A randomized complete block arrangement with four replicates per treatment was used. Replicated plots were a single row wide, and 5 m or 7.6 m long, with 25 cm spacing between seed pieces. Plots were fertilized with 20-10-10 NPK blend at 896 kg ha^{-1} at planting. The insecticide Admire (imidacloprid, 7.5–12 mL 100 m^{-1} row in furrow) and fungicide Quadris (azoxystrobin, 4–6 mL 100 m^{-1} row in furrow) were applied to the potato tubers at the time of planting. Herbicide application was: pre-emergence Sencor (thiophanate-methyl, 1.2–2.2 L ha^{-1}) + Dual Magnum (S-metolachlor, 1.25–1.75 L ha^{-1}). Curzate (Cymoxanil 60%, 225 g ha^{-1}) and Mancozeb (dithiocarbamates, 1.35–1.6 kg ha^{-1}) were applied to prevent late blight (caused by *Phytophthora infestans*) as needed based on weather conditions and potential for disease outbreak. Insecticides and fungicides were applied as needed during the season following OMAFRA recommendations (Ontario Ministry of Agriculture, Food and Rural Affairs 2010). Vines were top killed with Reglone (diquat, 1.7–2.3 L ha^{-1}) on 2008 Sep. 10 and 2009 Sep. 01. The trials were harvested mechanically between 2008 Sep. 22 and 26 and 2009 Oct. 13 (Sangre was harvested on Oct. 01). Immediately after harvest, tubers were stored in a temperature-controlled container ($\sim$13°C) during curing time. The temperature and

rainfall were measured at Elora Meteorological Research Station (located approximately 0.5 km from the potato plot) and the weather data are presented in Table 5. Temperature was measured using a thermistor [Campbell Scientific (Canada) Corp, Edmonton, AB, Canada] and the rainfall was collected using a tipping bucket rain gauge [Campbell Scientific (Canada) Corp, Edmonton, AB, Canada].

Potato tubers were stored at ambient temperature at the Elora Research Station for 3 wk, and then transferred to and stored at 4°C in a walk-in refrigerator at the Guelph Food Research Centre for another 2 wk prior to sampling. Descriptions of each cultivar for flesh and skin colours, shape, maturity, yield and storage quality of potato tubers used in this study are included in Table 1. A randomized complete block arrangement with four replicates per cultivar was used. Five medium-sized tubers were randomly selected from each replicate. Potato tubers were washed and surface-dried. Each whole clean potato tuber was cut into four to siz pieces and a total of 50–100 g wedges were collected from the five tubers, which were frozen in liquid nitrogen and subsequently stored in plastic bags at −20°C. Tubers were processed with their skin on due to the difficulty of uniformly peeling certain potato tubers. The frozen samples were freeze-dried in a bulk tray freeze dryer (Labconco Corporation, Kansas City, MO) for several days (4–7 d) until thoroughly lyophilized, and the freeze-dried samples were then finely ground with a mortar and pestle. The resulting powders were stored in 50-mL screw-capped plastic tubes (SARSTEDT) at −20°C until analysis.

Chemicals and Solvents

Gallic acid, *L*-ascorbic acid, 2,4,6-tripyridyl-S-triazine (TPTZ), the Folin-Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (trolox), fluorescein and 2,2′-azobis(2-amidinopropane) dihydrochloride were purchased from Sigma Chemical Co. (Oakville, ON). Ferric chloride ($FeCl_3$), ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) and sodium acetate were from Aldrich Chemical Co. (Milwaukee, WI). All HPLC grade solvents were from Caledon Laboratories Ltd (Georgetown, ON).

Sample Preparation and Extraction

For the extraction of phenolic compounds, 0.5 g freeze-dried powder of each potato cultivar was mixed with 10 mL 1% acetic acid in 80% methanol (methanol: distilled water: acetic acid = 80:19:1, vol/vol/vol) in a 15 mL screw-capped plastic tube (SARSTEDT), vortexed and sonicated in an ultrasonic bath twice, each for 5 min, before being loaded on an orbital shaker (ROTO-SHAKE GENIE, Scientific Industries) for 1 h. The tubes were then centrifuged at 3000 rpm for 10 min. The supernatant was filtered through a 0.45 μm syringe filter, and stored at −20°C until analysis.

Total Phenolic Content

A modified Folin-Ciocalteu method (Slinkard and Singleton 1977; Tsao et al. 2005) was used for the determination of TPC of the samples. Briefly, the extract (0.2 mL) was first mixed with 1 mL of the Folin-Ciocalteu reagent and allowed to react at room temperature for 30 min, and then 0.8 mL of 7.5% sodium carbonate solution

Table 1. General Characteristics of the 11 potato cultivars used in this study[z]

	Brief description						
	Colour						
Cultivars	Skin	Flesh	Maturity	Tuber shape	Yield potential	Storage potential	Sources
Mackintosh Black	Purple	Purple	Mid-to-late	Long	Low-medium	Good	Squirrell Farms[y]
Purple Majesty	Purple	Purple	Mid-to-late	Oval	Medium	Good	Research Material, U of G[x]
F04038	Purple	White	Mid	Round to oval	Low	Good	AAFC Breeding line[w]
Red Thumb	Red	Red	Late	Fingerling	Medium	Good	Research material, U of G[x]
Y38	Red	Red	Mid-to-late	Oval to long	High	Good	Breeding program research material, U of G[x]
Chieftain	Red	White	Late	Round to oval	High	Good	AAFC
Norland	Red	White	Early	Round	High	Good	AAFC
Sangre	Red	White	Late	Round to oval	Medium-High	Good	AAFC
Banana	Yellow	White	Mid-to-late	Long	Medium-High	Good	Squirrell Farms
Yukon Gold	Tan	Yellow	Mid-season	Round to oval	Medium	Good	AAFC
Snowden	Buff	White	Late	Round	Medium	Good	AAFC

[z]Potatoes were provided and grown at the Elora Research Station in 2008 and 2009 and all general information was based on long-term trials at the Elora Research Station.
[y]The cultivar originated from the Potato Research Centre and was distributed by Squirrel Farms, Glen Squirrell 477081 3rd line, R. R. #2 Shelburne, Ontario, Canada L0N 1S6.
[x]University of Guelph.
[w]AAFC, Agriculture and Agri-Food Canada, National Potato Breeding Program, Fredericton, NB.

was added to the mixture. A 200 μL mixture was added into the 96-well plate for each replicate. The absorbance was measured at 765 nm in a UV-Visible microplate kinetics reader (EL 340, Bio-Tek Instruments, Inc., Winooski, VT). A standard curve was generated with gallic acid, at concentrations ranging from 20 to 100 μg mL^{-1}, from which TPCs of the various samples were calculated and expressed as milligrams of gallic acid equivalent per gram of dry weight potato [gallic acid equivalent (GAE) g^{-1} dry weight (DW)]. Aqueous methanol (80%) was used as the blank control.

Total Anthocyanins Content

The total anthocyanins content (TAC) was estimated by a modified pH differential method (Cheng and Breen 1991). Extract of each potato sample (10 μL) was mixed separately with 272 μL of buffer at pH 1.0 (0.1 M HCl/4.9 m M KCl) and another at pH 4.5 (24.8 m M NaAC). The buffer was adjusted to pH 1.0 or 4.5 by hydrochloric acid if necessary. Absorbance was measured in a UV-visible microplate kinetics reader at 510 nm and at 700 nm in buffers of pH 1.0 and pH 4.5, respectively. The net total absorbance (A) was calculated using:

$$A = [(A_{510} - A_{700})\ pH_{1.0} - (A_{510} - A_{700})\ pH_{4.5}].$$

The total anthocyanins content was derived using cyanidin-3-glucoside (Cy3g) whose molar extinction coefficient was 26 900 L cm^{-1} mol^{-1} and molecular weight was 449.2 g mol^{-1}. Results were expressed as milligrams of Cy3g equivalent per gram of dry weight potato (Cy3g E g^{-1} DW). The detection limit for the plate reader is 0.017 μg mL^{-1} of cyanidin-3-glucoside concentration.

Antioxidant Activity Assays

The ferric reducing/antioxidant power (FRAP) method (Tsao et al. 2003) was used to measure the ability of the antioxidants in the hydrophilic fraction of extracted potato samples to reduce the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to the blue coloured ferrous form (Fe^{2+}) which absorbs light at 593 nm. A standard or sample extract (10 μL) was mixed with 300 μL of ferric-TPTZ reagent (prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ in a ratio of 10:1:1, vol/vol/vol) and added to the wells of a microplate. The plate was incubated at 37°C for 1 h. The absorbance readings were taken at 593 nm using a UV-Visible microplate kinetic reader. The total antioxidant activity (TAA) of each sample was calculated as FRAP value on the basis of 500 mM *L*-ascorbic acid and expressed as μmol ascorbic acid equivalents per gram of dry weight potato (AAE g^{-1} DW).

The oxygen radical absorption capacity (ORAC) method (Cao et al. 1997) was used to evaluate the antioxidant activity of the hydrophilic fraction in this study. All reagents were prepared in 75 mM phosphate buffer (pH 7.4). The 50-fold diluted (by 80% methanol) samples (25 μL) were added to wells of a 96-well plate and each mixed with 150 μL of 8.68×10^{-5} mM fluorescein working solution. Trolox and distilled water were used as the calibration standard and blank, respectively. Linear regression of trolox was used in the range of 3.125–100 μmol L^{-1} for the standard. The plate was allowed to equilibrate by incubating for a minimum of 30 min in the Synergy HT Multi-Detection Microplate Reader at 37°C. Reactions were initiated by the addition of 25 μL of 153 mmol L^{-1} 2,2′-azobis (2-amidinopropane) dihydrochloride followed by shaking at maximum intensity for 10 s. The fluorescence was monitored kinetically and data were recorded at 1 min intervals. The excitation and emission wavelengths were set at 485 and 528 nm, respectively. The final results were calculated using the differences of area under the fluorescence decay curves between the blank and a sample and the results were expressed as micromoles trolox equivalent (TE) per gram dry weight potato (μmol TE g^{-1} DW). The area under the curve (AUC) was calculated using the following equation:

$$AUC = (0.5 + f_5/f_4 + f_6/f_4 + f_7/f_4 + f_8/f_{4...} + f_i/f_4) \times CT$$

Where f_4 is the initial fluorescence reading at cycle 4, f_i is the fluorescence reading at cycle I, and CT is the total minutes of the cycle.

The hydrophobic fraction was not analyzed in this study, even though there may be some antioxidant activity from carotenoids in the yellow flesh or skin of some potato cultivars. Further analysis of the yellow pigments is underway in addition to an analysis of the specific anthocyanins in the purple and red potatoes.

Statistical Analysis

Data were analyzed using an analysis of variance (ANOVA) with the PROC MIXED procedure of SAS (Version 9.1.3, SAS Institute, Cary, NC). Means separation was obtained using Tukey's method of multiple comparisons. Pearson's correlation coefficient was used to analyze the relationship between TPC and FRAP values, as well as the correlation between TPC and ORAC values and between FRAP values and ORAC values. Significance was determined at the 5% level of probability.

RESULTS

Total Phenolic Content

There were differences in the TPC of the 11 potato cultivars each year ($P < 0.0001$, 2008; $P < 0.0001$, 2009) and there was a significant cultivar × year interaction ($P < 0.0001$). The TPC ranged from 1.2 to 3.6 mg GAE g^{-1} DW in 2008 and from 1.0 to 2.8 mg GAE g^{-1} DW in 2009 (Table 2). Cultivars could be grouped into one of three categories depending on pigmentation in

Table 2. Total phenolic content and total anthocyanin content in 11 potato cultivars

	Total phenolic content[z] (mg GAE g^{-1} DW)		
Cultivars	2008	2009	Difference between years
Mackintosh Black	3.6*a*	2.8*a*	0.7*
Purple Majesty	3.4*a*	2.7*a*	0.7*
Red Thumb	2.9*ab*	2.6*a*	0.2
Y38	2.1*bc*	3.1*a*	−1.0**
F04038	1.8*cd*	1.6*b*	0.2
Chieftain	1.7*cd*	1.1*c*	0.6
Norland	1.6*cd*	1.3*bc*	0.3
Banana	1.6*cd*	1.3*bc*	0.3
Sangre	1.6*cd*	1.1*c*	0.5
Yukon Gold	1.4*d*	0.9*c*	0.5
Snowden	1.2*d*	1.0*c*	0.2
Mean	2.1	1.8	
	Total anthocyanin content[y] (mg Cy3gE g^{-1} DW)		
Cultivars	2008	2009	Year difference
Purple Majesty	1.92*a*	1.52*b*	0.32*
Mackintosh Black	1.27*b*	0.86*c*	0.33*
Y38	1.16*b*	1.86*a*	−0.71**
Red Thumb	0.78*c*	0.95*c*	−0.26
F04038	0.20*d*	0.14*e*	0.06
Chieftain	0.10*d*	0.05*e*	0.04
Norland	0.08*d*	0.06*e*	0.02
Sangre	0.06*d*	0.06*e*	0.002
Banana	ND[x]	ND	–
Yukon Gold	ND	ND	–
Snowden	ND	ND	–
Mean	0.70	0.69	–

[z]Total phenolic content expressed as milligrams of gallic acid equivalent (GAE) per gram dry weight ($n=4$).
[y]Total anthocyanin content expressed as milligrams of cyanidin-3-glucoside equivalent (Cy-3-gE) per gram dry weight; Anthocyanins were not detected in cultivars Banana, Yukon Gold and Snowden ($n=4$).
[x]Not detected: samples contained anthocyanins less than 0.0096 mg Cy3gE g^{-1} DW.
a–e Numbers in a column followed by the same letter are not significantly different at $P<0.05$, according to Tukey's Test.
*, ** $P<0.05$ and $P<0.01$, respectively.

the skin and flesh of the tuber. Mackintosh Black (purple skin and purple flesh) had the highest overall TPC (3.6 mg and 2.8 mg GAE g^{-1} DW in 2008 and 2009, respectively) followed by the cultivars Purple Majesty, Red Thumb, and Y38, although statistically they were similar (Table 2). This group of potatoes (with both red/purple skin and flesh) had the highest TPC, followed by those with white flesh and red or purple skin, such as Chieftain and F04038, and cultivars with pale yellow or white skin/flesh (Table 2). There was significant difference in TPC among the three distinct groups ($P<0.0001$).

The significant cultivar by year interaction ($P<0.0001$) appears to be caused by three of the 11 cultivars which responded differently between the 2 yr (Table 2) for TPC values. The TPC values for Mackintosh Black and Purple Majesty were both 0.74 mg lower in 2009 ($P=0.02$), while TPC of Y38 was higher in 2009 ($P=0.0002$).

Total Anthocyanins Content

Anthocyanins were only found in pigmented potatoes with purple or red skin and/or flesh (Table 2). Significant differences in TAC among cultivars were also found over 2 yr ($P<0.0001$, 2008; $P<0.0001$, 2009) (Table 2). The cultivar Purple Majesty generally contained the highest TAC [1.92 mg cyanidin-3-glucoside equivalent (Cy3gE) g^{-1} DW] in 2008, whereas in 2009 Y38 had the highest TAC (1.79 mg Cy3gE g^{-1} DW). However, despite the slightly different ranking orders in TAC, there was a significant positive ranking correlation between 2 yr ($R^2=0.86$, $P<0.0001$). The average TACs were not significantly different between 2 yr for the 11 cultivars studied. However, there was a significant cultivar by year interaction for TAC ($P<0.0001$). The two purple fleshed cultivars (Mackintosh Black and Purple Majesty) had significantly lower TAC in 2009 as compared with 2008 (Mackintosh Black: $P=0.01$, $t=4.18$; Purple Majesty: $P=0.02$, $t=4$), while the TAC content of the red fleshed cultivar Y38 increased by 0.71 mg Cy3gE g^{-1} (61%) in 2009 ($P<0.0001$, $t=-8.96$). Between the two purple fleshed cultivars, the TAC content of Purple Majesty was always significantly higher than that of Mackintosh Black (by 0.65 and 0.66 mg Cy3gE g^{-1} DW in 2008 and 2009, respectively) ($P<0.0001$, $t=8.21$, 2008; $P<0.0001$, $t=8.39$, 2009). The potatoes with purple skin and/or flesh had the higher TAC compared with the red potatoes except for Y38, which, for unknown reasons, had the highest TAC in 2009 (Table 2).

Total Antioxidant Activity

Significant cultivar differences for TAA were found using both FRAP and ORAC assays (Table 3). Differences in antioxidant activity were also found between the 2 yr, but there was a significant genotype by year effect in both assays ($P<0.0001$). In the FRAP assay, the antioxidant activity of potato extracts ranged from 12 to 64 μmol AAE g^{-1} DW and from 6 to 20 μmol AAE g^{-1} DW, in 2008 and 2009, respectively (Table 3). Meanwhile, the antioxidant activity of potato in 2008 was 1–2.2 times higher than the potato from 2009. The ORAC assay showed a similar trend, i.e., lower in 2009, with ORAC values ranging from 42 to 168 μmol TE g^{-1} DW and from 75 to 121 μmol TE g^{-1} DW in 2008 and 2009, respectively (Table 3), However, cultivars Y38, F04038, Banana and Snowden had a higher antioxidant activity in 2009. Generally, the purple flesh potato cultivars Purple Majesty and Mackintosh Black had the highest TAA, while the white and yellow fleshed cultivars Yukon Gold and Snowden had the lowest TAA. As a comparison, Mackintosh Black had a TAA (ORAC in 2008) that was four times greater than Snowden. The red pigmented cultivars fell in the

Table 3. Total antioxidant activity[z] as measured by oxygen radical absorption capacity (ORAC) and ferric reducing/antioxidant power (FRAP) assays for different potato cultivars

	ORAC (μmol TE g^{-1} DW)		
Cultivars	2008	2009	Difference between years
Mackintosh Black	168*a*	121*b*	47.3*
Purple Majesty	176*a*	138*ab*	35.7
Red Thumb	141*ab*	130*b*	13.1
Y38	137*ab*	174*a*	−36.6
F04038	91*cd*	105*bc*	−14.6
Chieftain	99*bc*	55*d*	43.9
Norland	83*ced*	61*d*	21.1
Banana	60*cde*	67*d*	−6.9
Sangre	55*de*	45*d*	9.5
Yukon Gold	67*cde*	40*d*	27.3
Snowden	42*e*	75*cd*	−30.7
Mean	102	92	
	FRAP (μmol AAE g^{-1} DW)		
	2008	2009	Difference between years
Purple Majesty	68*a*	22*a*	46.1**
Mackintosh Black	64*a*	20*a*	44.1**
Red Thumb	54*a*	18*a*	35.9**
Y38	37*b*	20*a*	17.5**
Norland	24*bc*	8*b*	16.1**
Sangre	24*bc*	8*b*	16.4**
F04038	23*bc*	8*b*	15.3*
Banana	22*bc*	7*b*	14.4*
Chieftain	21*bc*	8*b*	13.2
Yukon Gold	16*c*	5*b*	11.3
Snowden	12*c*	6*b*	6
Mean	33	12	

[z]Antioxidant activity was investigated by the ORAC method [results were expressed as μmol Trolox equivalent (TE) per gram dry weight] and FRAP method presults were expressed as μmol Ascorbic acid (AAE) per gram dry weight] ($n=4$).
a–e Numbers in a column followed by the same letter are not significantly different at 0.05, according to Tukey's Test.
*,** Denotes significance at $P<0.05$ and $P<0.01$, respectively.

middle in terms of the antioxidant activity. TAA between the purple and the red fleshed cultivars was significant in the FRAP assay ($P<0.0001$, $t=6.15$), but not in the ORAC assay ($P=0.3391$, $t=0.96$) (Table 3).

Relationship Between Phenolic Content, Anthocyanins and Antioxidant Activity

There was a positive correlation between the TAA and TPC or TAC in both the FRAP and ORAC assays (Table 4). The two measurers of phenolic content, TPC and TAC, were positively correlated ($R^2=0.84$, $P<0.0001$). Similarly, the two antioxidant assay methods FRAP and ORAC were also a correlated ($R^2=0.67$, $P<0.0001$). The antioxidant activity as expressed in ORAC value was highly correlated with both TAC ($R^2=0.85$, $P<0.0001$) and TPC ($R^2=0.87$, $P<0.0001$), whereas the FRAP value had slightly lower correlation coefficients of $R^2=0.53$ and 0.73, respectively (Table 4).

The weather conditions were different in the 2 yr of the trial (Table 5). Generally, the 2008 growing season

Table 4. Pearson correlation coefficients (R^2) between total phenolics (TPC), anthocyanins (TAC) and total antioxidant activity (TAA)[z]

			TAA[z]	
	TAC	TPC	FRAP	ORAC
TAC ($n=64$)	–	0.84	0.53	0.85
TPC ($n=88$)	–	–	0.73	0.87
FRAP ($n=88$)	–	–	–	0.67
ORAC ($n=88$)	–	–	–	–

[z]Total antioxidant activity: antioxidant activity was detected by oxygen radical absorption capacity (ORAC) and ferric reducing/antioxidant power (FRAP) methods.
Note: All correlations are significant at $P<0.0001$.

was cool and wet and had fewer sunny days than normal (data not reported) and the 2009 growing season was even cooler, but not as wet as 2008. It also had fewer sunny days than normal in 2009. The average monthly air temperature from May to September in 2008 was 2.4°C higher compared with 2009 and the total rainfall was also higher in 2008 (517 mm) compared with 2009 (327 mm) at the Elora Research Station. The average air temperature and total rainfall in 2008 were higher than the long-term (10-yr) average; however, the average monthly air temperature and total rainfall in 2009 were both lower than the long-term (10-yr) average. No supplemental irrigation was applied in either year. Soil pH (7.9) and soil organic matter (3.0%) were similar in both years. The year (2008) with the higher rain fall was accompanied by higher TPC, TAC and TAA.

DISCUSSION

Results of the present study indicated that potato cultivars with pigmented flesh colour had significantly higher antioxidant activity than commonly consumed white or yellow fleshed potatoes; and the darker the colour, the higher the antioxidant activities. That is, the purple fleshed cultivars had higher antioxidant activity than those with red flesh. This was consistent with the results that have already been noted with TPC and TAC, suggesting phenolic compounds, particularly the anthocyanins may be the principle phytochemicals contributing to the total antioxidant activity of potato. Good correlations between the TPC/TAC and TAA (FRAP and ORAC) also support this observation. Similar results have been reported in a US study (Reyes et al. 2005) in which significant correlations were found between TAC, TPC and antioxidant activity using different purple and red flesh potato genotypes, such as All Blue, NDC 4069-4 and Russian Blue. Another study showed the similar levels of TPC (1.6 to 8.4 mg GAE g^{-1} DW) among 20 potato clones and higher TPC values were related to the presence of anthocyanins in the peel and tuber (Ji et al. 2011). Meanwhile, there are differences in anthocyanins between red and purple potato cultivars. The results from another study in our

Table 5. Summary of monthly precipitation, mean air temperature data and long-term averages at the Elora Research Station, Elora, ON, 2008 and 2009

	Temperature (°C)			Rainfall (mm)		
	2008	2009	LTA[z]	2008	2009	LTA
May	11.8	12.0	11.9	71	77	97.4
Jun.	19.6	15.9	17.8	88	70	75.0
Jul.	21.5	16.8	19.5	126	45	76.1
Aug.	19.7	18.1	18.8	125	89	66.3
Sep.	16.9	14.5	15.4	106	46	73.1
Mean	17.9	15.5	16.7	/	/	/
Total	/	/	/	517.2	327	387.9
Soil						
	2008	2009				
pH	7.9	7.8				
Organic matter (%)	3.40	2.50				

[z]10-yr averages in Elora Research Station, Environment Canada.

lab, which was focused on the anthocyanins composition of highly pigmented vegetables, indicated that petunidin was the major anthocyanin for purple potato cultivars, such as Purple Majesty and Mackintosh Black. Pelargonidin was the major component for red potato cultivars, such as cultivar Y38 and Red Thumb (Li et al. 2011).

White fleshed potato cultivars with red skin, such as Chieftain and Norland, had higher TPC and TAA than the pale skin, white fleshed cultivars such as Banana and Snowdon, again suggesting the differences are mainly made by the anthocyanins in the skin of these potatoes. Even though the skin or peel was not analysed separate from the flesh of the potato samples in this trial, there is evidence from other studies showing the antioxidant activity of potato is mainly from the peel (Sotillo et al. 1994; Kahkonen et al. 1999). More than 50% of the total phenolic content was found in the peel and the adjoining tissues of the potato tuber (Nara et al. 2006; Al-Weshahy and Venket Rao 2009). It has also been reported that the hydroxybenzoic and hydroxycinnamic acids such as gallic and chlorogenic acid, are the major phenolic compounds in potato, particularly in the peel (Sotillo et al. 1998).

Among the pale skin, white/yellow fleshed potatoes, the cultivar Banana had the highest TPC and TAA (Tables 2 and 3). This could be explained at least partly by the relatively small tuber size of Banana as compared with Snowden or Yukon Gold. Smaller tuber size means larger surface area to volume ratio. Considering the fact that the peel accounts for only 10% of the sample total weight (Reyes et al. 2005), but 50% of the phenolic content (Al-Weshahy and Venket Rao 2009) this effect of the surface area to volume ratio on the TPC and antioxidant activity of a potato tuber would be significant. In the present study, all potato samples were processed without separating the skin from the flesh. Thus cultivars with smaller tubers, such as Banana, would likely have more skin content in the total sample weight as compared with similar cultivars with larger tubers, such as Snowden.

Any differences associated with tuber size were due to genotypic differences as all potatoes were grown in the same experimental field and with the same agronomical practices. For this reason, the so-called dilution effect caused by tuber size, which is often used to explain the effect of fertilizer application (Jarrell and Beverly 1981; Maier et al. 1994), is not considered a contributor in this study. A dilution effect is mostly seen in cases where the same cultivar is grown under different agronomic practices such as organic and conventional, which produce increased crop yields under heavy fertilizer uses but reduced concentration of nutrients on the same volume or weight basis (Davis 2004).

There was a significant cultivar by year interaction for many traits as most cultivars had lower TPC and TAA in 2009. The biggest changes were seen for purple and red fleshed cultivars, particularly Mackintosh Black. Non-pigmented potatoes with white flesh and white/yellow skin, such as Yukon Gold and Snowden, did not undergo significant changes between the 2 trial years. These observations suggest that anthocyanin production in pigmented potatoes is influenced by environmental conditions. The soil type and production practices were very similar in the 2 yr, thus, changes of TPC, TAC and TAA in pigmented potatoes in particular may have been caused solely by the environmental conditions in which the potatoes were grown over the two seasons. The 2008 season had higher mean temperatures and rainfall compared with 2009. This implies that anthocyanin content may be improved by choice of location and agronomic practices, specifically, a region with higher temperatures and higher rainfall or where irrigation is possible.

Temperature can influence the level of phytochemicals in plants. Cooler temperatures and longer days (higher solar radiation) resulted in higher TAC but not TPC in potatoes grown in Colorado compared with those in Texas (Reyes et al. 2004). In the present study, however, a higher average growing temperature resulted in both higher TPC and TAC content in purple and red fleshed potato cultivars, a result that is opposite to what

was reported by Reyes et al. (2005). However increased flavonoids content has been reported for certain fruits such as strawberry under warmer temperatures during fruit development (Wang and Zheng 2001).

In this study, the TAC content of purple and red fleshed potato cultivars ranged from 0.78 to 1.92 mg Cy3gE g^{-1} DW, which was equivalent to 16.38 to 40.32 mg Cy3gE per 100 g fresh weight based on average potato water content of 79% (Bland and Tanner 1985). This result is within the range of anthocyanins contents of the purple and red fleshed potatoes reported in a study conducted in the United States which showed the TAC varied from 11 to 174 mg cyanidin-3-glucoside per 100 g fresh weight (Reyes et al. 2005). However, the TAC content in Ontario potatoes is relatively low. The cultivars were different in the Reyes et al. (2005) trial, so it is not possible to determine if cultivar or environment was responsible for the generally lower TAC in this trial.

Blueberry is often regarded as one of the richest sources of anthocyanins, which are considered to be the major contributors to the antioxidant activity and other health benefits of blueberry. TAC in blueberries ranged from 138 to 385 mg Cy3gE per 100 g fresh weight (Cevallos-Casals and Cisneros-Zevallos 2003). Although the TAC per gram in blueberry is much higher than in the potato tubers, purple and red potato cultivars may have greater health implications compared with blueberry because: (1) potatoes are available year round, (2) they are a staple food that is produced in bulk and consumed by a large population, (3) they are much less expensive on a fresh weight basis than blueberries and (4) they have a much longer shelf life than blueberries and require no refrigeration at home.

CONCLUSION

This is the first study to determine the antioxidant activity and phenolic content of potatoes grown in Ontario, including a range of popular white and yellow fleshed cultivars and relatively new cultivars with purple and red flesh. The cultivars with purple and red flesh had high antioxidant activity compared with those with white or yellow flesh or skin. Differences in anthocyanin and total phenolic content were related to antioxidant activity and varied considerably between the 2 yr of trials. These differences could be due to the influence of temperature and rainfall during the growing season, but further work is needed to determine how growing conditions influence anthocyanin content. Purple coloured potato cultivars, rich in anthocyanins, are a good source of antioxidants and can potentially be developed further as functional foods with higher antioxidant activity levels. Potato breeders wishing to increase antioxidant levels need to focus on improving the anthocyanin content in the flesh and skin of the tuber while maintaining eating quality. TAC and TPC were highly correlated and could be used during selection to improve quality. Potatoes with white skin and flesh had little antioxidant activity. Consumers wishing to increase their intake of antioxidant rich foods should choose the more intensely coloured potato cultivars.

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