Antioxidant capacity and polyphenolic content of potato tubers are affected by cultivar and hormetic treatment

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ABSTRACT

This thesis involved an investigation of potato *(Solanum tuberosum* L.*)* tuber antioxidant capacity, particularly the phytonutrients vitamin C and polyphenolics. Tubers of 12 Canadian-grown and 5 foreign cultivars were screened for antioxidants qualitatively and quantitatively following 1 and 7 months of storage (5±1 ^{o}C). Significant differences were seen in quality and quantity of tuber antioxidants (including vitamin C and polyphenolics) between cultivars, storage intervals, and tuber tissues (skin, cortex, and pith). A potato microtuber model system was developed to test the effects of hormesis on microtuber antioxidant capacity. Hydrogen peroxide sprays (2 and 4 mM) increased the antioxidant capacity of microtubers in 2 cultivars by 12-26 %, showing the potato microtuber model system to be sensitive and useful for manipulating phytonutrient composition via hormetic treatments. Information in this thesis may have future applications for better cultivar selection and cultivation practices for improved phytonutrient content of field-grown potato tubers.

RÉSUMÉ

La capacité antioxydante des tubercules de pomme de terre (*Solanum tuberosum* L.), particulièrement celle conférée par la vitamine C et les polyphénoliques, fit l'objet de cette étude. La teneur et la gamme des antioxydants présents dans les tubercules de 12 cultivars de pomme de terre sélectionnés au Canadiens et 5 cultivars sélectionnés à l'étranger, ont été.evalués après 1 ou 7 mois d'entreposage à 5±1^{o}C. Le cultivar, la durée d'entreposage, et le tissu échantillonné (peau, chair, ou moelle) eurent tous un effet significatif sur la teneur et la gamme des antioxydants (vitamine C et les polyphénoliques) présents. Un système modèle avec microtubercules servit à l'examen d'effets d'hormèse sur la teneur en antioxydants des microtubercules de deux cultivars. Une aspersion au péroxide (2 ou 4 mM) augmenta de 12% à 26% la capacité antioxydante de microtubercules de deux cultivars différents, indiquant que ce système modèle est sensible et se prête bien à la manipulation du contenu nutritif des tubercules par traitements hormetiques. Ces connaissances pourraient s'avérer utile dans la sélection de cultivars et la mise en oeuvre de pratiques culturales pouvant améliorer, au champ, le contenu nutritif des pommes de terre.

DEDICATION

I dedicate my thesis to my beloved mother Mrs. Swarna Latha Vunnam.

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CONTRIBUTIONS OF AUTHORS

This thesis has been prepared in regular thesis form according to "Thesis preparation and submission guidelines" recommended and approved by Graduate and Postdoctoral Studies (GPS). This thesis consists of five chapters with General Introduction and Literature Review in Chapters I and II, respectively. Chapters III and IV describe two different studies on field- and laboratory-grown potato tubers, respectively. Chapter V contains the summary, conclusions and suggestions for future research work in this field of study.

Chapter III of this thesis was part of on-going research in my supervisor's (Dr. Danielle J. Donnelly, Plant Science Department; Dr.Stan Kubow, School of Dietetics and Human Nutrition) laboratories. All the experiments for this study were conducted by me under the plan and supervision of Dr. Danielle J. Donnelly and Dr. Stan Kubow. Mr. Andre Piccolomini and Dr. Kebba Sabally (School of Dietetics and Human Nutrition) who conducted a preliminary round of experiments for this study, contributed by guiding me in the laboratory work. Dr. Atef Nassar (Plant Science Department) helped with the statistical analysis for this study.

Chapter IV of this thesis was a novel study with the experimental plan and set-up developed by myself and Dr. Danielle J. Donnelly. All the experimental work and statistical analyses were conducted by me. All the chapters in this thesis were prepared by me with extensive editorial help by Dr. Danielle J. Donnelly, Dr. Stan Kubow, and Dr. Mark Lefsrud.

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LIST OF ABBREVIATIONS

~	Approximately
α-TOC	α-tocopherol
AA	Ascorbic acid
AAE	Ascorbic acid equivalents
ABA	Abscisic acid
AOC	Antioxidant capacity
AOS	Active oxygen species
APX	Ascorbate peroxidase
ASC	Ascorbate
CAR	Carotenoid
CAT	Catalase
CFA	Caffeic acid
CGA	Chlorogenic acid
Cv(s).	Cultivar(s)
DPPH	2,2 Diphenyl-1-Picryl hydrazyl
DM	Dry Mass
Equiv.	Equivalents
FA	Ferulic acid
F-C	Folin-Ciocalteu
FM	Fresh Mass
FRAP	Ferric Reducing Antioxidant Power
GR	Glutathione reductase
GSH	Reduced glutathione
Ha	Hectare
HAT	Hydrogen Atom Transfer
MS	Murashige-Skoog
MT	Metric Tonne
PAL	Phenylalanine ammonia-lyase
ROS	Reactive Oxygen Species
RT	Rutin
SET	Singlet Electron Transfer
SNC	Single-Node Cutting
SOD	Superoxide dismutase
TP	Total Phenolics

CHAPTER I

GENERAL INTRODUCTION

1.1 Introduction

Potato *(Solanum tuberosum)* is the world's number one non-grain food commodity, with production reaching a record 315 million MT in 2008 (FAOSTAT, 2008). Potato has been consumed in the Andes of South America since it originated there about 8,000 years ago (FAO, 2008). It was taken by the Spanish to Europe in the 16th century. Potato quickly spread across the globe and today potatoes are grown on an estimated 192,000 sq km (74,000 sq. miles) in more than 160 countries (FAO STAT, 2008). The year 2008 was declared "International Year of the Potato" (IYP) by the United Nations General Assembly. This was done to increase the profile of this globally important crop in terms of its biological and nutritional attributes, and promote its production, processing, consumption, marketing, and trade.

The production of potato in the world has increased at an annual average rate of 4.5 % in the last 10 years (FAO, 2008). It has exceeded the growth in production of many other major food commodities in the developing countries, particularly in Asia. China, the leading producer of potato with 72,000,000 MT in 2007, contributing 22 % of the world production of 321,696,483 MT (FAOSTAT, 2008). "The potato produces more nutritious food more quickly, on less land, and in harsher climates than any other major crop - up to 85 % of the plant is edible human food, compared to around 50 % in cereals" (FAO, 2008). Potato is often identified as a rich source of calories which are no longer so essential for the sedentary lifestyle of the modern man. However, it remains a staple crop for a large number of humans. This provides a strong reason to study and characterize the phytonutrient content of the tuber. Potato is composed of a highly diverse list of phenolic compounds, flavonoids, carotenoids, vitamins, and minerals (Woolfe, 1987). Many of these compounds in potato are antioxidants, and potato has only

recently gained recognition for both its phytonutrient and antioxidant benefits (Brown et al., 2003; Brown, 2005; Brown et al., 2007). Humans cannot synthesize these antioxidant compounds which must be supplied through the diet (Andre et al., 2007). Different potato cultivars have different antioxidant capacity (AOC), which may show diverse and interesting effects on the risk factors for developing long-term chronic problems including cardiovascular diseases, certain cancers, and diabetes (Brown, 2005; 2008). The relative nutritional benefits of different potato cultivars grown in Canada were not entirely known when this project was initiated.

During normal metabolism, plants produce significant quantities of reactive oxygen species (ROS) and this is enhanced during stress, leading to oxidative damage of the cells (Andre et al., 2009). These ROS act as signalling molecules for the production of protective molecules including enzymatic and non-enzymatic scavengers which regulate ROS (Scandalios, 2005; Andre et al., 2009). Environmental conditions and genotype significantly affect antioxidant production quantitatively and qualitatively (Andre et al., 2009; Brown 2005; 2008). Controlled application of stress which causes mild oxidative damage in plants can be used as a tool to enhance the synthesis of phytochemicals which have nutraceutical activities (Cisneros-Zevallos, 2003). To minimize the damaging effects caused by the stress, plants tend to produce non-enzymatic and enzymatic antioxidant defences (Scandalios, 2005). Application of low doses of controlled stress to obtain beneficial effects is called hormesis (Luckey, 1980).

1.2 Hypotheses

1. Potato cultivars have different antioxidant capacities and can also differ qualitatively and quantitatively in phytonutrient components such as polyphenolics.
2. Antioxidants are not always uniformly distributed in potato tuber tissues and may vary in the periderm (skin), cortex, and pith (perimedula and medulla area within and including the vascular ring).

3. Mild stress (hormetic) treatments to micropropagated potato plantlets will affect the phytonutrient content and antioxidant capacity of the microtubers.

1.3 Objectives

1. To identify the potato cultivars with greater phytonutrient benefits (greater antioxidant capacity and polyphenolic content) when fresh, or stored for 7 months, among 12 Canadian-grown and 5 foreign cultivars (from Korea and the United Kingdom).
2. To study the effect of hormetic treatment with the oxidizing agent hydrogen peroxide (H_2O_2) on the phytonutrient composition (antioxidant capacity and polyphenolic content) of microtubers of two potato cultivars.

1.4 Scope

This study enables informed nutritional recommendations for consumers. It identifies potato cultivars with superior antioxidant capacity and polyphenolic content among 12 common Canadian-grown and 5 foreign cultivars, and provides an understanding of the distribution of phytonutrients within different tuber tissues and how tuber phytonutrients content is affected by storage (1 or 7 months). This study also contributes to the understanding of physiological processes in potato microtubers that are sensitive to hormetic stress. This information may have future applications for better cultivar selection and cultivation practices for improved phytonutrient content of field-grown potato tubers destined for human or animal consumption.

CHAPTER II

LITERATURE REVIEW

Historically, potato tubers were one of the top four major food commodities consumed in the world. In recent years, with the diversion of maize from human consumption to biofuels and animal feed, potato ranks third globally, following rice and wheat (Table 2.1) (FAOSTAT, 2008; Camire et al., 2009). In Canada, potato ranks first with a consumption of 2,816,723.00 MT, followed by wheat, maize, and rice (2003 data; FAOSTAT, 2008).

The potato is an herbaceous annual that grows up to 100 cm tall with prostrate, decumbent, semi-erect or erect growth habit and produces underground geophytic structures known as stem tubers (potato tubers or potatoes) at the tip of each of many stolons (Huaman, 1986). The root system of potato plants is comprised of a slender tap root with lateral branches or adventitious roots from the base of each sprout and from above the nodes of underground parts of the stem, depending on development of the plant from seed or seed tuber, respectively. Potato plants have compound leaves with midrib (rachis) and several leaflets attached to the midrib, and cymose inflorescences. The fertilized ovary develops into a berry fruit containing numerous seeds.

As the plant grows, the compound leaves manufacture the food material and towards the end of the season, following flowering, photosynthates are transported into the tubers. Each plant produces 10-20 tubers, depending on the availability of soil moisture, nutrients, and other components of ideal growth. Each tuber may weigh up to 300 g with as many as 2 to 10 buds (“eyes”) which are the growing points for the next season (FAO, 2008). The tuber is mainly differentiated into three layers from the outside in: periderm (skin), cortex, pith or medulla (Figure 2.1). Collectively, the cortex and pith are the “flesh” of the tuber and the primary storage areas for the potato tuber. Food storage material produced by the potato plant is transported to the pith and cortex via the vascular tissue (vascular ring; Figure 2.1). Cells in the flesh increase in number and size as they

are supplied with materials, causing the tuber to increase in size (Manitoba Agriculture, Food and Rural Initiatives, 2006).

2.1 Cultivation of potato

Potato is grown in approximately 160 countries (AAFC, 2007) with over 4,000 cultivars (Hils and Pieterse, 2007) under different climatic conditions, including temperate, subtropical, and tropical (FAO, 2008). It is essentially a "cool weather crop", with temperature being the main limiting factor for production. Optimum tuber yields are obtained when the mean daily temperature is 18 to 20 ^{o}C (FAO, 2008; Manitoba Agriculture, Food and Rural Initiatives, 2006). At temperatures below 10 °C and above 30 °C, the tuber growth is sharply inhibited. With shorter days (14 hours) and a moderate ground temperature of 15-18 °C, tuber formation is enhanced (Beukema and van der Zaag, 1979; Burton, 1989). With longer days (14-16 hours) and higher day temperatures (20-25 °C) flowering and seed formation is enhanced.

2.2 Potato production and consumption

The potato sector is undergoing major changes globally. Until the early 1990's, potatoes were mostly grown and consumed in Europe, North America, and countries of the former Soviet Union. Since then, potato production and demand in Asia, Africa, and Latin America has been increasing dramatically with an output of less than 30 million MT in the early 1960s to more than 165 million MT in 2007 (FAO, 2008). In 2005, for the first time, potato production from the developing world exceeded that of the developed world (Table 2.2**)**. China is now the biggest potato producer and almost a third of the world's potatoes are harvested in China and India. In 2007, the top three world producers of potato were China (72 million MT) followed by the Russian Federation (35.7 million MT) and India (26.3 million MT) (Camire et al., 2009). The overall Asian potato production is increasing at 6 % annually (Bamberg and Del Rio, 2005). Asia and Europe are now the major potato-producing regions of the world, accounting for more than 80 % of world production in 2007 while production from Africa and

Latin America were far smaller (FAOSTAT, 2008). The average yields from North America were significantly greater, with more than 36 MT per hectare (ha) compared with the world's average of 16.64 MT per ha (Table 2.3).

2.3 Potato production in Canada

In the year 2010, the estimated Canadian potato growing area was 145,449 ha, 3 % less than in 2009 which was 150,184 ha (Table 2.4; Statistics Canada, 2010). This decline in potato growing area over the past year mainly occurred in Manitoba which showed the greatest decrease (11 %) followed by British Columbia (7 %) and Alberta (3.8 %). The area fell by 2.7 % in New Brunswick and 0.6 % in Prince Edward Island. Ontario and Quebec showed an increase of 4 % and 0.6 % respectively. The other five provinces remained stable in potato production.

Prince Edward Island is the leading potato-producing province in Canada, with 24 % of the national area, followed by Manitoba, with 22 % in 2010 (Statistics Canada, 2010). Alberta and New Brunswick are in third and fourth position, with 16 % and 14 %, respectively.

2.4 Nutritional value of potato

Potato tubers are better understood for their traits having to do with processing quality than for their nutrient composition (Gordon and Katz, 2005). Potato often fails to appear as a "health benefiting food" due to its marginalization from "vegetable" status. Though potatoes are a good source of complex carbohydrates, and minerals such as potassium, and phosphorus, they are often neglected or ignored for their value within the human diet (Casanas et al., 2002). Potato has fairly low protein content, but is of excellent quality with biological value of 90-100 (Camire et al., 2009). On a dry mass basis, the protein content of potato (1-1.5 %) is similar to that of cereals and is very high in comparison with other tuberous crops (root and stem tubers). Potatoes are often believed to be high in calories and fat in spite of the fact that they have negligible fat and low energy density, which is similar to legumes. A freshly harvested potato contains about 80

% water and 20 % dry matter. About 60 to 80 % of the dry matter is carbohydrate with starch as the major carbohydrate. Potatoes are rich in several mineral elements, especially potassium, and phosphorous, and are a moderate source of iron (Table 2.5) (FAO, 2008). Potato is a good source of vitamin C, and its high vitamin C content promotes iron absorption. It is also a good source of B6 (pyridoxine). Potatoes contain antioxidants, apart from the mentioned vitamins, which may play a part in preventing metabolic diseases related to ageing.

2.5 Antioxidants

An antioxidant is a molecule that when present at low concentrations is capable of slowing or preventing the oxidation of other molecules (Halliwell et al., 1999; Frankel and Meyer, 2000; Brown, 2005). Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which can start free radical-mediated chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents. Based on their mechanism, antioxidants are mainly categorised into primary and secondary antioxidants (Maisuthisakul et al., 2007). Primary antioxidants scavenge free radicals by donation of an electron and/or hydrogen atom and stabilizing them. Secondary antioxidants function by mechanisms like deactivating singlet oxygen, binding of metal ions, scavenging oxygen, converting hydroperoxides to non-radical species, etc. Diets rich in antioxidant flavonoid and carotenoid compounds have been associated with a lower incidence of atherosclerotic heart disease, certain cancers, macular degeneration, and cataracts (Hertog et al., 1993; Knekt et al., 1996; Cao et al., 1998, 1999; Wang et al., 1999; Kruezer, 2001).

2.6 Antioxidants in potato

Potato contributes dietarily much more than just starch. In the last two decades, potato has gained increased attention as an important source of secondary phytochemical metabolites and antioxidant compounds (Stushnoff et

al., 2008). Potato contains a highly diverse list of phenolic acids, flavonoids (anthocyanins), carotenoids, vitamins, and proteins, which contribute to antioxidant capacity (Table 2.6) (Woolfe, 1987; Camire et al., 2009). These antioxidants are capable of efficiently scavenging superoxide and peroxyl radicals, show ferrous ion-chelating effects, and strong reducing capacity. Polyphenolics are the major compounds with antioxidant capacity in potato, scavenging and neutralizing free radicals, decomposing lipid peroxides, and quenching singlet oxygen (Cao et al., 1997; Brown, 2005). Phenolic compounds like chlorogenic acid, caffeic acid, and ferulic acid are also reported to be present in potato tubers at relevant concentrations. Chlorogenic acid can compose up to 80 % of the total phenolic acid content (Brown, 2005). Purple and red skinned tubers contained twice the concentration of phenolic acids as white-skinned tubers (Brown, 2005), and tubers with purple or red flesh contained three to four times the concentration of phenolic acids as white-fleshed tubers (Lewis et al. 1998). The total phenolic content of potato was positively correlated with its total antioxidant capacity (Brown, 2005).

Some potato cultivars have higher levels of anthocyanins, which are water soluble antioxidants conferring red to purple coloration in the skin and flesh of potato (Brown, 2008). Anthocyanins are potent antioxidants with a half-life of several hours in the body once ingested. Some of these anthocyanins are strong anti-inflammatory agents (Mazza et al., 2002; Brown, 2008). Cultivars with solidly pigmented flesh (red or purple) may have as much as 40 mg/100 g FM of total anthocyanins compared to a red or purple skinned white fleshed cultivar with only 1.5 mg/100 g FM (Mazza et al., 2002; Brown, 2005, 2008). They show a total antioxidant capacity (ORAC), ranging from 7.6 to 14.2 μmole Trolox equiv. per g FM (Brown, 2005). The purple fleshed potato cultivars were high in antioxidant activity due to their relatively high anthocyanin concentrations (Hale et al., 2008). The tubers of purple or red fleshed cultivars had twice the flavonoid concentration of white-fleshed cultivars (Lewis et al., 1998). The range of hydrophilic oxygen radical absorbance capacity (ORAC) values in potato ranges from 200 to 1,400 μmol Trolox equiv./100 g FM (Brown, 2008).

All potatoes have carotenoids, of which lutein and zeaxanthin are important nutritionally, as they are essential for proper eye health (Rein et al., 2007; Brown, 2008). Xanthophylls, the most abundant carotenoids of potato are fat soluble, and have half-lives of several days in the human body (Brown, 2008; Camire et al., 2009). These xanthophylls are antioxidants that associate with membranes in the cell (Brown, 2008).

Vitamin C is a crucial compound in the human diet (Love and Pavek, 2008). It is a reducing agent that can neutralize reactive oxygen species such as hydrogen peroxide. It also plays an important role in the absorption of iron which is often limiting in the human diet (Love and Pavek, 2008; Camire et al., 2009). It cannot be synthesized in humans and must be obtained in the diet. Potato is an important source of vitamin C. It contributes up to 20 % of the overall vitamin C content of the European diet (Love and Pavek, 2008). A medium sized white potato of 150 g FM, consumed with skin, can provide up to half of the daily adult requirement of vitamin C (FAO, 2008). On average, potato contains 20 mg/100 g FM of vitamin C (Brown, 2005). Vitamin C can contribute up to 13 % of the total antioxidant capacity of the potato (Chu et al., 2002; Brown, 2005).

Potatoes may play an important role in increasing the intake of antioxidants in the human diet. Even though potato is not among the top antioxidant capacity food items (Table 2.7) (Wu et al., 2004), the quantity and regularity of potato consumption as a staple food makes it an important source of antioxidants in the human diet (Stushnoff et al., 2008). Hence, the quantity of potato eaten by the US consumer makes it a good source of required phytonutrients (Brown, 2008). As potato is consumed as a staple food in Canada (Table 2.1) (FAO, 2008), this makes it an important source of antioxidants in the Canadian diet.

2.7 Methods to determine the antioxidant capacity and to measure phenolics

There are several well established methods to determine the AOC and phenolic content of food samples. Any method considered for use in determining

the AOC and phenolic content should have the following characteristics: analytical range, recovery, repeatability, reproducibility, and recognition (Prior et al., 2005). The free radicals are deactivated by antioxidants via two major mechanisms, the hydrogen atom transfer (HAT) and singlet electron transfer (SET). The AOC of antioxidants that quench the free radicals by hydrogen donation are measured by HAT-based methods, while the AOC of antioxidants that transfer one electron to reduce any compound, including metals, carbonyls, and radicals, are measured by SET-based methods. HAT-based methods include Oxygen Radical Absorbance Capacity (ORAC), Total Radical-trapping Antioxidant Parameter (TRAP), Total Oxidant Scavenging Capacity (TOSC), Chemiluminescence (CL), Photochemiluminescence (PCL), Croton or β-Carotene Bleaching, or Low Density Lipoprotein (LDL) oxidation. SET-based methods include Ferric Reducing Antioxidant Power (FRAP) and Copper Reduction assay (CUPRAC). The methods which are based on both HAT and SET include: Trolox Equivalent AOC (TEAC), 2, 2- Diphenyl-1- Picrylhydrazyl (DPPH) assay, and Folin-Ciocalteu (F-C). Choosing a method for measuring the AOC of a sample should be based on the expected type of antioxidants present in the sample, rapidness, simplicity of the method. DPPH and FRAP assays are simple, quick, and quite extensively used for determining the total AOC, while the F-C assay for total phenolics and High Performance Liquid Chromatography (HPLC) for assessment of specific phenolics are well established and extensively used for determining the AOC and phenolic content of plant samples.

DPPH is a stable free radical, commercially available, and most effective method for measuring free radical-scavenging materials (Prior et al., 2005; Maisuthisakul et al., 2007). The deep violet coloured DPPH molecule reacts with hydrogen-donating antioxidant molecules and reduces to a yellow coloured hydrazine; its decrease in absorption is measured spectrophotometrically at 515-528 nm (Sanchez-Moreno, 2002). The DPPH assay is a relatively easy and well validated assay to measure the scavenging activity of antioxidants, as it is quite stable and does not need to be generated like some other radical scavenging assays (Sanchez-Moreno, 2002; Prior et al., 2005). DPPH provides a rapid way of

evaluating the AOC of the samples spectrophotometrically (Brand-Williams et al., 1995; Prior et al., 2005; Hale et al., 2008). The DPPH assay is considered to be mainly SET-based, but DPPH may be neutralized either by electron transfer or hydrogen atom transfer (Prior et al., 2005; Hale et al., 2008).

DPPH reacts with the hydrogen donating antioxidants but does not react with free radical intermediates and oxidative chain reaction products (Nair et al., 2007). DPPH cannot measure the antioxidants which quench singlet oxygen (Prior et al., 2005). The DPPH assay is complicated for the compounds like carotenoids, which have a spectral overlap with DPPH at 515 nm. Many antioxidants that react with peroxyl radicals may either react slowly or remain inert to DPPH due to steric inaccessibility. Smaller molecules like ascorbic acid, which have better access to the DPPH radical site, react very quickly. Some cautions are necessary during data interpretation as antioxidant interaction with DPPH is dependent on the structure of the compound (Brand-Williams et al., 1995). Some compounds react very rapidly with DPPH, reducing a number of DPPH molecules and some react slowly depending on the complexity (size and arrangement) of the compound. The reduction of DPPH by antioxidant molecules depends on the number of hydroxyl molecules available. Though DPPH has some drawbacks, its simplicity for broad screening of sample AOC makes it one of the more extensively used antioxidant assays.

FRAP was originally developed to measure the reducing power of plasma, and subsequently adapted to measure antioxidants in botanicals (Prior et al., 2005). The FRAP assay is SET-based rather than a mix of SET- and HAT-based mechanism like DPPH. FRAP measures the antioxidants which reduce free radicals by electron donation (Benzie and Strain, 1996). The pale yellow coloured ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex upon reacting with the electron donating antioxidant reduces to a blue-coloured ferrous complex (Fe^{2+}-TPTZ). Its increase in absorbance is measured spectrophotometrically at 593 nm (Frankel and Meyer, 2000). FRAP can also be utilized to measure the non-enzymatic antioxidants (Nair et al., 2007). However, FRAP cannot detect the antioxidant compounds that act by hydrogen atom transfer (Prior et al., 2005). The FRAP

assay is based on redox reactions which proceed rapidly, completing in 4-6 min. Some polyphenols react too slowly and would require more time for detection than provided by this assay. FRAP may therefore not identify all polyphenolic antioxidants and single endpoint absorption may not represent the complete reaction. The FRAP assay measures the reducing capability of antioxidants based on the ferric ion, which may not be relevant to antioxidant activity mechanistically and physiologically (Frankel and Meyer, 2000; Prior et al., 2005). However, FRAP is simple, fast, inexpensive, and does not require any specialized equipment except a spectrophotometer.

The Folin-Ciocalteu (F-C) assay, to determine the total phenolics in samples of natural products, has been one of the most popularly used for many years (Prior et al., 2005). There are not many other well established methods for determining the total phenolic content of botanical samples. The basic mechanism of the F-C assay is an oxidation/reduction reaction which can be considered as an AOC determination method. The F-C method was originally developed in 1927 by O. Folin, and originated from tyrosine analysis where phenols were oxidized by a molybdotungstate reagent which yielded a coloured product. This method was slow at acid pH and lacked specificity. This was improved by Singleton and Rossi (1965) with a molybdotungston-phosphoric heteropolyanion reagent. The F-C method is simple, quick, inexpensive, and useful and does not require specialized equipment in establishing the phenolic status of the botanical samples (Prior et al., 2005). However the F-C method of determining total phenolics has an interference problem with several substances, including sugars, aromatic compounds, sulfur dioxide, ascorbic acid, enediols, reductones, organic acids, and Fe (II) for which correction should be made. The F-C method of analyzing total phenolics is usually positively correlated with AOC-measuring methods.

High Performance Liquid Chromatography (HPLC) is one of several chromatographic techniques used for the separation and analysis of mixtures of chemicals (Snyder et al., 2009a). It has evolved into a highly sophisticated technology since its advent in the early 1970's (Nollet, 1992). It has several of the following advantages compared with other separation procedures (Snyder et al.,

2009a): 1. Universal applicability in sample separation with very few exceptions; 2. Remarkable precision (± 0.5 % or better); 3. Commercially available in a wide range of equipment and accessories which allows its use in almost all applications; and 4. Usually the first choice for most laboratories that are involved in analyzing mixtures of chemicals. This makes HPLC one of the most used analytical techniques for a wide range of applications.

The HPLC is made of components that include a solvent inlet system, pumps, an injector or autosampler device, column, a detector, connecting tubes, and data acquisition system (computer, software, etc.) among which the column is the heart of the system (Nollet, 1992). The retention and resolution of a compound analysed depends on the column (stationary phase) characteristics (C-loading, chain length, porosity, etc.), the eluent (mobile phase), scheme characteristics (eluent pH, polarity, flow rate, temperature), and the solubility and polarity of the sample analysed. The columns usually have a solid support, a back bone for the bonded phase which is commonly made of silica based particles. This solid support is firmly linked (chemically bound) with functional groups which are bonded phases. These bonded phases are extremely stable and reproducible. The components of a mixture in a sample are separated in the column by pumping the eluent solvent (mobile phase) and samples together through the column. Samples analysed by HPLC are usually separated by two kinds of modes and bonded phases; the normal phase and reverse phase. In the normal phase, the mobile phase is non-polar (hexane, methylene chloride, etc.) and the stationary phase is polar (silica). In this mode the less polar molecules are eluted first followed by more polar molecules. The reverse phase uses polar mobile phase (water, polar solvents, or mixtures), and a non-polar stationary phase (C-18 coated silica). In this mode, elution order of the molecules is reversed. Depending on the specific/unique affinity of each compound (analyte) with the mobile and stationary phases, they move in the column at different speeds and elute out of the column at different times, resulting in separation (Snyder et al., 2009b). Analytes with higher affinity for the mobile phase move down the column more quickly than those having higher affinity for the stationary phase. Consequently, elution

times (retention times) are unique for each analyte and are the basis of analyte identification. The analytes identified are quantified based on their response to a detector.

HPLC is a very powerful and popular tool used in identification and purification of a wide range of compounds, including amino acids, peptides, proteins, lipids, phospholipids, carbohydrates, fat and water soluble vitamins, organic acids, phenolic compounds, etc. (Nollet, 1992). It is a well established and most commonly used method for characterization of phenolic compounds in potato tuber samples (Shakya and Navarre, 2006; Nair et al., 2007; Reddivari et al., 2007; Hale et al., 2008; Stushnoff et al., 2008).

2.8 Plant tissue culture

Plant tissue culture broadly refers to the cultivation of all plant parts, including single cells, tissues, and organs, under aseptic conditions (Thorpe, 1981). Plant tissue culture, which is an essential component of plant biotechnology, provides novel approaches to plant production, propagation, and preservation (Bhojwani, 1990). Tissue culture has greatly contributed to the understanding of factors responsible for growth, metabolism, differentiation and morphogenesis of plant cells (Razdan, 2002a). It has been utilized as an important aid in conventional methods of plant improvement. For example, it has been a useful tool to produce specific pathogen-free plants in many crops. Significant progress has been made in recent years in the application of culture systems for the synthesis of secondary metabolites (including pharmaceuticals). Over the past 2 decades tissue culture applications have been focused and utilized in plant breeding, horticulture, forestry, industrial production of compounds, and conservation of natural genetic resources (Razdan, 2002b). The facilities required for establishing a plant tissue culture unit depends on the kind of research done and the amount of funds available (Razdan, 2002d). The basic requirements of any plant tissue culture unit include: 1. Washing and storage facilities; 2. Media preparation, sterilization and storage area; 3. Aseptic transfer area; 4. Culture

rooms or incubators for maintenance of cultures under controlled atmospheric conditions of light, temperature, humidity; and 5. Transplantation area.

2.9 Potato tissue culture

Tissue culture techniques have been extensively utilized in the propagation of potato (Wang and Hu, 1985). Potato can be multiplied rapidly under in vitro conditions using micropropagation techniques. Potato has been described as a model plant for tissue culture (Espinosa et al., 1986). Under appropriate conditions, potato, like other Solanaceous crops, is quite responsive and regenerative to many tissue culture techniques, producing adventitious shoots both from organ tissue and from callus (Hussey and Stacey, 1981; Estrada et al., 1986). Different micropropagation techniques are used for propagation of specific pathogen tested material including, single-node cuttings (Hussey and Stacey, 1981), multimeristems (Roca et al., 1978), layered plantlets (Wang, 1977), or shoot tips (Goodwin et al., 1980).

Single node cuttings (SNC) are the most commonly used propagule for multiplication of potato, which are grown on growth regulator-free or gibberellin-containing MS medium (Hussey and Stacey, 1981; Murashige and Skoog, 1962). The medium is amended with 30 gl^{-1} (Hussey and Stacey, 1981; Estrada et al., 1986) or 20 gl^{-1} (Wattimena, 1983) of sucrose, various levels of inositol, thiamine-HCl (Estrada et al., 1986), and amino acids (Hussey and Stacey, 1981), solidified with agar (2-9 gl^{-1} depending on the brand), with pH adjusted to 5.6 to 5.8 before autoclaving (Wattimena, 1983; Estrada et al., 1986). The cultures are incubated at a temperature ranging from 22 to 30 ^{o}C, under 20 to 133 $\mu molm^{-2}s^{-1}$ illumination with 16:8 to 24:0 h day:night cycle (Wattimena, 1983; Wang and Hu, 1985).

2.10 Potato Microtuberization

The tubers produced from in vitro microplants of potato are called microtubers. Microtuberization of potato was first reported by Barker (1953). Microtubers are used as seed tubers, as a source of medium- and long-term

conservation of germplasm, and as ideal material for international germplasm exchange between countries and seed certification schemes (Slimmon et al., 1989; Dodds and Watanabe, 1990; Thieme, 1992). In many countries, microtuber technology is a vital component of seed potato production (Donnelly et al., 2003). In countries like China, India, and other parts of Asia, where the availability of high-quality seed tubers is a constraint, microtubers may provide a solution. Microtubers provide seed tubers generally of greater quality compared with those produced in the field. Use of microtubers can also be a good replacement for in vitro plantlets as the former can be stored, and are handled easily with no acclimatization required before transferring them to the field.

Historically, propagules used for microtuberization were excised buds placed in vitro (Baker, 1953), sprouts or stolon segments (Palmer and Smith, 1970), single-node cuttings from in vitro plantlets (Wattimena, 1983), or layered shoots (Wang and Hu, 1982). Then came development of two phase microtuberization systems (vegetative and tuber induction phases) (Estrada et al., 1986). The most commonly used propagules are single-node cuttings or layered shoots (Donnelly et al., 2003). Among these two, layered shoots seems to be more efficient and less tedious, producing 3-5 times greater microtuber mass after 56 d of inductive environment (basal medium with 8 % sucrose under 8:16 h day:night cycle at 50 μmol $m^{-2}s^{-1}$ and 15 ^{o}C) than single node cuttings.

Potato tuberization is influenced by several factors; environmental conditions including light and temperature, medium components including sucrose, nitrogen, and growth regulators (Wang and Hu, 1985; Donnelly et al., 2003), and explant utilized (Wang and Hu, 1985). Several approaches have been made to improve tuber induction, number of tubers per plant, and to increase the size of the tubers by manipulating the factors discussed above. Though there have been several studies done to understand the factors effecting tuber induction and subsequent microtuber growth and dry matter accumulation, the later is less well understood (Donnelly et al., 2003).

Photoperiod at different stages of microtuberization plays a vital role in tuber induction. Total darkness induced tuberization on nodal cuttings when

preceded by short but not long days (Garner and Blake, 1989). Micropropagated source plants grown under long days (16:8 h day:night) followed by microtuber induction under short days (8:16 h day:night) or continuous darkness, showed greater efficiency than micropropagated source plants grown under short days followed by the same microtuber induction conditions (8:16 h day:night or continuous darkness) (Seabrook et al., 1993). Photoperiod from longer to shorter days promoted earlier (Garner and Blake, 1989), more numerous (Wang and Hu, 1982), and bigger microtubers (Seabrook et al., 1993). Total darkness induced rapid tuberization in single-node cuttings, but bigger tubers were achieved by using 8 h photoperiod (Slimmon et al., 1989). In contrast, Hussey and Stacey (1984) reported that photoperiod had no effect on tuberization. These reported discrepancies are explained by differences in cultivars, culture media, growth regulators, type of explants, and incubation conditions (Wang and Hu, 1985).

Temperatures of 20 to 25 ^{o}C during the vegetative growth phase and temperatures of 15 to 18 ^{o}C during tuber induction are optimal for microtuberization (Wang and Hu, 1982; Leclerc et al., 1994). Temperatures above 35 ^{o}C inhibit microtuberization (Palmer and Smith, 1970). Tuber induction in single-node cuttings was more effected by growth regulators, especially anti-gibberellin agents, than temperature (Levy et al., 1993).

Sucrose is the most critical component for tuberization in intact potato plants (Wang and Hu, 1982). Increasing the concentration of sucrose from 1 to 8 % increased early and overall percentage, of microtuberization (Wang and Hu, 1985). However sucrose concentrations above 8 % inhibited microtuberization (Palmer and Smith, 1970; Garner and Blake, 1989). Sucrose plays an inductive role in signalling for microtuber formation (Simko, 1994; Struik and Wiersema, 1999).

The total nitrogen levels or relative concentrations of nitrate:ammonium in the medium shows more effect on some cultivars than others (Avila et al., 1998). Greater nitrate:ammonium (50 mM nitrate:10 mM ammonium) promoted microtuber growth compared with lesser nitrate:ammonium (40 mM nitrate:20 mM ammonium) (Chen and Liao, 1993).

2.11 Use of potato microtubers as a model system

Although potato microtuberization was first reported by Barker (1953), its application as a reliable research model system has been slow to develop (Coleman et al., 2001). Most of the studies which used microtuberization have used complex media with plant growth regulators. This affected induction and dormancy of the microtubers, which led to uncertainty in considering microtubers as a model system for studying tuber physiology. Microtubers were used as a research system to examine plant metabolism, ranging from pigment biosynthesis in tubers (Lewis et al., 1998) to tuber protein gene expression (Bourque et al., 1987; Ulloa et al., 1997). They can be used as a tool to understand tuberization in field potatoes, since environmental factors stimulating tuberization and the reaction of the plant to these factors can be well studied in controlled in vitro conditions (Leclerc et al., 1994).

Plant breeders evaluate hundreds or thousands of seedlings each year at different field locations which involve laborious and time-consuming processes (Donnelly et al., 2003). Use of effective in vitro microtuber systems for screening could reduce the time and cost for evaluation. For example, screening (with ELISA tests) putative virus resistant transformants growing in vitro was considerably faster, less costly, and as effective as screening greenhouse-grown ex vitro transplants (Russo and Slack, 1998). Strong similarities were evident in growth response between field grown seed tubers and microtubers (Donnelly et al., 2003). In vitro screening was done for salinity tolerance using single-node cuttings and microtuber bioassays, which were analogous with field lysimeter results (Zhang and Donnelly, 1997). Gopal and Minocha (1997) investigated the use of crops raised from microtubers compared to crops raised from normal seed tubers in selection for agronomic characters. Selection at the microtuber crop level was found to be highly effective for tuber colour and general impression, moderately effective for plant vigour and tuber shape, and less effective for tuber yield, tuber number, average tuber mass, and number of eyes. However, Naik et al. (1998) compared 37 potato cultivars in vitro and in the field for genetic parameters and character association related to tuberization. They concluded that

in vitro performance is not an accurate measure of its field performance. However, the efficiency of in vitro selection can be increased by identifying and stimulating in vitro conditions that increase the phenotypic expression as found in the field (Gopal and Minocha, 1998).

Microtubers resemble field-grown tubers in characters including tuber induction, growth and development, and even abiotic stress tolerances (Coleman et al., 2001), which makes microtubers a usable tool for basic research (Donnelly et al., 2003). Microtubers grown in a controlled environment can be a model for biochemical and physiological studies for conventional and transformed potatoes (Coleman et al., 2001). Their induction in response to exogenous triggers, similar protein and starch composition, and small size, are all important characteristics of a model system.

2.12 Hormesis

Hormesis has been defined as stimulation of living organisms by low doses of any potentially harmful agents in order to induce beneficial stress responses (Shama and Alderson, 2005; Luckey, 1980). The word "hormesis" is derived from the Greek word "hormaein" which means "to excite". Hormetic agents are highly diverse, and include heavy metals, polychlorinated biphenyls, insecticides, alcohols, cyanide, antibiotics, ionizing radiation such as gamma radiation, electromagnetic radiation, and ultraviolet radiation (Smith-Sonneorn, 1993). Despite the fact that high doses of hormetic agents are harmful, sub lethal doses can show beneficial effects such as enhancement of nutraceuticals, including polyphenolic compounds within plants used for human foods (Cisneros-Zevallos, 2003). For example, postharvest treatment of tomato fruits with UV-C doses of 3.6 and 4.8 kJm^{-2} delayed ripening while ten-fold doses of 40 kJm^{-2} resulted in skin decolourization (Liu et al., 1993). UV irradiation of fruit and vegetables can increase their antioxidant levels. For example, irradiation increased anthocyanin levels in apples (*Malus domestica* Borkh.) and strawberries (*Fragaria x ananassa* Duch.), vitamin D2 content in mushrooms (*Agaricus*

bisporus), and polyphenolics in grapes (*Vitis vinifera* L.) (Dong et al., 1995; Mauch et al., 1998; Cantos et al., 2003).

During normal cellular metabolism, plants produce significant quantities of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), superoxide radical (O_2^-), and hydroxide radical (OH^-), which are all oxidizing molecules (Guan et al., 2000). To minimize the damage caused by these ROS, plants have evolved antioxidant defences to scavenge these free radicals (Scandalios, 2005). Plants produce even greater ROS levels when exposed to biotic stress, such as herbivores or pathogenic agents, and abiotic stress, including water stress, extreme temperatures, excessive light, pollution, etc. Stress agents affect genetically determined biosynthesis of the three principal groups of secondary metabolites: terpenes, phenolics, and nitrogen-containing compounds and promote increased endogenous AOC to minimize the damage caused by ROS (Zevallos, 2003).

Abiotic stress can sometimes be used in preharvest activities to enhance the quality and yield of plant produce (Kalt et al., 2001). For example, water stress can lead to the accumulation of different kinds of enzymatic (peroxidises, superoxide dismutase, and catalase) and non-enzymatic antioxidants (phytochemicals) to quench ROS induced by stress (Oh et al., 2010). For example, lettuce (cv. Baronet) growth chamber study plants exposed to -1.5 MPa water stress (determined by leaf water potential, measured using a thermocouple psychrometer) once at 6 weeks (before harvest) for 2 d, showed significant increase in total phenolic compounds and AOC compared to controls. Vitamin C levels can be enhanced in plants exposed to water stress, as vitamin C may protect against drought injury, although the mechanism is not understood (Lee and Kader, 2000). Ascorbic acid (AA) is among the most studied antioxidant in plants (Smirnoff, 1996; Smirnoff, 2000). Its ability to donate electrons in many enzymatic and non-enzymatic reactions makes it a powerful antioxidant (Blokhina et al., 2003).

The vitamin C content of broccoli is inversely related to the amount of precipitation during the head-development stage. In cv. Pardon pepper fruits the

pungency level increased with water stress due to the accumulation of capsaicinoids, a group of pungent phenolics derived from the phenlypropanoid pathway (Estrada et al., 1999). Biotic or abiotic stress factors induce the production of secondary metabolites in plant cells. Antioxidant enzymes like superoxide dismutase (SOD) often increase in response to stress related hormones such as abscisic acid (ABA) (Kwak et al., 1996). The plant hormone ABA acts as a stress signal and plays an important role in regulating plant stress responses (Jiang and Zhang, 2002). Greenhouse-grown maize seedlings at the second fully expanded leaf stage, exposed to -0.7 MPa water stress induced by polyethylene glycol (PEG 6000) for 24 h at 25 ^{o}C showed an increase in ABA of almost 6 times in leaf samples. In another experiment, cut leaf segments (3-cm-long) of greenhouse-grown maize seedlings at the same stage, exposed to 10, 100, and 1000 μM of ABA for 24 h at 25 ^{o}C showed a significant increase in the antioxidant enzymes SOD, catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) and the non-enzymatic antioxidants, ascorbate (ASC), reduced glutathione (GSH), α-tocopherol (α-TOC), and carotenoids (CAR) (Jian and Zhang, 2001). In vitro potato plantlets of cv. Alpha and Atlantic grown in medium containing 10 μM of ABA, showed up to double the survival rates ex vitro following cold exposure (Mora-Herrera and Lopez-Delgado, 2007). This increased survival rate was associated with increased level of the antioxidant enzyme APX by 1.6- and 1.2-fold in cv. Alpha and Atlantic, respectively. This clearly indicates that ABA exposure or induction leads to the induction of antioxidant defence responses.

However, not all abiotic stresses result in beneficial effects. Some stresses, like hot temperatures during the growing season, can reduce the vitamin C content of fruits. Grape fruit grown in the cooler coastal areas of California contain more vitamin C than those grown in the warmer desert areas of California and Arizona (Lee and Kader, 2000). Greenhouse-grown maize plants treated with ABA at low concentration (10 and 100 μM) induced antioxidant defences but ABA treatment at higher concentration (1000 μM) led to excess generation of active oxygen species (AOS) and resulted in oxidative damage (Jiang and Zhang, 2001). It is

clear that hormetic stress (biotic or abiotic) application must be optimized to enhance the nutraceutical potential of produce (Shama and Alderson, 2005; Zevallos, 2003).

Diets rich in natural colorants and antioxidants have positive effects on human health (Reyes and Zevallos, 2003). So, any method that can enhance the AOC by inducing polyphenols has potential utility. Phenolic compounds are one of the major antioxidants present in plant tissues and may be accumulated by different abiotic stresses. They are produced by phenyl-propanoid metabolism which involves the induction of the enzyme phenylalanine ammonia lyase (PAL).

In potato, several postharvest abiotic stresses (ethylene, methyl jasmonate, temperature, light and wounding) have been used as hormetic agents to induce accumulation of antioxidants (such as polyphenolic compounds) in purple fleshed potato (cv. All Blue) (Reyes and Zevallos, 2003). Overall, wounding stress increased total phenolics up to 60 % with a parallel 85 % increase in AOC. This wounding stress was done by surface sterilization of tubers with chlorinated water (250 ppm), followed by cutting into slices (~ 0.5 cm). These slices were stored in jars for 2 days and opened every 4-8 h to avoid CO_2 accumulation (> 0.15 %) prior to analysis of phenolics. These results suggest that abiotic stress could be utilized to enhance the nutritional value of potatoes.

2.13 Hydrogen peroxide-induced hormesis

Recently much attention has been devoted to the role of ROS; especially hydrogen peroxide (H_2O_2), which plays a multiple role in the defence mechanisms of biotic and abiotic stresses (Kuzniak and Urbanek, 2000). Hydrogen peroxide is one of the predominant ROS which is produced along with other oxidizing molecules during normal cellular metabolism (Guan et al., 2000). Superoxide radicals, the toxic by-products of oxidative metabolism, can interact with H_2O_2 to form highly reactive hydroxyl radicals which are the primary oxygen toxicants in the cells. Enzymatic antioxidant defences like superoxide dismutase, work to dismutase superoxide radicals into H_2O_2 and oxygen. Hydrogen peroxide in turn, is dismutased into oxygen and water by catalases (Prasad et al., 1994; Guan et al.,

2000). Mitochondria are the major producers of H_2O_2 in the cell (Prasad et al., 1994). H_2O_2 is known to induce the antioxidant mechanism in maize seedlings (Prasad et al., 1994). For example, H_2O_2 applied exogenously to 3-day-old maize seedlings induced catalase (CAT 3) transcription and chilling tolerance. Upon brief treatment with aminotriazole (AT) which inhibits CAT 3 activity, there was accumulation of H_2O_2. In the maize plants, abscisic acid (ABA), a hormone and abiotic stress agent, induced the catalase isozyme gene CAT 1. Due to the antioxidant nature of the maize CAT 1 gene, it is speculated that oxygen free radicals might be involved in the ABA signal transduction pathway leading to the activation of CAT 1. CAT 1 expression is induced by H_2O_2 (Guan et al., 2000). ABA induces antioxidant defence responses against oxidative damage in a range of crops including potato. Evidences suggest that ABA stress responses are mediated through increased H_2O_2 levels (Mora-Herrera and Lopez-Delgado, 2007). For example, micropropagated potato (cv. Atlantic and Alpha) grown in ABA-amended medium showed a significant increase in the enzymatic activity of peroxidase and ascorbate peroxidase along with H_2O_2. Apparently, endogenous H_2O_2 plays a regulatory role in the activation of genes encoding enzymes and other proteins involved in protection from oxidative stress. H_2O_2 applied to 4-week-old greenhouse-grown tobacco plants at a moderate dosage (5 mM x 1.7 mg/plant) resulted in increased AOC and better tolerance to oxidative stress (determined by a lipid peroxidation test) caused by excessive light stress treatment (14:10 h light:dark under 500 µmol $m^{-2}s^{-1}$) (Gechev et al., 2002). Although the mechanism of stress-signal perception and transduction in activation of antioxidants in the cell is not known, endogenous H_2O_2 is implicated in this process.

Field grown potato plants (cv. Alpha) treated twice a week with 5 and 50 mM H_2O_2 (1.5 L/18.4 m^2 area) from day-21 till harvest on day-90 showed significant increase in tuber starch (6.7-30 % greater than control) as determined by specific gravity (dry matter accumulation) (Lopez-Delgado et al., 2005). Quantification using image analysis confirmed that stems of H_2O_2-treated plants contained up to 3.4-fold more starch and 62 % more lignin. Phenolic compounds

like lignins and some lignin-related monomeric and dimeric compounds are recognized as antioxidants (Dizhbite et al., 2004). Though there was 62 % more lignin deposition in hydrogen peroxide-treated plants in the Lopez-Delgado et al., (2005) study, the phytonutrient status of these plants was not explored.

H_2O_2 has been utilized in different in vitro studies on potato to determine its role in inducing stress tolerance (Rusite and Gertnere, 2001). Potato microplants grown in hormone-free MS medium in vitro were soaked with 1 mM H_2O_2 for 2 h and showed significant tolerance to applied ethylene stress. The oxidizing agent hydrogen peroxide induced greater levels of endogenous peroxidase compared with the controls which aided in stress tolerance. These studies suggested that H_2O_2 is involved in the ethylene signal transduction pathway and in development of ethylene stress tolerance. Increased tolerance for one form of stress, caused by the treatment with another stress agent, is called "cross-tolerance" (Foyer et al., 1994). In another example of cross-tolerance, potato microplants soaked with 1 mM H_2O_2 for 1 h acquired increased thermotolerance (Lopez-Delgado et al., 1998). The H_2O_2-treated microplants were subcultured to MS medium and grown at 18±1 ^{o}C with 16:8 h day:night under 32 µmol $m^{-2}s^{-1}$ irradiation for 3 weeks to recover followed by a heat treatment for 15.25 h at 42 ^{o}C. The survival rate was established 10 d after treatment. Both endogenous and exogenously applied H_2O_2 enhanced the same antioxidant enzymes in maize seedlings, resulting in improved chilling tolerance (Prasad et al., 1994). In this thesis, a more controlled study system, the microtuber model system, was used to investigate the effects of hydrogen peroxide on the AOC of microtubers.

Table 2.1. Human consumption of major food crops (potato highlighted) in Metric tonnes for the year 2003 (modified from FAOSTAT, 2008).

Food crop	World	North America	Canada
Rice	510,503,843	4,244,341	282,039
Wheat	420,845,273	27,170,563	2,732,137
Potato	206,605,423	21,547,615	2,816,723
Maize	116,418,57	4,512,031	633,333

Table 2.2. Potato production in developed and developing nations and world total from 1991 to 2007 (modified from FAOSTAT, 2008).

Countries	1991	1993	1995	1997	1999	2001	2003	2005	2007
Developed	183.13	199.31	177.47	174.63	165.93	166.94	160.97	159.99	155.56
Developing	84.86	101.95	108.50	128.72	135.15	145.92	152.11	160.12	165.15
World	267.99	301.27	285.97	303.36	301.08	312.86	313.09	320.11	321.69

Table 2.3. Potato production by region (North America highlighted) in 2007 (modified from FAOSTAT, 2008).

	Harvested Area (ha)	Total Quantity (MT)	Mean yield (MT/ha)
Africa	1,502,695	16,323,530	10.86
Asia/Oceania	8,744,049	137,226,926	15.69
Europe	7,492,010	129,395,767	17.27
Latin America	971,935	16,124,302	16.58
North America	614,972	22,625,958	36.79
World	**19,325,661**	**321,696,483**	**16.64**

Table 2.4. Potato growing area of Canada and provincially (Quebec highlighted) from 2007 to 2010 (modified from Statistics Canada, 2010).

	Growing area (ha)			
	2007	**2008**	**2009**	**2010**
Canada	**161,556**	**153,746**	**150,184**	**145,449**
Newfoundland and Labrador	283	283	243	243
Prince Edward Island	38,851	37,435	34,400	34,197
Nova Scotia	1,012	890	931	931
New Brunswick	23,675	22,461	22,258	21,651
Quebec	18,495	17,200	17,888	18,009
Ontario	14,974	14,569	14,974	15,581
Manitoba	34,400	32,781	31,971	28,329
Saskatchewan	3,845	3,845	3,440	3,440
Alberta	22,582	21,247	21,247	20,437
British Columbia	3,440	3,035	2,833	2,631

Table 2.5. Nutritive value of white potato tuber (150 g), after boiling with skin and peeling before consumption (modified from FAO, 2008).

Component	Quantity
Calcium (mg)	7.50
Carbohydrates (g)	30.19
Energy (K cal)	130.50
Fat (g)	0.15
Fibre (g)	2.70
Iron (mg)	0.46
Niacin (mg)	2.16
Phosphorus (mg)	66.00
Protein (g)	2.80
Potassium (mg)	568.50
Riboflavin (mg)	0.03
Thiamine (mg)	0.16
Vitamin C (mg)	19.50
Water (g)	115.50

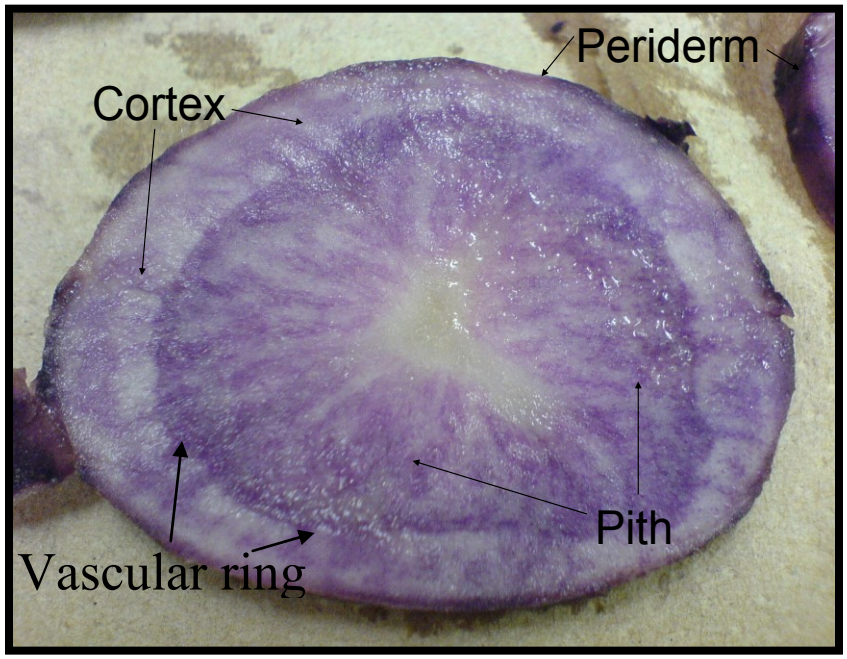
Table 2.6. Categorized antioxidant compounds present in potato highlighting the class of compound and specific antioxidants that were quantified in this study (modified from Camire et al., 2009).

Category	Compounds
Proteins	Patatin
Vitamins	Ascorbic acid Dehydroascorbic acid Folic acid
Phenolic acids	Caffeic acid Chlorogenic acid *p*-Coumaric acid Ferulic acid Gallic acid Protocatechuic acid Vanillic acid
Flavonoids (Flavonols and Anthocyanins)	Catechin Delphinidin Malvidin-3-(*p*-coumaroyl rutinoside) Pelargonidin Peonidin Petunidin Rutin
Carotenoids (Xanthophylls)	B-carotene (trace) Lutein Neoxanthin Violaxanthin Zeaxanthin

Table 2.7. Top Forty six foods (potato highlighted) in total antioxidant capacity (Oxygen Radical Absorbance Capacity (ORAC), in micromoles of Trolox equiv./g; (µmol of TE/g) (modified from Wu et al., 2004).

	Food name (variety)	**Total antioxidant capacity (µmol of TE/g)**		**Food name (variety)**	**Total antioxidant capacity (µmol of TE/g)**
1	Peacans	179.40	24	Red cabbages	31.46
2	Small red beans	149.21	25	Raab broccoli	30.84
3	Red kidney beans	144.13	26	Asparagus	30.17
4	Walnuts	135.41	27	Gala apple	28.28
5	Pinto beans	123.59	28	Beets	27.74
6	Hazelnuts	96.45	29	Golden delicious apple	26.70
7	Cranberry	94.56	30	Spinach	26.40
8	Artichoke	94.09	31	Fuji apple	25.93
9	Wild blue berry	92.60	32	Eggplant	25.33
10	Black beans	80.40	33	Navy beans	24.74
11	Pistachios	79.83	34	Cashew	19.97
12	Black plums	73.39	35	Avacado	19.33
13	Red plums	62.39	36	Green pear	19.11
14	Cultivated blue berry	62.20	37	Peaches	18.63
15	Black berry	53.48	38	Orange	18.14
16	Raspberry	49.25	39	Red leaf lettuce	17.85
17	Almonds	44.54	40	Macadamias	16.95
18	Black eye peas	43.43	41	Tangerines	16.20
19	Red delicious apple	42.75	**42**	**Russet potatoes**	**15.55**
20	Granny smith apple	38.99	43	Green leaf lettuce	15.50
21	Strawberry	35.77	44	Red grape fruit	15.48
22	Cherries	33.61	45	Brazil nuts	14.19
23	Peanuts	31.66	**46**	**Red potatoes**	**13.26**

Figure 2.1. Cross section of a purple pigmented potato tuber showing the three tissue layers (periderm or skin, cortex and pith). The vascular ring is evident in the flesh between the cortex and pith.



CHAPTER III

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY AND MAJOR PHENOLICS IN TWELVE CANADIAN–GROWN AND FIVE FOREIGN POTATO CULTIVARS

3.1 Introduction

There is considerable on-going research (and increased consumer awareness) regarding the health benefits of many phytonutrients, especially antioxidants, found in various fruits and vegetables (Hale et al., 2008). A wide range of phytochemicals are recognised to have antioxidant properties, including polyphenols (flavonoids, flavanols, flavones, and isoflavones), phenolic acids, carotenoids (carotenes and xanthophylls), and vitamins (Hale et al., 2008; Reddivari et al., 2007). Potato, which has been long recognised as a good dietary source of carbohydrates, has in the past 20 years gained recognition as a good source of secondary phytochemical metabolites, many of which are antioxidants (Hale et al., 2008; Stushnoff et al., 2008). Potato contains a highly diverse list of flavonoids, phenolic acids, carotenoids, vitamins, and proteins which could contribute to AOC (Woolfe, 1987). While potato does not rank greatest among foods for its phytonutrient content (or AOC), its relatively greater consumption as compared with other vegetables and fruits contributes to its importance as a source of phytonutrients in the human diet (Stushnoff et al., 2008; Hale et al., 2008). Potato, as the most consumed vegetable in the US and Canada (USPB-Potato NPD Consumption Trends 2010; Table 2.1), even with its modest phytonutrient levels, could contribute a major proportion of the phytonutrients supplied in the North American diet. Many of the secondary metabolites and antioxidants such as hydroxycinnamic compounds found in potato are known to offer health benefits against some age-related chronic diseases that include certain cancers, cardiovascular diseases, and diabetes (Camire et al., 2009).

The antioxidant capacities vary significantly among different cultivars (Brown, 2008; Li et al., 2006) and even among different tissue layers of potato tubers (Li et al., 2006). Potato cultivars screened in several previous studies have exhibited a wide range of variation in: (a) AOC as measured via DPPH assays; (b) total phenolic content assessed by Folin-Ciocalteu; and (c) polyphenolic composition as measured using HPLC (Reddivari et al., 2007). Polyphenolics were the compounds with major antioxidant activity in potato that positively correlated with total AOC (Brown, 2005). Phenolic compounds, including chlorogenic acid, caffeic acid, and ferulic acid, are reported to be present in potato tubers at significant concentrations whereby chlorogenic acid comprises up to 80 % of the total phenolic acids (Brown, 2005).

Potato cultivars with coloured flesh or skin pigmentation are known to have greater antioxidant capacities than white skinned or fleshed cultivars due to their high anthocyanin content (Brown, 2005). The purple and red skinned tubers contained three to four times the concentration of phenolic acids compared with white-fleshed cultivars (Lewis et al., 1998). Potatoes with solidly pigmented flesh of red and purple colour had total anthocyanin ranging from 9 to 38 mg per 100 g FM, and total AOC (ORAC), ranging from 7.6 to 14.2 µmole per g FM of Trolox equivalents (Brown, 2005). The total phenolic content of highly pigmented cultivars of potato have shown an increase after storage for 6 to 7 monthss at 5±1^{o}C, while there was no increase observed in the white or yellow fleshed cultivars stored under the same conditions (Stushnoff et al., 2008). Although potatoes are routinely stored for months before use in the fresh market or processing industries (Kerby et al., 2007), there is relatively little information regarding the impact of storage on AOC or polyphenolic content.

On average, potato contains 20 mg/100 g FM of vitamin C (ascorbic acid), which contributes up to 13 % of the total AOC of the potato (Chu et al., 2002; Brown, 2005). Several long-term plant breeding studies are underway to improve the AOC of potato, with particular focus on improving vitamin C content (Reddivari et al., 2007). The content of ascorbic acid is affected by storage, which

may decrease from 30 to 8 mg/100 g over a storage interval of 8-9 months at 10^{o}C (Love and Pavek, 2008).

The objective of the study was to identify the cultivars (and tissues) with greatest antioxidant capacities and to examine the impact of long-term storage on polyphenolic content and AOC. Twelve Canadian-grown and five foreign cultivars were screened for AOC and polyphenolic content when freshly harvested (within 1 month of harvest) and after a more extensive storage period (7 months). Due to their relative abundance in potato and their dietary importance as antioxidants, three phenolic acids (chlorogenic acid, caffeic acid, and ferulic acid), the flavonoid rutin, and ascorbic acid were assessed to identify the cultivars with relatively better phytochemical profiles.

3.2 Materials and methods

3.2.1 Potato Source Material

Tubers of twelve common cultivars grown in Canada: Atlantic, Goldrush, Green Mountain, Kennebec, Norland, Onaway, Red Pontiac, Russet Burbank, Sebago, Shepody, Superior, and Yukon Gold (Figure 3.1), were received from the Bon Accord Elite Seed Potato Centre (Bon Accord, NB, Canada). These were grown using conventional local field practices for New Brunswick and harvested in late September, 2008. The tubers were randomly selected from storage bins, packaged, and shipped to McGill University. Tubers were stored in their boxes in a walk-in fridge (5±1^{o}C) until analysis.

The foreign cultivars were chosen from a published list of USDA-held genotypes with "high antioxidant activity" or "deep purple colour". Cultivars Alwara (PI639204), Bora Valley (PI634776), Gogu Valley (PI634778), Gui Valley (PI642430), and Purple Valley (PI 634780) (Figure 3.2) were received from the USDA Potato Gene Bank, (Sturgeon Bay, WI, USA) as minitubers (small tubers collected from greenhouse-grown plants). Two minitubers per cultivar were planted at the Horticulture Research Center, Macdonald Campus, McGill University and grown, using conventional field practices for Quebec,

during the summer of 2008. Tubers from these plants were harvested in early October, 2008 and stored in a walk-in fridge (5±1^{o}C) until analysis.

3.2.2 Sample Preparation

The sample tubers of 12 Canadian-grown cultivars and 5 foreign cultivars were analyzed twice; after 1 month storage (November, 2008) and again after 7 months storage (May, 2009). For each cultivar, 5 average-sized tubers (5 replicates) were used for analysis at each sampling time. Tubers were separated into 3 different tissues, including periderm (skin), cortex, and pith. The tissue layers were separated using a domestic paring knife. Care was taken while separating the samples to avoid mixing between tissue samples. Tissues were collected into labeled 20 ml plastic vials (Fisher Scientific, ON, Canada) and weighed on an analytical balance (Denver Instrument Company, Arvada, CO) for fresh mass (FM). The vials were then dropped into liquid nitrogen to freeze the contents and transferred to a freeze-dryer (FTS Systems, NY, USA). Freeze-drying served to remove all moisture and concentrate the phytonutrients. After freeze-drying (72 h), vials were weighed to obtain a vial and sample (by subtraction) dry mass (DM). The freeze-dried samples were ground with a metal rod and stored in a – 80 ^{o}C freezer until analyzed.

3.2.3 Antioxidant determination assay- 2,2 Diphenyl-1-picryl hydrazyl (DPPH)

The AOC of the tuber samples was estimated using the 2,2 Diphenyl-1-picryl hydrazyl (DPPH) assay of Nair et al. (2007). DPPH is a stable free radical, which upon reaction with an antioxidant molecule which donates a hydrogen, so is reduced from a violet colour to form a yellow coloured diphenylpicrylhydrazine. This colour change is measured spectrophotometrically at 517 nm. Ascorbic acid was used as a standard and the results were represented as ascorbic acid equivalents (AA equiv.). An ascorbic acid dilution series was prepared from 10 mM ascorbic acid stock solution (0.0088 g of ascorbic acid in 50 ml distilled water) using distilled water. Using a micropipette, 50 µl of each

ascorbic acid dilution was transferred into a 2 ml microcentrifuge tube. To these, 1.5 ml of 1 mM DPPH (3.94 mg of DPPH into 100 ml methanol) reagent was added and vortexed for 60 s and the tubes were left for 20 min at room temperature for the reaction to proceed. The blank consisted of 1.5 ml distilled water. The control consisted of 1.5 ml DPPH reagent + 50 µl distilled water. The samples were pipetted into 2.5 ml cuvettes and read at 517 nm in the spectrophotometer (Beckman DU 640, Beckman Instruments, Fullerton, CA). A standard curve was prepared from the spectrophotometer readings and used to calculate the quantity of antioxidants in tissue samples based on mg of ascorbic acid equivalents (AA equiv.).

The tuber samples were prepared by placing 10 mg of freeze-dried powdered tissue into 1 ml microcentrifuge tubes to which 1 ml of distilled water was added, vortexed for 60 s, then centrifuged at 4 ^{0}C for 15 min at 3000 x g. After centrifuging, 50 µl of the supernatant was collected into a 2 ml microcentrifuge tube into which 1.5 ml of DPPH solution was added and vortexed for 60 s, then left for 20 min at room temperature for the reaction to proceed. The samples were pipetted into 2.5 ml labeled cuvettes and read at 517 nm in the spectrophotometer.

3.2.4 Antioxidant determination assay - Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay is used to determine the total AOC of the sample through the reduction of the ferric tripyridyltriazine complex to a ferrous complex (Benzie and Strain, 1996). When pale yellow coloured ferric tripyridyltriazine complex is reduced to the ferrous form by electron donation from the antioxidant molecules, an intense blue colour is developed which is measured spectrophotometrically by a change in the absorbance at 593 nm. The FRAP reagent was prepared by mixing acetic acid buffer: TPTZ solution:ferric chloride solution in 10:1:1. Acetic acid buffer was prepared by mixing 16 ml of glacial acetic acid and 3.1 g of sodium acetate trihydrate ($C_2H_3NaO_2.3H_2O$) in 1 L distilled water. TPTZ solution was prepared by mixing 0.3123 g of TPTZ (2,4,6-tripyridyl-s-triazine), and 0.33 ml

HCl (1 M) in 100 ml distilled water. Ferric chloride solution (0.2 M) was prepared by dissolving 0.54 g of ferric chloride in 100 ml distilled water.

Ascorbic acid was used as a standard and the results were represented in AA equiv. Using a micropipette, 50 µl of each ascorbic acid dilution (refer to section 3.2.3) was transferred into a 2 ml microcentrifuge tube. To these, 1.5 ml of FRAP reagent was added and vortexed for 60 s and the tubes were left for 6 min at room temperature for the reaction to proceed. The blank consisted of 1.5 ml distilled water. The control consisted of 1.5 ml FRAP reagent + 50 µl distilled water. The samples were pipetted into 2.5 ml cuvettes and read at 593 nm in the spectrophotometer. A standard curve was prepared from the spectrophotometer readings and used to calculate the quantity of antioxidants in tissue samples based on AA equiv.

The tuber samples were prepared by placing 10 mg of freeze-dried powdered tissue into 1 ml microcentrifuge tubes to which 1 ml of distilled water was added, vortexed for 60 s, then centrifuged at 4 ^{0}C for 15 min at 3000 x g. After centrifuging, 50 µl of the supernatant was collected into a 2 ml microcentrifuge tube into which 1.5 ml of FRAP solution was added and vortexed for 60 s, then left for 6 min at room temperature for the reaction to proceed. The samples were pipetted into 2.5 ml labeled cuvettes and read at 593 nm in the spectrophotometer.

3.2.5 Quantification of antioxidants, including polyphenolics - High Performance Liquid Chromatography (HPLC)

Polyphenolic acids were identified and quantified using HPLC (Shakya and Navarre, 2006; Vipin et al., 2007). HPLC (Varian 9012, Varian Chromatography Systems, Walnut Creek, CA) was used to identify and quantify three polyphenolic acids (chlorogenic acid, caffeic acid, and ferulic acid) the flavonoid rutin, and ascorbic acid. HPLC identification of a compound is based on the retention time of a particular compound within the column compared with purchased pure standards. Two different buffers (Buffer A and Buffer B) were used as mobile phases. Buffer A had 10 mM formic acid (0.4603 g of formic acid

in 1 L distilled water adjusted to pH 3.5 using 1 M NH_4OH solution). Buffer B had 5 mM ammonium formate (0.3153 g of ammonium formate in 1 L 100% methanol with agitation on a magnetic stirrer as ammonium formate is highly insoluble). An extraction buffer was used for the polyphenol extractions. This extraction buffer was composed of 50 % methanol, 2.5 % metaphosphoric acid, and 1 mM ethylenediaminetetraacetic acid (EDTA) (50 ml of 100 % methanol, 2.5 g of metaphosphoric acid, and 0.442 g of EDTA in 100 ml distilled water with stirring followed by filtering using a cup filter (Millipore Corporation, MS, USA)).

The microtuber samples for HPLC were prepared by placing 50 mg of freeze-dried powered sample into a 1.5 ml microcentrifuge tube along with 0.9 ml of extraction buffer. The tubes were vortexed for 60 s and centrifuged at 4 ^{o}C for 15 min at 3000 x g. The supernatant was micropipetted into a 1.5 ml glass vial. The samples were re-extracted by adding 0.6 ml of extraction buffer to the same sample, vortexed for 60 s, centrifuged, and the supernatant was again collected into the same 1.5 ml glass vial. The 1.5 ml glass vials with supernatant were kept in a speedvac for 6-8 h for vacuum-drying. Following the vacuum-drying, 500 µl of extraction buffer was added to the samples and vortexed for 30 min to solublize them. The sample was filtered into a 1 ml HPLC vial using a 1 ml syringe and topped with a 0.2 µm nylon filter (Fisher Scientific, Ottawa, ON). The HPLC vials were sealed with rubber-topped metal lids using a sealer. The samples were run in the HPLC and the compounds of interest were identified and quantified based on the retention time and area of the peaks in the chromatographs compared to pure standards.

3.2.6 Experimental design and statistical analysis

The experiment was designed as a Completely Randomized Design (CRD) with two main factors; cultivars and storage time (1 and 7 months), three sub-factors within each cultivar; skin, cortex and pith. For each cultivar, five replicates were tested and each replicate was represented by one tuber. The tubers for analysis in each cultivar were selected based on confidence interval values of

tuber masses. Results were analyzed for variance (ANOVA) test using the General Linear Model (GLM) of Statistical Analysis System (SAS) (SAS v 9.2, 2010; SAS Institute Inc., Cary, NC, USA). Means of the results were compared using Duncan's multiple comparison test ($P \leq 0.05$). The results from 1 and 7 months storage were compared using t-tests to investigate the effect of storage on AOC and polyphenolics in individual cultivars. Pearson's correlation test was conducted to determine the correlation among different analysis means.

Concentration data (mg/g DM) for each tissue was transformed into whole/virtual tuber data (mg/100 g FM) using unique conversion factors recommended for specific cultivars which were based on volume measurements (Ortiz-Medina et al., 2009). Conversion-factor mean values for 20 cvs. (Ortiz-Medina et al., 2009) were used for the foreign cultivars as specific conversion factors were not available. This enables logical comparison across the cultivars with different tuber shapes and masses.

3.3 Results

3.3.1 Antioxidant determination assay- 2,2 Diphenyl-1-picryl hydrazyl (DPPH)

a. Twelve Canadian-grown cultivars

DPPH analysis on potato tubers stored for 1 month, showed significant variation in AOC among Canadian-grown cultivars, which ranged from 431.75±59.076 to 128.16±10.936 mg AAE/100 g FM (Table 3.1). Cultivars Onaway and Red Pontiac had the greatest tuber DPPH values, while Green Mountain, Yukon Gold, Russet Burbank, Superior and Norland had the least.

Storage period (1 and 7 months) affected tuber AOC; with significant reduction in all the cultivars except Norland and Yukon Gold (Table 3.1). Response to storage, however, varied with cultivar. Three of the cultivars with the greatest AOC at 1 month (Onaway, Red Pontiac and Kennebec) demonstrated major decreases in AOC by 7 months whereas cultivars with lower values at 1 month showed a lesser effect of storage on AOC (Table 3.1). However, two cultivars with among the least AOC at 1 month had either the greatest (Norland;

205.28±1.541 mg AAE/100 g FM) or the least AOC (Russet Burbank; 58.94±3.288) at 7 months, underlining cultivar-specific effects of storage on AOC. Tuber tissues showed significant variation in AOC as skin showed the greatest AOC (DPPH) values, followed by pith and cortex, at both storage intervals (Table 3.5).

At 1 month, tuber AOC measured using DPPH was significantly positively correlated with AOC measured using FRAP, and with chlorogenic acid, caffeic acid, and rutin but not ascorbic acid or ferulic acid (Table 3.12). After 7 months storage, tuber AOC as assessed by the DPPH assay was significantly positively correlated with AOC measured with FRAP as well as with chlorogenic acid, rutin, and ascorbic acid and not with caffeic acid demonstrating altered relationships between AOC measures and phytochemical content with storage (Table 3.13).

b. Five foreign cultivars

As seen with the Canadian-grown potatoes, the foreign cultivars showed that AOC (DPPH) varied among cultivars after 1 month of storage (Table 3.2). Tuber AOC was greatest in cvs. Bora Valley and Purple Valley (359.66±17.629 and 307.72±9.894 mg AAE/100 g FM, respectively) whereas tuber AOC was not significantly different among the other three foreign cultivars. Cultivar variation in tuber AOC was also evident at 7 months storage with cv. Bora Valley still showing the greatest AOC (210.42±3.252 mg AAE/100 g FM) and the smallest % decline in DPPH AOC with storage. Purple Valley had an intermediate AOC value at 7 months (180.85±8.042 mg AAE/100 g FM), while the other three cultivars that had the lowest AOC values at 1 month. Purple Valley also demonstrated the greatest decrease in AOC with storage time. Overall, a significant reduction in AOC occurred from 1 to 7 months storage in tubers of all cultivars with average AOC values at 7 months of 121.612±12.614 mg AAE/100 g FM as compared with an average AOC at 1 month of 244.55±17.690 mg AAE/100 g FM (Table 3.2). As observed with the Canadian-grown potatoes, the skin in foreign cultivars also had the greatest AOC at both 1 and 7 months storage. The cortex had a higher DPPH AOC value than the pith at 1 month

storage whereas they showed no differences in DPPH AOC at 7 months storage in the foreign cultivars (Table 3.5).

For the foreign cultivars at 1 month of storage, AOC measured by DPPH was significantly positively correlated with the FRAP AOC, chlorogenic acid and rutin. DPPH AOC was negatively correlated with ferulic acid content in the 1 month stored tubers (Table 3.14). At 7 months storage, tuber AOC measured with DPPH was again significantly positively correlated with FRAP AOC, chlorogenic acid, and also with caffeic acid, and no longer with rutin and ferulic acid (Table 3.15).

3.3.2 Antioxidant determination assay - Ferric Reducing Antioxidant Power (FRAP)

a. Twelve Canadian grown cultivars

A wide range of variation was also found in tuber AOC of cultivars analyzed using the FRAP assay, which showed a 6-fold variation between the cultivars with the greatest (Red Pontiac) and least (Atlantic) AOC (694.27±104.735 and 116.62±9.960 mg AAE/100 g FM respectively at 1 month storage (Table 3.3). The cv. Red Pontiac showed the greatest AOC. Cultivars Shepody, Onaway, and Sebago had significantly greater FRAP AOC relative to cvs. Atlantic, Green Mountain, Norland, Russet Burbank, Superior, and Yukon Gold. Cultivars Atlantic, Norland, and Yukon Gold showed the least AOC. The order of the cvs. with greatest and least AOC measured using FRAP and DPPH was the same. At 7 months, the AOC of cultivars varied widely, although the relative order was maintained with storage as Red Pontiac showed the greatest AOC (185.56±2.816) whereas Atlantic and Russet Burbank had the least AOC of 28.61±5.651 and 56.74 ±2.172 mg AAE/100 g FM, respectively.

Storage of 7 months significantly reduced FRAP AOC in all the cvs. except Norland and Yukon Gold. A mean decline of 59.09 % occurred in the AOC between 1 and 7 months (361.45±24.615 and 124.67±6.579 mg AAE/100 g FM at 1 and 7 months, respectively; Table 3.3). Cultivars showed varied response to storage. As cvs. Russet Burbank and Sebago had the greatest decline in AOC,

with 76.93 and 75.63 % respectively (Table 3.3), cvs. Norland and Yukon Gold showed the least decline in AOC, with 2.58 and 4.07 %, respectively.

Significant variation was found at 1 and 7 months storage in the FRAP AOC among the tuber tissues, with skin showing the greatest AOC values versus the pith and cortex (Table 3.5). The pith had significantly greater FRAP AOC versus the cortex at both 1 and 7 months storage (Table 3.5).

The AOC (FRAP) of tubers, measured at 1 month storage, was significantly positively correlated with AOC (DPPH), and with chlorogenic acid, caffeic acid, and rutin, but not ascorbic acid (Table 3.12). At 7 months storage, AOC (FRAP) showed a significant positive correlation with DPPH, ascorbic acid, and rutin (Table 3.13).

b. Five foreign cultivars

Variation in AOC (FRAP) of the tubers from the foreign cultivars ranged from 386.45±18.576 to 179.83±5.571 mg AAE/100 g FM (Table 3.4). While cv. Bora Valley (386.45±18.576 mg AAE/100 g FM) showed the greatest AOC, followed by cv. Purple Valley (319.58±25.428 mg AAE/100 g FM); cv. Gogu Valley (179.83±5.571 mg AAE/ 100 g FM), cv. Alwara (218.87 ± 13.517 mg AAE/ 100 g FM) and cv. Gui Valley (192.70 ± 9.478 mg AAE/ 100 g FM) showed the least AOC. Cultivar variation in AOC of the tubers was also evident with storage period. The cv. Bora Valley still showed the greatest AOC value for FRAP after 7 mo storage, whereas cv. Purple Valley had a greater FRAP AOC than cvs. Alwara, Gogu Valley, and Gui Valley.

Significant reduction in tuber AOC with prolonged storage was evident in all the foreign cultivars (Table 3.4). Cultivars varied widely in storage impact on AOC as cv. Alwara showed the greatest reduction due to long-term storage (67.27 %) while cvs. Gogu Valley and Bora Valley showed the least reduction (38.20 and 38.73 %, respectively). Significant variation in AOC of the tuber tissues was found, with skin showing greatest AOC and no significant variation in FRAP AOC was observed between cortex and pith after short- or long-term storage (Table 3.5).

The FRAP AOC showed significant positive correlation with chlorogenic acid, rutin, and DPPH AOC in 1-month stored tubers (Table 3.14) and with chlorogenic acid, caffeic acid and DPPH AOC in the 7-months stored tubers (Table 3.15). The above correlations were similar to those found with DPPH AOC in 1- and 7-months stored tubers.

3.3.3 Quantification of the major antioxidants - High Performance Liquid Chromatography (HPLC)

a. Twelve Canadian cultivars

Five antioxidant compounds, including ascorbic acid, chlorogenic acid, caffeic acid, ferulic acid, and rutin were present in tubers of all 12 cvs. (Table 3.6). Ascorbic acid content varied significantly among cultivars; Goldrush and Shepody had the greatest levels (43.40±3.722 and 41.34±0.541 mg/100 g FM, respectively) and Russet Burbank and Green Mountain the least levels (14.39±5.818 and 18.91±0.469 mg/100 g FM, respectively) at 1-month storage. Chlorogenic acid also varied significantly between cultivars; cv. Onaway had the greatest content (2.66±0.232 mg/100 g FM) and cvs. Russet Burbank, Goldrush, Green Mountain, and Yukon Gold the least, with much lower values (0.98±0.069, 1.01±0.065, 1.25±0.061, and 1.28±0.204 mg/100 g FM, respectively). Cultivar Onaway tubers had relatively greater ascorbic acid content and significantly greatest chlorogenic acid, caffeic acid, and rutin content, at 1-month storage. Curiously, cv. Red Pontiac, which showed the greatest tuber AOC in both FRAP and DPPH assays, had relatively low ascorbic and chlorogenic acid contents at both 1- and 7-months storage.

Significant reduction in quantity of the five antioxidant compounds analysed was shown from 1- to 7-months storage except for chlorogenic acid in cvs. Atlantic, Norland and Yukon Gold, and caffeic acid in cv. Green Mountain. While storage appeared to have the greatest impact on caffeic and ferulic acid content (average reduction of 92.16 and 90.21%, respectively), ascorbic acid and rutin (average decline of 80.48 and 81.50 %, respectively) and chlorogenic acid levels (average reduction of 60.27 %) appeared to be less affected (Table 3.10).

Significant variation in the quantity of the compounds analyzed was found in different tissues of the tuber with skin consistently showing the greatest concentration for all five phytochemicals measured (Table 3.8). The cortex and pith did not differ significantly in terms of the five phytochemicals measured (Table 3.8).

In tubers stored for 1 month, significant positive correlations were found between ascorbic acid and caffeic acid; between chlorogenic acid and caffeic acid, rutin, FRAP, and DPPH; between caffeic acid and all other compounds, FRAP and DPPH (Table 3.12). Significant negative correlations were found between ascorbic and ferulic acids in tubers stored for 1 month.

b. Five foreign cultivars

HPLC results showed the presence of significant quantities of ascorbic acid, chlorogenic acid, caffeic acid, ferulic acid, and rutin in all five foreign cultivars at 1 month of storage, but no caffeic acid was observed in cv. Gogu Valley (Table 3.7). Chlorogenic acid was the predominant compound present in the foreign cultivars and ranged widely from 35.05±1.411 to 3.20±0.235 mg/100 g FM. The purple coloured cv. Bora Valley which showed the greatest tuber AOC in both FRAP and DPPH assays also showed significantly greater quantities of chlorogenic acid, caffeic acid, and rutin among the five cultivars. Similarly, cv. Purple Valley that had relatively higher FRAP and DPPH AOC values relative to cv. Gogu Valley, Alwara, and Gui Valley, also had significantly higher concentrations of chlorogenic acid and rutin in comparison to the above cultivars. In contrast, cv. Gui Valley had the greatest concentrations of ferulic acid relative to the other five cultivars with cv. Purple Valley showing the lowest concentrations.

Significant reduction in quantity of these compounds occurred as storage progressed from 1 to 7 months. While cvs. Bora Valley, Gogu Valley, and Purple Valley had well-retained ascorbic acid content, cvs. Alwara and and Gui Valley showed significant reduction (Table 3.7). Cultivar Bora Valley stored relatively well in comparison with the rest of the foreign cultivars screened with no

significant reduction in ascorbic acid, caffeic and ferulic acids. Among the phytochemicals analysed, ascorbic and ferulic acid content of tubers showed the least and greatest reduction, respectively, with time in storage (Table 3.11).

Significant variation was found in the distribution of phytochemicals in different tuber tissue layers (Table 3.9). Skin showed significantly greatest polyphenolic concentration, whereas cortex and pith showed no significant differences between tissues in the concentrations of chlorogenic acid, caffeic acid, and rutin. Pith showed significantly greater concentrations of ascorbic acid. The ascorbic acid concentrations did not differ between cortex and skin. Ferulic acid was present only in the skin tissue.

At 1 month storage, ascorbic acid content was positively and negatively correlated with ferulic acid and rutin, respectively (Table 3.14). Chlorogenic acid was positively correlated with rutin, and with both FRAP and DPPH AOCs, and negatively correlated with ferulic acid content. Rutin was positively correlated with chlorogenic acid, FRAP and DPPH assays, and was negatively correlated with ferulic acid content.

3.4 Discussion

AOC of the potato tubers measured using DPPH and FRAP showed a wide range of variation both in the Canadian-grown and foreign cultivar groups, which was in accordance with our first hypothesis and previous studies (Reddivari et al., 2007). Six cultivars, Onaway, Red Pontiac, Goldrush, Kennebec, Sebago, and Shepody were consistently greater in AOC than the other six cultivars. It is interesting that cv. Onaway has both white skin and flesh while cv. Red Pontiac has red coloured skin and white flesh (Figure 3.1). So, cultivars with the greatest AOC capacity are not necessarily brightly pigmented. Notably, the cvs. Yukon Gold and Norland, with the lowest tuber AOC contained yellow coloured skin and flesh, and red coloured skin and white flesh, respectively. This suggests that brightly coloured skin or flesh is not necessarily correlated with elevated AOC. However, the FRAP and DPPH assays used water as extraction solvent (Andre et al., 2007), considered only the AOC affected by hydrophilic antioxidant

components. Thus cultivars with yellow skinned or/and yellow flesh such as Goldrush, Shepody, and Yukon Gold containing relatively greater amounts of lipophilic carotenoids would have their AOC underestimated. Among the foreign cultivars, Bora Valley and Purple Valley tubers with purple coloured skin and flesh showed greater AOC, while the red skinned cv. Gogu Valley (Figure 3.2) had the least AOC with both DPPH and FRAP assays.

The DPPH and FRAP assays were highly correlated (Table 3.12; 3.13; 3.14; 3.15). Although the order of the cultivars (Canadian-grown and foreign) from greater to least tuber AOC was not exactly identical, both the tests showed more or less a similar trend in dividing them into groups with high, medium and low tuber AOC (Tables 3.1; 3.3). As the two different tests have different mechanisms, different kinds of antioxidants react differently with DPPH and FRAP reagents. The AOC of the tubers measured using FRAP analysis seemed to show greater values than DPPH analysis among the Canadian-grown cultivars, but little variation for the foreign cultivars. Lesser tuber AOC values for DPPH in comparison to FRAP may be due to the fact that DPPH does not react with free radical intermediates and oxidative chain reaction products (Nair et al., 2007) or measure the antioxidants which quench singlet oxygen (Prior et al., 2005). The six Canadian-grown cultivars Goldrush, Kennebec, Onaway, Red Pontiac, Shepody, and Sebago had the greatest tuber AOC analyzed by DPPH and FRAP at 1 month storage. Onaway, Red Pontiac, and Shepody maintained the greatest AOC at 7 months storage (Table 3.1, 3.3), which would suggest that the latter cultivars would provide the best antioxidant benefits among the tested Canadian cultivars. Among the foreign cvs., Bora Valley had the greatest tuber AOC after 1 month storage, which was maintained as the best AOC over 7 months storage, which indicates that Bora Valley could provide of the best antioxidant benefits among the tested foreign cultivars.

The skin tissue showed significantly greater AOC both in Canadian-grown and foreign cultivars at two storage time intervals (1 and 7 months) (Table 3.5). The AOC of the skin was approximately 1.5 to 2 times and 2.5 to 4 times greater than the inner flesh in Canadian and foreign cultivars respectively. This was in

accordance with our second hypothesis and previous study (Li et al., 2006) which showed two-fold greater AOC of the skin than inner flesh. Significant differences in tuber AOC were not found between cortex and pith in foreign cultivars, but pith showed greater AOC compared with cortex in Canadian-grown cultivars (Table 3.5). In most cases, pith occupies a greater total volume of the tuber than cortex and both are significantly greater in volume than the skin.

Wide range was found in the quantities of different phenolic compounds analysed both in Canadian-grown and foreign cultivars. Interestingly, the white skinned cv. Onaway had significantly greater amount of phenolic compounds than red skinned cv. Red Pontiac (Table 3.6), which is not in accordance with previous studies showing that purple and red skinned tubers contained twice the concentration of phenolic acids than white-skinned tubers (Brown, 2005). Among the foreign cultivars, purple skinned and fleshed Bora Valley and Purple Valley showed 6 to 10 times the concentration of chlorogenic acid compared with the white fleshed cv. Gogu Valley (Table 3.7). This latter result is indicative of a greater differential than suggested by earlier work whereby tubers with purple or red flesh contained 3 to 4 times the concentration of phenolic acids compared with white-fleshed tubers (Lewis et al. 1998). Chlorogenic acid, the major phenolic compound of potato (80 % of total phenolics; Brown, 2005) accounted up to 70 % (Canadian cultivars; Table 3.6) and 98 % (foreign cultivars; Table 3.7) of mean summative values of the three phenolic compounds (chlorogenic acid, caffeic acid and ferulic acid) analysed in our study. The ascorbic acid content of Canadian-grown cultivars (14.39±5.818 to 43.40±3.722 mg/100 g FM; Table 3.6) and foreign cultivars (1.32±0.171 to 8.82±0.608 mg/100 g FM; Table 3.7) at 1 month storage showed a wide range, similar to the earlier work done on North American cultivars and breeding lines (11.5 to 29.8 mg/ 100 g FM; Love et al., 2003).

Cultivars with greater tuber AOC when fresh and that show better retention of tuber AOC over longer storage periods would likely provide nutritionally better antioxidant related benefits. In our study, ascorbic acid and phenolic antioxidants were reduced significantly in quantity when storage was extended from 1 to 7 months (Table 3.10; 3.11). This contrasts with a previous

study (Stushnoff et al., 2008) that showed an increase in total phenolic content in highly pigments cultivars stored for 6-7 months at 5±1 ^{o}C. The degree of antioxidant reduction varied with the phytonutrient compound. Generally, chlorogenic acid and caffeic acid showed the least and greatest % reduction, respectively, with storage among the 12 Canadian-grown cultivars. Among the 5 foreign cultivars, ascorbic acid and ferulic acid showed the least and greatest % reduction respectively. There was a huge difference in the % reduction of ascorbic acid with storage among Canadian-grown cultivars (80.5 %) and foreign cultivars (36.1 %) (Tables 3.10, 3.11). This lower percentage reduction in foreign cultivars could be due to the presence of a greater concentration of ascorbic acid in relatively less effected inner pith tissue (Table 3.9) in comparison with skin tissue in the Canadian-grown cultivars (Table 3.8).

Both the Canadian-grown and foreign cultivars showed significant variation in the distribution of the antioxidant phytonutrients in different tissue layers of the tubers, which was in accordance with our second hypothesis and previous studies (Li et al., 2006). Skin tissue showed greater AOC than inner flesh, which could be due to greater accumulation of phenolic compounds and pigmentation molecules in the skin compared with inner flesh. The skin tissue showed 0.9 to 1.6-fold greater phenolics than inner flesh in purple and red-fleshed potatoes (Reyes et al., 2005); it may accumulate up to 50 % of the phenolics (Friedman, 1997). Our study showed similar results but with a greater degree of phenolic compound accumulation in the skin. The major potato phenolic compound, chlorogenic acid showed up to 35 to 45 times, and 6 to 7 times greater accumulation in skin than the inner tissues in Canadian cultivars (Table 3.8) and foreign cultivars (Table 3.9) respectively. Similar trends were observed for other phenolic antioxidants analysed. While skin showed greater ascorbic acid, chlorogenic acid, caffeic acid, ferulic acid, and rutin in Canadian-grown cultivars, ascorbic acid was uniquely greater in pith of the foreign cultivars.

3.5 Conclusions

In the Canadian-grown cultivar group, Goldrush, Kennebec, Onaway, Red Pontiac, Shepody, and Sebago had the greatest tuber AOC and are therefore recommended as healthiest nutritionally in terms of AOC for the Canadian-grown group. Among these, cv. Onaway tubers had relatively greater ascorbic acid content and significantly greater chlorogenic acid, caffeic acid, and rutin content, at 1 month storage. For this reason, it is the best cultivar nutritionally among the cultivars tested, despite its lack of bright pigmentation in skin or flesh. Skin or flesh colour is not necessarily the best indicator of AOC, since of these twelve, only cv. Red Pontiac which had deeply pigmented tissue (skin) showed greater AOC (Figure 3.1). Conversely, cv. Norland tubers also had deeply pigmented red skin, but it was among the group of cultivars with the least AOC. This underlines the potential discrepancies between skin colour and AOC.

In the foreign cultivar group, cvs. Bora Valley and Purple Valley were greatest for tuber AOC and had deeply purple pigmented skin and flesh (Figure 3.2). Cultivar Bora Valley had approximately twice the AOC and ten times the chlorogenic acid content of red skinned and white fleshed cv. Gogu Valley, indicating that cultivars with tubers that have solidly pigmented flesh can have much greater AOC than those with tubers that have coloured skin and white flesh.

The tubers of both Canadian-grown and foreign cultivars had greater accumulation of phenolic antioxidants in the skin than inner flesh of cortex or pith. This underlines the importance of consuming potato skin. Though the skin tissue composes only 2 % of the tuber volume (Ortiz-Medina et al., 2009), its higher accumulation of phenolic antioxidants makes the skin tissue an important dietary contributor of antioxidants. However, long-term storage (7 months) had a large negative impact on AOC; fresh tubers should be consumed where possible.

Table 3.1 Antioxidant capacity (mean ± SE) values of 12 Canadian-grown cultivars analyzed after 1 and 7 months in storage and expressed as mg/100 g FM of Ascorbic Acid Equivalents (AAE) using the 2,2 Diphenyl-1-Picryl Hydrazyl (DPPH). (n=5).

Cultivars	**1 month storage (mg AAE/100 g FM)**	**7 month storage (mg AAE/100 g FM)**	***Significance between 1 and 7 months storage**	**(% reduction from 1 to 7 months storage)**
Atlantic	235.29 ± 15.955 ^{cd}	124.14 ± 5.781 ^{cd}	0.0002	47.24
Green Mountain	210.04 ± 14.944 ^{de}	114.65 ± 6.173 ^{d}	0.0004	45.41
Goldrush	327.18 ± 17.482 ^{b}	140.84 ± 12.321 ^{c}	<.0001	56.95
Kennebec	380.61 ± 32.542 ^{ab}	112.79 ± 5.553 ^{d}	<.0001	70.37
Norland	189.49 ± 11.669 ^{de}	205.28 ± 1.541 ^{a}	0.2165	-8.33
Onaway	431.75 ± 59.076 ^{a}	172.39 ± 6.505 ^{b}	0.0024	60.07
Russet Burbank	186.41 ± 10.294 ^{de}	58.94 ± 3.288 ^{e}	<.0001	68.38
Red Pontiac	419.28 ± 40.445 ^{a}	128.54 ± 3.999 ^{cd}	<.0001	69.42
Sebago	315.24 ± 22.329 ^{bc}	119.88 ± 7.366 ^{cd}	<.0001	61.97
Shepody	306.61 ± 27.460 ^{bc}	128.86 ± 5.409 ^{cd}	0.0002	57.97
Superior	198.67 ± 12.777 ^{de}	114.17 ± 4.372 ^{d}	0.0002	42.53
Yukon Gold	128.16 ± 10.936 ^{e}	137.70 ± 9.351 ^{c}	0.5191	-7.58
Mean	277.51 ± 14.335	129.90 ± 4.706		47.03

** T-test significance at P < 0.05; Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test (P < 0.05).*

Table 3.2 Antioxidant capacity (mean ± SE) values of 5 foreign cultivars analyzed after 1 and 7 months in storage and expressed as mg/100 g FM of Ascorbic Acid Equivalents (AAE) using the 2,2 Diphenyl-1-Picryl Hydrazyl (DPPH). (n=5).

Cultivars	**1 month storage (mg AAE/100 g FM)**	**7 month storage (mg AAE/100 g FM)**	***Significance between 1 and 7 months storage**	**(% reduction from 1 to 7 months storage)**
Alwara	204.29 ± 6.662 [b]	75.75 ± 1.561 [c]	<.0001	62.92
Bora Valley	359.66 ± 17.629 [a]	210.42 ± 3.252 [a]	<.0001	41.49
Gogu Valley	171.93 ± 3.576 [b]	72.40 ± 1.847 [c]	<.0001	57.89
Gui Valley	179.16 ± 10.163 [b]	68.64 ± 3.219 [c]	<.0001	61.69
Purple Valley	307.72 ± 9.894 [a]	180.85 ± 8.042 [b]	<.0001	48.40
Mean	244.55 ± 17.690	121.612 ± 12.614		54.47

** T-test significance at P < 0.05; Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test (P < 0.05).*

Table 3.3 Antioxidant capacity (mean ± SE) values of 12 Canadian-grown cultivars analyzed after 1 and 7 months in storage and expressed as mg/100 g FM of Ascorbic Acid Equivalents (AAE) using the Ferric Reducing Antioxidant Power (FRAP). (n=5).

Cultivars	**1 month storage (mg AAE/100 g FM)**	**7 month storage (mg AAE/100 g FM)**	***Significance between 1 and 7 months storage**	**(% reduction from 1 to 7 months storage)**
Atlantic	116.62 ± 9.960 ^{g}	28.61 ± 5.651 ^{f}	<.0001	75.47
Green Mountain	322.25 ± 6.097 ^{cd}	123.21 ± 10.532 ^{cde}	<.0001	61.69
Goldrush	424.09 ± 28.432 ^{bc}	145.62 ± 12.627 ^{cde}	<.0001	65.59
Kennebec	425.67 ± 21.507 ^{bc}	116.14 ± 2.409 ^{de}	<.0001	72.66
Norland	128.14 ± 16.866 ^{fg}	124.82 ± 9.942 ^{cde}	0.8684	02.58
Onaway	499.95 ± 51.985 ^{b}	150.27 ± 1.478 ^{abcd}	0.0001	69.88
Russet Burbank	246.44 ± 26.484 ^{def}	56.74 ± 2.172 ^{f}	<.0001	76.93
Red Pontiac	694.27 ± 104.735 ^{a}	185.56 ± 2.816 ^{a}	0.0013	73.72
Sebago	493.54 ± 54.421 ^{b}	120.01 ± 5.484 ^{cde}	0.0001	75.63
Shepody	516.00 ± 38.574 ^{b}	157.90 ± 21.842 ^{abc}	<.0001	69.34
Superior	284.98 ± 14.030 ^{de}	109.30 ± 21.437 ^{e}	0.0001	61.57
Yukon Gold	185.43 ± 17.480 ^{efg}	177.87 ± 22.846 ^{ab}	0.7982	04.07
Mean	361.45 ± 24.615	124.67 ± 6.579		59.09

** T-test significance at P < 0.05; Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test (P < 0.05).*

Table 3.4 Antioxidant capacity (mean ± SE) values of 5 foreign cultivars analyzed after 1 and 7 months in storage and expressed as mg/100 g FM of Ascorbic Acid Equivalents (AAE) using the Ferric Reducing Antioxidant Power (FRAP). (n=5).

Cultivars	**1 month storage (mg AAE/100 g FM)**	**7 month storage (mg AAE/100 g FM)**	***Significance between 1 and 7 months storage**	**(% reduction from 1 to 7 months storage)**
Alwara	218.87 ± 13.517 [c]	71.49 ± 2.355 [c]	<.0001	67.27
Bora Valley	386.45 ± 18.576 [a]	236.28 ± 37.793 [a]	0.0075	38.73
Gogu Valley	179.83 ± 5.571 [c]	110.91 ± 17.614 [bc]	0.0059	38.20
Gui Valley	192.70 ± 9.478 [c]	89.29 ± 19.042 [c]	0.0013	53.57
Purple Valley	319.58 ± 25.428 [b]	163.43 ± 18.626 [b]	0.0011	48.76
Mean	259.49 ± 17.646	134.28 ± 15.181		49.31

** T-test significance at $P < 0.05$; Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test ($P < 0.05$).*

Table 3.5 Tuber tissue (skin, cortex, and pith) concentrations (per g Dry Mass (DM)); 2,2 Diphenyl-1-Picryl Hydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) means ± SE of 12 Canadian-grown and 5 foreign cultivars analyzed 1 and 7 months after storage and expressed as mg/g DM of Ascorbic Acid Equivalents (AAE) (n=60 and 25 in Canadian and foreign cultivars respectively).

	12 Canadian-grown cultivars (mg AAE/g DM)		**5 Foreign cultivars (mg AAE/g DM)**	
		1 month storage		
	DPPH	**FRAP**	**DPPH**	**FRAP**
Cortex	11.25 ± 0.508 [c]	13.73 ± 0.977 [c]	12.53 ± 0.587 [b]	11.04 ± 0.403 [b]
Pith	12.84 ± 0.816 [b]	17.89 ± 0.137 [b]	10.85 ± 0.518 [c]	11.80 ± 0.636 [b]
Skin	33.25 ± 0.894 [a]	28.77 ± 0.908 [a]	27.42 ± 0.583 [a]	45.93 ± 0.165 [a]
		7 months storage		
	DPPH	**FRAP**	**DPPH**	**FRAP**
Cortex	4.90 ± 0.202 [c]	5.18 ± 0.317 [c]	5.38 ± 0.370 [b]	6.13 ± 0.489 [b]
Pith	6.18 ± 0.258 [b]	5.79 ± 0.354 [b]	5.53 ± 0.402 [b]	5.96 ± 0.517 [b]
Skin	19.46 ± 0.886 [a]	16.01 ± 0.433 [a]	16.39 ± 0.674 [a]	19.76 ± 0.133 [a]

Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test ($P < 0.05$).

Table 3.6 High Performance Liquid Chromatography (HPLC) results showing the virtual tuber (100 g Fresh Mass (FM)) means ± SE of ascorbic acid, chlorogenic acid, caffeic acid, ferulic acid, and rutin of 12 Canadian-grown cultivars analyzed 1 and 7 months after storage (n=5).

Cultivars	Ascorbic acid (mg/100 g FM)			Chlorogenic acid (mg/100 g FM)		
	1 month	7 months	*Sig between 1 & 7 months	1 month	7 months	*Sig between 1 &7 months
Atlantic	34.59 ± 0.916 ^{bcd}	6.52 ± 0.073 ^{cd}	<.0001	1.33 ± 0.244 ^{bcd}	0.93 ± 0.017 ^{b}	0.1462
Green Mountain	18.91 ± 0.469 ^{fg}	4.47 ± 0.172 ^{f}	<.0001	1.25 ± 0.061 ^{cd}	0.31 ± 0.020 ^{e}	<.0001
Goldrush	43.40 ± 3.722 ^{a}	7.05 ± 0.181 ^{bc}	<.0001	1.01 ± 0.065 ^{d}	0.38 ± 0.036 ^{de}	<.0001
Kennebec	27.45 ± 1.179 ^{de}	6.38 ± 0.111 ^{d}	<.0001	1.56 ± 0.136 ^{bc}	0.29 ± 0.008 ^{e}	<.0001
Norland	30.30 ± 0.521 ^{cde}	6.51 ± 0.058 ^{cd}	<.0001	1.75 ± 0.109 ^{bc}	1.61 ± 0.020 ^{a}	0.2405
Onaway	34.72 ± 0.845 ^{bcd}	6.04 ± 0.542 ^{de}	<.0001	2.66 ± 0.232 ^{a}	0.43 ± 0.023 ^{d}	<.0001
Russet Burbank	14.39 ± 5.818 ^{g}	3.06 ± 0.203 ^{g}	0.0875	0.98 ± 0.069 ^{d}	0.16 ± 0.06 ^{f}	<.0001
Red Pontiac	25.28 ± 0.433 ^{ef}	4.26 ± 0.058 ^{f}	<.0001	1.46 ± 0.054 ^{bcd}	0.36 ± 0.023 ^{de}	<.0001
Sebago	35.66 ± 2.479 ^{bc}	5.46 ± 0.038 ^{e}	<.0001	1.83 ± 0.163 ^{b}	0.46 ± 0.052 ^{d}	<.0001
Shepody	41.34 ± 0.541 ^{ab}	10.03 ± 0.263 ^{a}	<.0001	1.76 ± 0.204 ^{bc}	0.76 ± 0.074 ^{c}	0.0017
Superior	31.97 ± 2.655 ^{cde}	4.71 ± 0.053 ^{f}	<.0001	1.76 ± 0.199 ^{bc}	0.76 ± 0.046 ^{c}	0.0012
Yukon Gold	29.99 ± 0.307 ^{cde}	7.34 ± 0.048 ^{b}	<.0001	1.28 ± 0.204 ^{cd}	0.93 ± 0.020 ^{b}	0.1272
Mean	30.67 ± 1.209	5.99 ± 0.281		1.55 ± 0.071	0.62 ± 0.052	

** T-test significance at P < 0.05; Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test (P < 0.05).*

Table 3.6 continued

Cultivars	Caffeic acid (mg/100 g FM)			Ferulic acid (mg/100 g FM)		
	1 month	7 months	*Sig between 1 & 7 months	1 month	7 months	*Sig between 1 & 7 months
Atlantic	0.30 ± 0.063 ^{bcd}	0.02 ± 0.002 ^{b}	0.0022	0.13 ± 0.023 ^{gh}	0.01 ± 0.000 ^{d}	0.0008
Green Mountain	0.18 ± 0.012 ^{d}	0.15 ± 0.006 ^{a}	0.0623	0.18 ± 0.014 ^{fg}	traces ^{d}	0.0005
Goldrush	0.18 ± 0.008 ^{d}	traces ^{c}	<.0001	0.02 ± 0.005 ^{h}	Nil	
Kennebec	0.17 ± 0.010 ^{d}	traces ^{c}	<.0001	0.36 ± 0.056 ^{de}	0.14 ± 0.020 ^{a}	0.0059
Norland	0.20 ± 0.008 ^{d}	0.01 ± 0.000 ^{c}	<.0001	0.39 ± 0.018 ^{cd}	0.04 ± 0.000 ^{c}	<.0001
Onaway	0.88 ± 0.091 ^{a}	0.02 ± 0.002 ^{b}	<.0001	0.84 ± 0.089 ^{b}	Nil	
Russet Burbank	0.19 ± 0.014 ^{d}	traces ^{c}	<.0001	1.07 ± 0.082 ^{a}	0.07 ± 0.006 ^{b}	<.0001
Red Pontiac	0.37 ± 0.059 ^{bc}	Nil		0.49 ± 0.039 ^{c}	0.02 ± 0.000 ^{d}	<.0001
Sebago	0.39 ± 0.051 ^{b}	Nil		0.23 ± 0.033 ^{efg}	traces ^{d}	0.0001
Shepody	0.36 ± 0.032 ^{bc}	Nil		0.31 ± 0.014 ^{def}	0.02 ± 0.002 ^{d}	<.0001
Superior	0.22 ± 0.002 ^{cd}	0.01 ± 0.001 ^{c}	<.0001	0.24 ± 0.014 ^{efg}	Nil	
Yukon Gold	0.27 ± 0.084 ^{bcd}	traces ^{c}	0.0125	0.27 ± 0.005 ^{def}	0.01 ± 0.000 ^{d}	<.0001
Mean	0.31 ± 0.032	0.02 ± 0.011		0.38 ± 0.040	0.04 ± 0.010	

** T-test significance at P < 0.05; Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test (P < 0.05).*

Table 3.6 continued

Cultivars	Rutin (mg/100 g FM)		
	1 month	**7 month**	***Sig between 1 & 7 months**
Atlantic	0.74 ± 0.018 ^{de}	0.05 ± 0.013 ^{e}	<.0001
Green Mountain	0.95 ± 0.120 ^{bcde}	Nil	
Goldrush	0.83 ± 0.064 ^{cde}	0.25 ± 0.008 ^{bc}	<.0001
Kennebec	0.76 ± 0.027 ^{cde}	0.18 ± 0.012 ^{cd}	<.0001
Norland	0.87 ± 0.036 ^{bcde}	0.17 ± 0.015 ^{cd}	<.0001
Onaway	2.64 ± 0.121 ^{a}	0.47 ± 0.017 ^{a}	<.0001
Russet Burbank	1.24 ± 0.189 ^{b}	Nil	
Red Pontiac	1.14 ± 0.163 ^{bc}	0.16 ± 0.011 ^{d}	0.0003
Sebago	0.78 ± 0.036 ^{cde}	0.18 ± 0.008 ^{cd}	<.0001
Shepody	1.10 ± 0.223 ^{bcd}	0.27 ± 0.081 ^{b}	0.0083
Superior	0.70 ± 0.035 ^{e}	0.11 ± 0.016 ^{de}	<.0001
Yukon Gold	0.90 ± 0.113 ^{bcde}	0.12 ± 0.008 ^{de}	0.0001
Mean	1.05 ± 0.072	0.19 ± 0.022	

** T-test significance at P < 0.05; Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test (P < 0.05).*

Table 3.7 High Performance Liquid Chromatography (HPLC) results showing the virtual tuber (100 g Fresh Mass (FM)) means ± SE of ascorbic acid, chlorogenic acid, caffeic acid, ferulic acid, and rutin of 5 foreign cultivars analyzed 1 and 7 months after storage (n=5).

Cultivars	Ascorbic acid (mg/100 g FM)			Chlorogenic acid (mg/100 g FM)		
	1 month	7 months	*Sig between 1 & 7 months	1 month	7 months	*Sig between 1 & 7 months
Alwara	8.50 ± 0.635 [a]	3.10 ± 0.670 [b]	0.0004	8.74 ± 1.071 [c]	1.37 ± 0.379 [c]	0.0002
Bora Valley	2.31 ± 0.873 [c]	3.41 ± 0.662 [ab]	0.3424	35.05 ± 1.411 [a]	13.91 ± 0.524 [a]	<.0001
Gogu Valley	1.32 ± 0.171 [c]	1.56 ± 0.026 [c]	0.2094	3.20 ± 0.235 [d]	0.99 ± 0.007 [c]	<.0001
Gui Valley	8.82 ± 0.608 [a]	4.36 ± 0.084 [ab]	<.0001	3.87 ± 0.545 [d]	0.50 ± 0.017 [c]	0.0003
Purple Valley	5.51 ± 0.955 [b]	4.49 ± 0.034 [a]	0.3158	21.41 ± 2.037 [b]	5.30 ± 0.040 [b]	<.0001
Mean	5.29 ± 0.426	3.38 ± 0.181		14.45 ± 1.643	4.41 ± 0.671	

** T-test significance at P < 0.05; Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test (P < 0.05).*

Table 3.7 continued

Cultivars	Caffeic acid (mg/100 g FM)			Ferulic acid (mg/100 g FM)		
	1 month	**7 months**	***Sig between 1 & 7 months**	**1 month**	**7 months**	***Sig between 1 & 7 months**
Alwara	0.16 ± 0.004 [c]	0.03 ± 0.006 [b]	<.0001	0.07 ± 0.014 [b]	traces [b]	0.0143
Bora Valley	0.25 ± 0.023 [a]	0.21 ± 0.039 [a]	0.4489	0.06 ± 0.017 [bc]	traces [b]	0.1273
Gogu Valley	Nil	0.02 ± 0.001 [b]		0.05 ± 0.007 [bc]	traces [b]	0.0001
Gui Valley	0.20 ± 0.002 [b]	0.05 ± 0.001 [b]	<.0001	0.16 ± 0.022 [a]	0.01 ± 0.002 [a]	0.0002
Purple Valley	0.15 ± 0.014 [c]	traces [b]	<.0001	0.01 ± 0.002 [c]	Nil	
Mean	0.19 ± 0.010	0.06 ± 0.011		0.07 ± 0.013	0.01 ± 0.001	

** T-test significance at P < 0.05; Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test (P < 0.05).*

Table 3.7 continued

Cultivars	Rutin (mg/100 g FM)		
	1 month	**7 month**	***Sig between 1 & 7 months**
Alwara	0.97 ± 0.218 [c]	0.09 ± 0.012 [bc]	0.0040
Bora Valley	2.96 ± 0.168 [a]	0.19 ± 0.068 [ab]	<.0001
Gogu Valley	0.76 ± 0.042 [c]	0.03 ± 0.001 [c]	<.0001
Gui Valley	0.69 ± 0.044 [c]	0.25 ± 0.013 [a]	<.0001
Purple Valley	2.24 ± 0.264 [b]	0.15 ± 0.012 [b]	<.0001
Mean	1.52 ± 0.126	0.14 ± 0.011	

** T-test significance at P < 0.05; Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test (P < 0.05).*

Table 3.8 Tuber tissue (skin, cortex, and pith) concentrations (per g Dry Mass (DM)); High Performance Liquid Chromatography (HPLC) means ± SE of 12 Canadian-grown cultivars analysed after 1 and 7 months storage (n=120).

	Ascorbic acid (mg/g DM)	Chlorogenic acid (mg/g DM)	Caffeic acid (mg/g DM)	Ferulic acid (mg/g DM)	Rutin (mg/g DM)
Cortex	0.86 ± 0.060 [b]	0.04 ± 0.003 [b]	0.01 ± 0.001 [b]	0.01 ± 0.001 [b]	0.03 ± 0.003 [b]
Pith	0.79 ± 0.056 [b]	0.03 ± 0.003 [b]	0.01 ± 0.001 [b]	0.01 ± 0.001 [b]	0.03 ± 0.002 [b]
Skin	1.42 ± 0.101 [a]	1.40 ± 0.093 [a]	0.06 ± 0.010 [a]	0.22 ± 0.022 [a]	0.17 ± 0.031 [a]

Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test (P < 0.05).

Table 3.9 Tuber tissue (skin, cortex, and pith) concentrations (per g Dry Mass (DM)); High Performance Liquid Chromatography (HPLC) means ± SE of 5 foreign cultivars analysed after 1 and 7 months storage (n=50).

	Ascorbic acid (mg/g DM)	Chlorogenic acid (mg/g DM)	Caffeic acid (mg/g DM)	Ferulic acid (mg/g DM)	Rutin (mg/g DM)
Cortex	0.17 ± 0.012 [b]	0.45 ± 0.044 [b]	0.01 ± 0.000 [b]	Nil	0.04 ± 0.004 [b]
Pith	0.22 ± 0.013 [a]	0.40 ± 0.048 [b]	0.01 ± 0.000 [b]	Nil	0.05 ± 0.005 [b]
Skin	0.16 ± 0.011 [b]	2.90 ± 0.200 [a]	0.16 ± 0.023 [a]	0.14 ± 0.015 [a]	0.44 ± 0.032 [a]

Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test (P < 0.05).

Table 3.10 Virtual tuber (100 g Fresh Mass (FM)) High Performance Liquid Chromatography (HPLC) means ± SE of 12 Canadian-grown cultivars analyzed after 1 and 7 months storage. (n=60).

	1 month (mg/100 g FM)	7 months (mg/100 g FM)	% reduction; from 1 to 7 months storage
Ascorbic acid	30.67 ± 1.210 [a]	5.99 ± 0.231 [b]	80.48
Chlorogenic acid	1.55 ± 0.070 [a]	0.62 ± 0.051 [b]	60.27
Caffeic acid	0.31 ± 0.027 [a]	0.02 ± 0.006 [b]	92.16
Ferulic acid	0.38 ± 0.039 [a]	0.04 ± 0.006 [b]	90.21
Rutin	1.05 ± 0.072 [a]	0.19 ± 0.016 [b]	81.50

Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test ($P < 0.05$).

Table 3.11 Virtual tuber (100 g Fresh Mass (FM)) High Performance Liquid Chromatography (HPLC) means ± SE of 5 foreign cultivars analyzed after 1 and 7 months storage. (n=25).

	1 month (mg/100 g FM)	7 months (mg/100 g FM)	% reduction; from 1 to 7 months storage
Ascorbic acid	5.29 ± 0.447 [a]	3.38 ± 0.178 [b]	36.09
Chlorogenic acid	14.45 ± 1.640 [a]	4.41 ± 0.669 [b]	69.47
Caffeic acid	0.19 ± 0.006 [a]	0.06 ± 0.011 [b]	68.21
Ferulic acid	0.07 ± 0.007 [a]	0.01 ± 0.001 [b]	90.94
Rutin	1.52 ± 0.128 [a]	0.14 ± 0.013 [b]	90.63

Values with same superscript in the same column are not significantly different based on Duncan's New Multiple Range Test ($P < 0.05$).

Table 3.12 Virtual tuber mean correlations among ascorbic acid (AA), chlorogenic acid (CGA), caffeic acid (CFA), ferulic acid (FA), rutin (RT), Ferric Reducing Antioxidant Power (FRAP) and 2,2 Diphenyl-1-Picryl Hydrazyl (DPPH) in 12 Canadian-grown cultivars after 1 month storage (Pearson's Correlation Coefficient test).

	AA	**CGA**	**CFA**	**FA**	**RT**	**FRAP**	**DPPH**
AA	1.00	(0.24) NS	(0.26) *	(-0.40) **	(0.02) NS	(0.16) NS	(0.23) NS
CGA	(0.24) NS	1.00	(0.63) ***	(0.23) NS	(0.46) ***	(0.27) *	(0.40) **
CFA	(0.26) *	(0.63) ***	1.00	(0.38) **	(0.72) ***	(0.33) *	(0.50) ***
FA	(-0.40) **	(0.23) NS	(0.38) **	1.00	(0.57) ***	(0.11) NS	(0.14) NS
RT	(0.02) NS	(0.46) ***	(0.72) ***	(0.57) ***	1.00	(0.27) *	(0.35) **
FRAP	(0.16) NS	(0.27) *	(0.33) *	(0.11) NS	(0.27) *	1.00	(0.81) ***
DPPH	(0.23) NS	(0.40) **	(0.50) ***	(0.14) NS	(0.35) **	(0.81) ***	1.00

NS – Not significant; * Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level.

Table 3.13 Virtual tuber mean correlations among ascorbic acid (AA), chlorogenic acid (CGA), caffeic acid (CFA), ferulic acid (FA), rutin (RT), Ferric Reducing Antioxidant Power (FRAP) and 2,2 Diphenyl-1-Picryl Hydrazyl (DPPH) in 12 Canadian-grown cultivars after 7 months storage (Pearson's Correlation Coefficient test).

	AA	**CGA**	**CFA**	**FA**	**RT**	**FRAP**	**DPPH**
AA	1.00	(0.41) **	(-0.30) *	(-0.18) NS	(0.15) NS	(0.27) *	(0.38) **
CGA	(0.41) **	1.00	(-0.24) NS	(-0.30) NS	(-0.31) *	(0.01) NS	(0.64) ***
CFA	(-0.30) NS	(-0.24) NS	1.00	(-0.36) NS	(0.23) NS	(0.00) NS	(-0.09) NS
FA	(-0.18) NS	(-0.30) NS	(-0.36) NS	1.00	(0.14) NS	(-0.17) NS	(-0.21) NS
RT	(0.15) NS	(-0.31) *	(0.23) NS	(0.14) NS	1.00	(0.38) **	(0.38) **
FRAP	(0.27) *	(0.01) NS	(0.00) NS	(-0.17) NS	(0.38) **	1.00	(0.40) **
DPPH	(0.38) **	(0.64) ***	(-0.09) NS	(-0.21) NS	(0.38) **	(0.40) **	1.00

NS – Not significant; * Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level.

Table 3.14 Virtual tuber mean correlations among ascorbic acid (AA), chlorogenic acid (CGA), caffeic acid (CFA), ferulic acid (FA), rutin (RT), Ferric Reducing Antioxidant Power (FRAP) and 2,2 Diphenyl-1-Picryl Hydrazyl (DPPH) in 5 foreign cultivars after 1 month storage (Pearson's Correlation Coefficient test).

	AA	**CGA**	**CFA**	**FA**	**RT**	**FRAP**	**DPPH**
AA	1.00	(-0.35) NS	(-0.23) NS	(0.45) *	(-0.43) *	(-0.36) NS	(-0.27) NS
CGA	(-0.35) NS	1.00	(0.41) NS	(-0.42) *	(0.94) ***	(0.94) ***	(0.89) ***
CFA	(-0.23) NS	(0.41) NS	1.00	(0.33) NS	(0.30) NS	(0.29) NS	(0.22) NS
FA	(0.45) *	(-0.42) *	(0.33) NS	1.00	(-0.48) *	(-0.40) NS	(-0.52) **
RT	(-0.43) *	(0.94) ***	(0.30) NS	(-0.48) *	1.00	(0.91) ***	(0.88) ***
FRAP	(-0.36) NS	(0.94) ***	(0.29) NS	(-0.40) NS	(0.91) ***	1.00	(0.92) ***
DPPH	(-0.27) NS	(0.89) ***	(0.22) NS	(-0.52) **	(0.88) ***	(0.92) ***	1.00

NS – Not significant; * Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level.

Table 3.15 Virtual tuber mean correlations among ascorbic acid (AA), chlorogenic acid (CGA), caffeic acid (CFA), ferulic acid (FA), rutin (RT), Ferric Reducing Antioxidant Power (FRAP) and 2,2 Diphenyl-1-Picryl Hydrazyl (DPPH) in 5 foreign cultivars after 7 months storage (Pearson's Correlation Coefficient test).

	AA	**CGA**	**CFA**	**FA**	**RT**	**FRAP**	**DPPH**
AA	1.00	(0.10) NS	(-0.10) NS	(0.59) *	(0.31) NS	(-0.08) NS	(0.30) NS
CGA	(0.10) NS	1.00	(0.79) ***	(-0.50) NS	(0.27) NS	(0.76) ***	(0.90) ***
CFA	(-0.10) NS	(0.79) ***	1.00	(-0.33) NS	(0.38) NS	(0.69) ***	(0.54) **
FA	(0.59) *	(-0.50) NS	(-0.33) NS	1.00	(0.30) NS	(-0.43) NS	(-0.50) NS
RT	(0.31) NS	(0.27) NS	(0.38) NS	(0.30) NS	1.00	(0.18) NS	(0.20) NS
FRAP	(-0.08) NS	(0.76) ***	(0.69) ***	(-0.43)	(0.18)	1.00	(0.71) ***
DPPH	(0.30) NS	(0.90) ***	(0.54) **	(-0.50) NS	(0.20) NS	(0.71) ***	1.00

NS – Not significant; * Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level.

Figure 3.1 Field grown tubers of the 12 Canadian cultivars used in this study: (A) Atlantic, (B) Green Mountain, (C) Goldrush, (D) Kennebec, (E) Norland, (F) Onaway, (G) Russet Burbank, (H) Red Pontiac, (I) Sebago, (J) Shepody, (K) Superior, and (L) Yukon Gold.

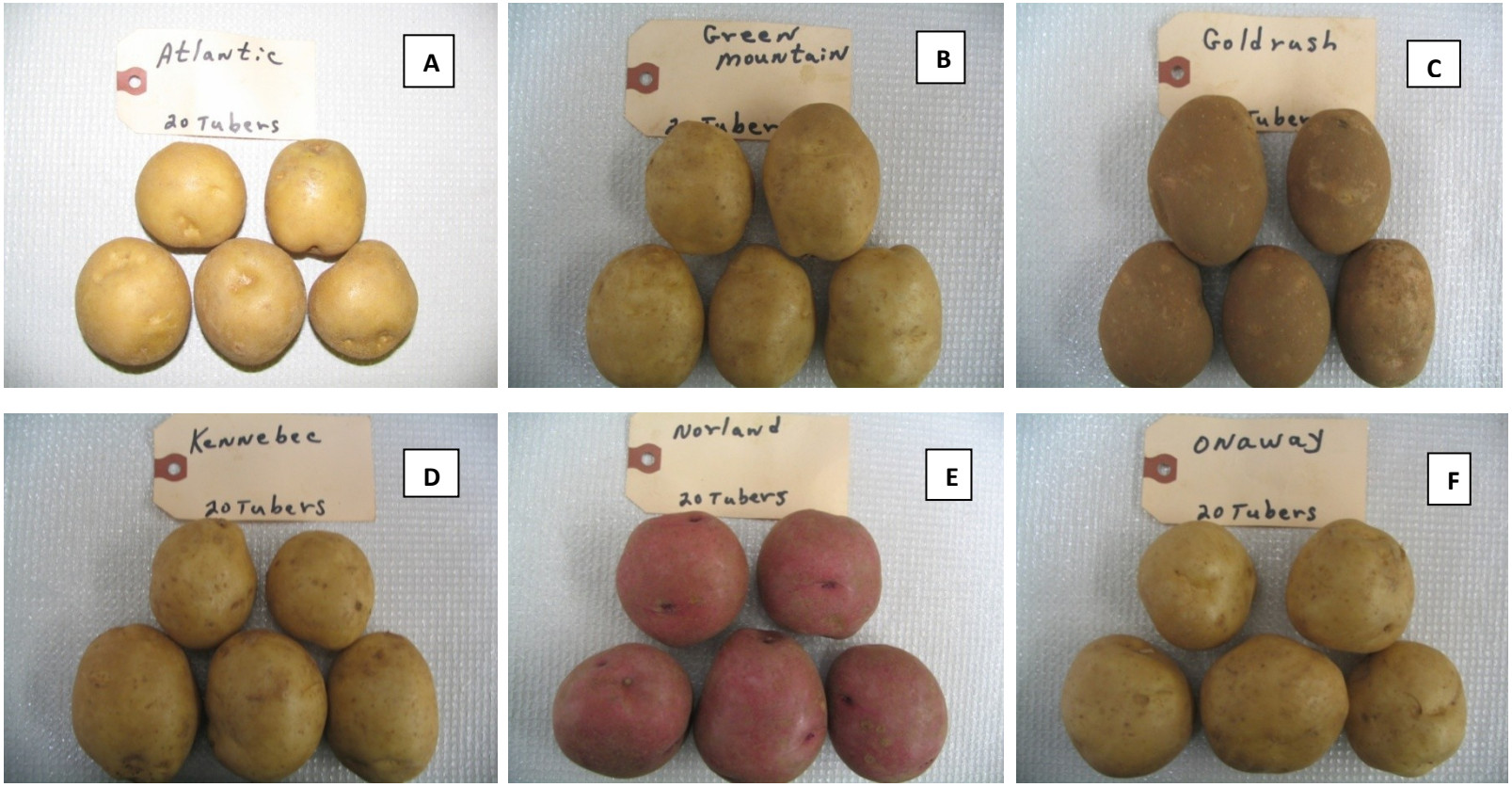
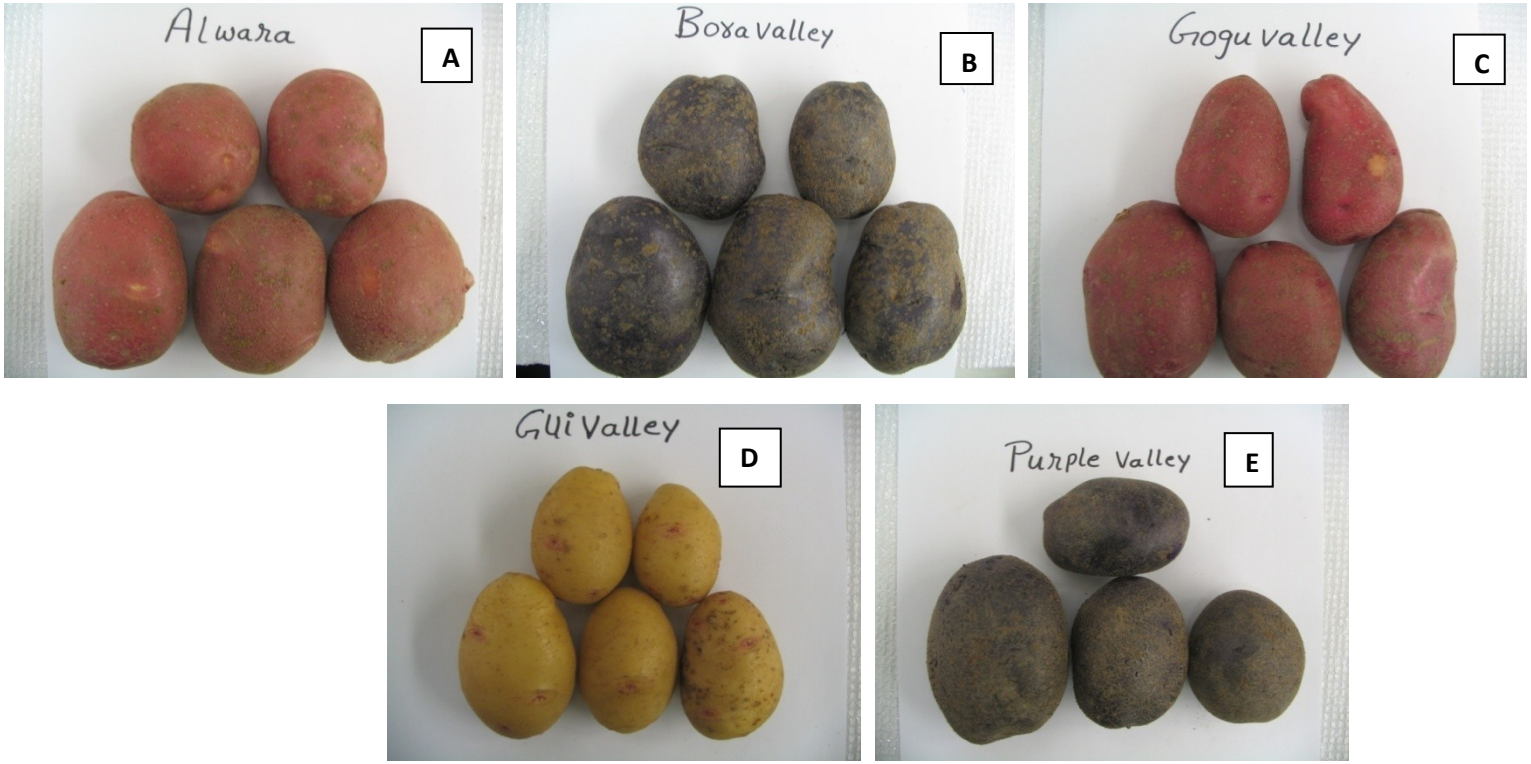


Figure 3.1 Continued

Figure 3.2 Field grown tubers of the 5 foreign cultivars used in this study: (A) Alwara, (B) Bora Valley, (C) Gogu Valley, (D) Gui Valley, and (E) Purple Valley.



CONNECTING STATEMENT

Chapter IV of this thesis, entitled "Chemical (H_2O_2) hormesis increases antioxidant capacity and major polyphenols in potato microtubers", reports the effect of chemical hormesis by H_2O_2 spray (2 and 4 mM) on potato microtuber total antioxidant capacity. For this study, cultivars Goldrush and Onaway were selected based on their relatively greater ascorbic acid and chlorogenic acid contents compared with field-grown tubers of 10 other Canadian-grown cultivars, as reported in Chapter III.

CHAPTER IV

CHEMICAL (H_2O_2) HORMESIS INCREASES ANTIOXIDANT CAPACITY AND MAJOR POLYPHENOLS IN POTATO MICROTUBERS

4.1 Introduction

With greater awareness that consumption of dietary antioxidants has a positive influence on human health, there is increased interest in methods to promote these antioxidant phytochemicals in plants (Reyes and Zevallos, 2003). When exposed to biotic stress, such as pathogen attack, and abiotic stress, including water stress, extreme temperatures, air pollution, extreme light etc., plants produce more Reactive Oxygen Species (ROS) molecules (Scandalios, 2005). However plants have developed antioxidant defence mechanisms to scavenge these ROS molecules. Polyphenolics are secondary metabolites produced by plants which help them in the defence against environmental stresses, including ultraviolet radiation, pathogen attack, etc. Polyphenols include phenolic acids, flavonoids, and anthocyanins (Andre et al., 2007).

Abotic stressors can be used, under controlled conditions, to promote the synthesis of phytochemicals with nutraceutical activity (Cisneros-Zevallos, 2003). The use of potentially harmful agents under controlled conditions to obtain beneficial effects is known as hormesis. The application of hormesis has spread to a wide range of living organisms since it was first reported in the early 1880's (Shama and Alderson, 2005). Hormetic agents and stress agents have been used in applications that include protection of post-harvest fruits and vegetables against pathogen attack (UV-C radiation) (Shama and Alderson, 2005) and increased tolerance to chilling temperatures during plant growth (ABA treatment) (Mora-Herrera and Lopez-Delgado, 2007).

Hydrogen peroxide (H_2O_2) is a predominant ROS detected in plants, and is believed to have a dual role as an element of oxidative stress causing deleterious

effects when accumulated in excess, and as an inducer of protective mechanisms against oxidative stress. (Kuzniak and Urbanek, 2000). Hydrogen peroxide has gained considerable attention in recent years for its involvement in induction of protective mechanisms against abiotic and biotic stresses (Rusite and Gertnere, 2001). Hydrogen peroxide has been studied in different field and in vitro studies in a range of plants for its effects on promoting thermotolerance (Lopez-Delgado et al., 1998) and freeze tolerance (Mora-Herrera and Lopez-Delgado, 2007). Hydrogen peroxide has been used to develop tolerance to oxidative stress in tobacco (Kuzniak and Urbanek, 2000) and ethylene stress in potato microplants in vitro (Rusite and Gertnere, 2001). It has been shown to affect the accumulation of tuber starch and stem lignin in field-grown potato (López-Delgado et al., 2005).

Mechanistically, hydrogen peroxide is a signal molecule that plays a key role in mediating plant responses to biotic and abiotic stresses (Gechev et al., 2002). It is involved in the ABA-signal transduction pathway for inducing antioxidant genes (Guan et al., 2000). The effects of H_2O_2 have also been investigated in studies for its role in protection against pathogen invasion as a microbicide, in the cell-wall reinforcement process (lignification), in triggering programmed cell death during pathogen invasion, and in signaling the induction of systemic acquired resistance (Kuzniak and Urbanek, 2000). Most recently, H_2O_2 was shown to play a regulatory role in the activation of genes encoding enzymes and other proteins involved in the protection from oxidative stress (Mora-Herrera and Lopez-Delgado, 2007).

Cultivated potatoes have significant quantities of important antioxidants including ascorbic acid, phenolic acids, flavonoids, and carotenoids (Hale et al., 2008). Ascorbic acid is among the most studied antioxidant in plants (Smirnoff, 1996; Smirnoff, 2000). Its ability to donate electrons in many enzymatic and non-enzymatic reactions makes it a powerful antioxidant (Blokhina et al., 2003). Ascorbic acid can directly reduce H_2O_2 to water via ascorbate peroxidase (Noctor and Foyer, 1998). Ascorbic acid may account for up to 13 % of the total AOC of potato (Brown, 2005). In potato, the total AOC has been positively correlated with the total phenolic content (Chirinos et al., 2007; Camire et al., 2009; Brown,

2005; Reddivari et al., 2007). Chlorogenic acid, caffeic acid, and ferulic acid are among the major phenolic compounds in potato (Brown, 2005). Phenolics are more effective antioxidants in vitro than ascorbic acid or tocopherols due to their ideal structural chemistry for free radical-scavenging activity (Blokhina et al., 2003). Phenolic compounds are involved in a hydrogen peroxide scavenging cascade in plant cells (Takahama and Oniki, 1997).

Until recently (Oh et al., 2010), few studies have utilized hormetic agents to increase antioxidant compounds in growing plants despite their importance in the human diet. This shows the need to develop reliable methods which can enhance health-benefiting phytochemicals in fresh fruit and vegetable produce prior to harvest. The objective of this study was to examine the effect of an oxidizing agent (H_2O_2; applied at 0, 2, and 4 mM) on the AOC and specific antioxidant phytonutrients, including ascorbic acid and the major phenolic compounds, of in vitro-grown tubers (microtubers). The long-term objective of the research is to ultimately develop a model system to explore the effects of hydrogen peroxide and other hormetic agents on AOC and phytonutrients in growing potatoes.

4.2 Materials and Methods

4.2.1 Micropropagation and microtuberization of potato

Plantlets of the potato cvs. Onaway and Goldrush were received from the Plant Propagation Centre (Fredericton, NB). These two cultivars were selected for their relatively high concentrations of antioxidants in field-grown tubers (Chapter 3). Plantlets were aseptically subcultured, using single-node cuttings, into 25 X 150 mm culture tubes containing 10 ml/tube MS medium (Murashige and Skoog, 1962). Medium consisted of basal salt solution and organic fraction solidified with 7 g l^{-1} agar (Anachemia, Lachine, QC) adjusted to pH 5.7 before autoclaving at 121 ^{o}C for 20 min. Cultures were maintained at 22 ± 2 ^{o}C under 85 µmol $m^{-2}s^{-1}$ cool white florescent illuminations with 16:8 h day:night cycle.

Microtubers were produced using the layering method of Leclerc et al. (1994). In Phase I, 5 root- and tip-severed plantlets, with 5 nodes each, were layered into 150 ml of liquid medium containing MS basal salt solution and organic fraction plus 20 g l^{-1} sucrose, 0.4 mgl^{-1} GA_3, 0.5 mgl^{-1} BAP, at pH 5.7 in 500 ml plastic containers (Better Plastics, Kissimmee, FL) (Fig. 4.1A). Cultures were placed into a growth chamber adjusted to 20 ± 2 ^{o}C under 85 µmol $m^{-2}s^{-1}$ cool white florescent illumination with 16:8 h day:night cycle. Phase I promoted the vegetative growth of plantlets which was luxurious after 4 weeks (Fig. 4.1B).

In Phase II, the residual medium was drained and replaced with 150 ml of liquid microtuber induction medium containing increased sucrose (80 gl^{-1}) and no growth regulators. Phase II incubation occurred at cooler temperature (15 ± 2^{o}C), under reduced illumination (50 µmol $m^{-2}s^{-1}$), and reversed (8:16 h) day:night cycle. Microtubers were initiated after 10-14 days, treated at 2- and 3-weeks and harvested at 4-weeks (Fig. 4.1D).

4.2.2 Hormetic treatment with hydrogen peroxide

A preliminary study was used to establish a suitable hydrogen peroxide dose for 2-week-old Phase II cultures. Phase II plantlets treated with a fine mist of ~ 6 ml of 1, 5, 50 mM H_2O_2 showed a concentration-dependent decrease in growth of the plantlets. Plantlets exposed to 5 or 50 mM had significantly shorter stems compared with control. Based on this preliminary study, concentrations of 2 and 4 mM H_2O_2 were selected for hormetic treatments. The treatments were applied to Phase II cultures at 2- and 3-weeks of ~ 6 ml with 0, 2, or 4 mM H_2O_2 using a spray (from a spray bottle) directed onto the leaves (Fig. 4.1C).

4.2.3 Experimental design and statistical analysis

Phase II plantlets of cvs. Onaway and Goldrush were treated with three concentrations (0, 2, 4 mM) of H_2O_2 with 5 replicates x 4 containers per replicate (20 containers per treatment). Results were analyzed for variance (ANOVA) test using the General Linear Model (GLM) of Statistical Analysis System (SAS) (SAS v 9.2, 2010) (SAS Institute Inc., Cary, NC, USA). Means were compared

using Duncan's multiple comparison test. Correlations among different variables were measured using Pearson's correlation coefficients. For all analyses, $P \leq 0.05$.

4.2.4 Sample harvest and preparation for analysis

At harvest, microtubers of both cvs. Onaway (Fig. 4.1E) and Goldrush (Fig. 4.1F) were weighed, and averaged from 20 separate containers. For each replicate, microtubers were pooled for analysis, for a total of 5 samples per treatment/cultivar. For each sample, microtubers were sliced into small pieces, freeze-dried (FTS Systems, NY, USA for 48 h, ground to a fine powder, and stored at – 80^{o}C until analyzed.

4.2.5 Antioxidant capacity measured by 2,2 Diphenyl-1-picryl hydrazyl (DPPH)

The AOC of the microtuber samples was estimated using the 2,2 Diphenyl-1-picryl hydrazyl (DPPH) assay of Nair et al. (2007). DPPH is a stable free radical, which on reaction with an antioxidant molecule changes color from violet to a yellow. This change in color is measured spectrophotometrically at 517 nm. The DPPH solution was prepared by mixing 3.94 mg of DPPH into 100 ml methanol. Ascorbic acid was used as a standard and the results were represented in ascorbic acid equivalents (AA equiv.). Ascorbic acid solution was prepared by mixing 0.0088 g of ascorbic acid in 50 ml distilled water. An ascorbic acid dilution series was prepared with distilled water. Using a micropipette, 50 µl of each ascorbic acid dilution was transferred into a 2 ml microcentrifuge tube. To these, 1.5 ml of DPPH reagent was added and vortexed for 60 s and the tubes were left for 20 min at room temperature for the reaction to proceed. The blank consisted of 1.5 ml distilled water. The control consisted of 1.5 ml DPPH reagent + 50 µl distilled water. The samples were pipetted into 2.5 ml cuvettes and read at 517 nm in the spectrophotometer (Beckman DU 640, Beckman Instruments, Fullerton, CA). A standard curve was prepared from the spectrophotometer readings and used to calculate the quantity of antioxidants in tissue samples based on mg of ascorbic acid equivalents (AAE).

Microtuber samples were prepared by placing 10 mg of freeze-dried powdered tissue into 1 ml microcentrifuge tubes to which 1 ml of distilled water was added, vortexed for 60 s, then centrifuged at 4 ^{0}C for 15 min at 3000 x g. After centrifuging, 50 µl of the supernatant was collected into a 2 ml microcentrifuge tube into which 1.5 ml of DPPH solution was added and vortexed for 60 s, then left for 20 min at room temperature for the reaction to proceed. The samples were pipetted into 2.5 ml labeled cuvettes and read at 517 nm in the spectrophotometer.

4.2.6 Determination of total polyphenolics using Folin-Ciocalteu

The total phenolic content of potato microtubers was measured spectrophotometrically by the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965; Chirinos et al., 2007; Andre et al., 2009). The phenolic compounds were extracted from the microtuber samples using acidified (0.1% HCl) 90% methanol (HPLC grade) as an extraction buffer. Chlorogenic acid, which is the major polyphenolic acid in potato, was used as a standard to determine the total phenolic content of the sample and the results were expressed in milligrams of chlorogenic acid equivalents (CAE) per gram DM. Chlorogenic acid standard stock solution was prepared by dissolving 50 mg of chlorogenic acid in 50 ml of extraction buffer. This standard stock solution was used to prepare a chlorogenic acid standard dilution series with extraction buffer. Using a micropipette, 150 µl each of chlorogenic acid dilution series samples were transferred into 4 ml microcentrifuge tubes, to which 1500 µl of 0.2 N Folin-Ciocalteau reagent was added. This was left to react for 3 min, then 300 µl 1 N Na_2CO_3 was added to this reaction mixture and vortexed for 30 s. The reaction mixture was allowed to react for 60 min at room temperature in the dark. The samples were pipetted into 2.5 ml cuvettes and the absorbance of the samples was measured using a spectrophotometer set at 725 nm. The control was extraction buffer. A standard curve was made using the spectrophotometric readings from which a standard curve equation was developed.

Extraction buffer (3 ml) was added to 50 mg of freeze-dried sample and agitated (using modified vortex equipment) for 60 min in the dark, then centrifuged at 4 ^{o}C for 15 min at 3000 x g. 150 µl of supernatant was filtered into 4 ml microcentrifuge tubes to which 1500 µl of 0.2 N Folin-Ciocalteu reagent was added, and left to react for 3 min. 300 µl of 1 N Na_2CO_3 was added to this reaction mixture and vortexed for 30 s. The reaction mixture was allowed to react for 60 min at room temperature in the dark. The samples were pipetted into 2.5 ml cuvettes and their absorbance measured in a spectrophotometer at 725 nm. The control was made up with 150 µl of extraction buffer. A chlorogenic acid standard curve equation was used to calculate the quantity of phenolic compounds in the samples and the results were represented in mg of CGA equivalents.

4.2.7 Quantification of antioxidants, including ascorbic acid and the major polyphenolics using High Performance Liquid Chromatography (HPLC)

HPLC (Varian 9012, Varian Chromatography Systems, Walnut Creek, CA) was used to identify and quantify ascorbic acid, three polyphenolic acids (chlorogenic acid, caffeic acid, and ferulic acid) and the flavonoid rutin. These five compounds were examined, based on their relative abundance in potato and their dietary importance. HPLC identification of a compound is based on the retention time of a particular compound within the column compared with purchased pure standards. Two different buffers (Buffer A and Buffer B) were used as mobile phases. Buffer A had 10 mM formic acid (0.4603 g of formic acid in 1 L distilled water adjusted to pH 3.5 using 1 M NH_4OH solution). Buffer B had 5 mM ammonium formate (0.3153 g of ammonium formate in 1 L 100% methanol (with agitation on a magnetic stirrer as ammonium formate is highly insoluble). An extraction buffer was used for the polyphenol extractions. This extraction buffer was composed of 50 % methanol, 2.5 % metaphosphoric acid, and 1 mM ethylenediaminetetraacetic acid (EDTA) (50 ml of 100 % methanol, 2.5 g of metaphosphoric acid, and 0.442 g of EDTA in 100 ml distilled water with stirring followed by filtering using a cup filter (Millipore Corporation, MS, USA).

The microtuber samples for HPLC were prepared by placing 50 mg of freeze-dried powered sample into a 1.5 ml microcentrifuge tube along with 0.9 ml of extraction buffer. The tubes were vortexed for 60 s and centrifuged at 4 ^{o}C for 15 min at 3000 x g. The supernatant was micropipetted into a 1.5 ml glass vial. The samples were re-extracted by adding 0.6 ml of extraction buffer to the same sample, vortexed for 60 s, centrifuged, and the supernatant was again collected into the same 1.5 ml glass vial. The 1.5 ml glass vials with supernatant were kept in a speedvac for 6-8 h for vacuum drying. Following the vacuum drying, 500 µl of extraction buffer was added to the samples, and vortexed for 30 min to solublize them. The sample was filtered into a 1 ml HPLC vial using a 1 ml syringe and topped with a 0.2 µm nylon filter (Fisher Scientific, Ottawa, ON). The HPLC vials were sealed with rubber-topped metal lids using a sealer. The samples were run in the HPLC and the compounds of interest were identified and quantified based on the retention time and area of the peaks in the chromatographs compared to the pure standards.

4.3 Results

4.3.1 Microtuber yield

Cultivar had a significant influence on microtuber yield. Cultivar Goldrush had significantly greater microtuber yield (control-10.232±0.429 g), twice the yield of cv. Onaway (control-5.098±0.316 g). However, hormetic treatment, at the dosage applied, did not affect microtuber yield (Table 4.1). This confirmed that the hormetic agent was applied at suitable dosages.

4.3.2 Antioxidant assay – 2, 2 Diphenyl-1-picryl hydrazyl (DPPH)

Cultivar had an influence on AOC (DPPH) of microtubers. This was evident with the control treatment of cv. Goldrush showing 27 % greater AOC compared with the control treatment of cv. Onaway (Table 4.1). The microtuber total AOC of both the cultivars increased significantly in response to hormetic treatment, although cv. Goldrush microtubers showed proportionally more increase (20-26 %), compared with cv. Onaway (12-14 %).

Cultivar Goldrush showed significantly greater AOC in plants treated with 2 mM H_2O_2 in comparison to 4 mM treated, both of which were in turn significantly greater than control (by 26 and 20 % increase, respectively). The microtubers of cv. Onaway also showed significantly greater AOC in response to H_2O_2 treatment with 2 or 4 mM H_2O_2, though there was no significant difference found between these two doses.

4.3.3 Total Phenolics in potato microtubers

Cultivar had an influence on total polyphenolic concentration as well. Control of cv. Goldrush showed 59 % greater total phenolics value (Folin-Ciocalteau) in comparison with control of cv. Onaway (Table 4.1). However, total polyphenolic levels were not significantly affected by hormetic treatments in both cultivars.

4.3.4 Quantification of antioxidants, including polyphenolics - High Performance Liquid Chromatography (HPLC)

The increased AOC observed in response to hormetic treatment could be explained in cv. Goldrush, by increased ascorbic acid (at both 2 and 4 mM dosage) and chlorogenic acid (at 2 mM dosage) while in cv. Onaway, it is explained by increased chlorogenic acid (at 2 mM dosage).

a. Ascorbic acid

Hydrogen peroxide treatment significantly increased the ascorbic acid content in microtubers of cv. Goldrush at both 2 and 4 mM, with an increase of 35 and 34 % respectively in comparison to control (Table 4.1). In cv. Onaway, treatment effects were not significant, though the trend was positive.

b. Phenolic acids and rutin

Hydrogen peroxide treatment at 2 mM significantly increased microtuber chlorogenic acid content of both the cvs. Goldrush (increase of 16.8 %) and Onaway (increase of 17.3 %) (Table 4.1). As 4 mM treatment depressed the

chlorogenic acid content in cv. Goldrush, it showed a similar effect to that of 2 mM treatment in cv. Onaway. Hydrogen peroxide treatment at 2 mM showed significant depression of microtuber caffeic acid content of cv. Goldrush, but showed no affect in its content in cv. Onaway. Although the 4 mM treatment apparently depressed caffeic acid content of microtubers in cv. Onaway, these levels approached the threshold for quantification. Similarly, ferulic acid and rutin were present in trace amounts, but not accurately quantifiable at these levels.

c. Correlations between antioxidant capacity and antioxidant compounds

In cv. Goldrush, AOC (DPPH) was positively correlated with ascorbic acid and chlorogenic acid (Table 4.2). Positive correlation was also found between total phenolics (F-C) and chlorogenic acid. Caffeic acid was negatively correlated with AOC (DPPH), ascorbic acid, and chlorogenic acid. In cv. Onaway, though similar correlation trends were found between AOC and antioxidant compounds, significant positive correlation was found only between total phenolics (F-C) and chlorogenic acid (Table 4.3).

4.4 Discussion and Conclusions

This is the first study to apply any hormetic agent to potato under defined growing conditions to investigate the antioxidant or phytonutrient impact on tubers. Significant positive effects on AOC (DPPH) of microtubers occurred in response to the hormetic agent H_2O_2 (Table 4.1). Increased DPPH was positively associated with ascorbic acid and chlorogenic acid content, and negatively associated with caffeic acid content in the cv. Goldrush (Table 4.2), although the above correlations were not evident in cv. Onaway (Table 4.3).

Increase in AOC could also be due to the up-regulation of enzymatic antioxidants like catalases and peroxidases along with low molecular mass antioxidants like ascorbic acid. As discussed earlier, 4-week-old greenhouse-grown tobacco plants treated with a moderate dosage (5 mM x 1.7 mg/plant) of H_2O_2 had increased AOC with increase in the antioxidant enzyme, catalase (Gechev et al., 2002). ROS formation in plant cells is controlled by a variety of

enzymes, including Superoxide Dismutase (SOD), peroxidases, and catalases. These regenerate the reduced forms of antioxidants and the potential antioxidant enzymes, which interact directly with ROS (Blokhina et al., 2003). SOD helps in dismutation of superoxide ($O_2^{\cdot -}$) yielding H_2O_2 which in-turn is converted to O_2 and H_2O by an array of catalases and peroxidases. These enzymes play an important role in regulation of H_2O_2 in the cell. In maize seedlings, both endogenous and exogenously-supplied H_2O_2 increased the same kind of antioxidant enzymes (Prasad et al., 1994). In our study, these enzymes were not analyzed, but this should be performed in the future for better-understanding of the observed hormetic effect (Chapter 5).

In the current study, abiotic stress treatment with H_2O_2 significantly enhanced chlorogenic acid content in both cvs. Goldrush and Onaway (Table 4.1). Chlorogenic acid was positively correlated with total AOC (DPPH) and total phenolics (Folin–Ciocalteu test) in cv. Goldrush (Table 4.2) and with total phenolics in cv. Onaway (Table 4.3). Chlorogenic acid has been described to comprise up to 80 % of the total phenolic content (Brown, 2005). Controlled mechanical wounding in potato increased phenolic compounds by up to 60 % compared with control, which was mostly associated with increased chlorogenic acid content (Reyes and Zevallos, 2003). However, in the current study though H_2O_2 treatment (2 mM) showed positive effect in chlorogenic acid content of the microtubers, it did not show an impact on total phenolic content. The total polyphenolic content was not affected by hormetic treatment, quite possibly due to an interesting inverse relationship between chlorogenic and caffeic acid, in response to hormetic stress (Table 4.1). Caffeic acid was significantly negatively correlated with total antioxidants, ascorbic acid, and chlorogenic acid (Tables 4.2; 4.3). The reason for this negative correlation is currently undetermined.

In conclusion, the potato microtuber system appears to be a sensitive and useful model for manipulating phytonutrient composition via hormetic stress treatments. Exogenous application of sprayed H_2O_2 appears to be an effective hormetic treatment as the plants stayed intact, without visible mechanical injury, and with no effect on microtuber yield, confirming the suitability of the dosage

applied. Cultivar Goldrush, with its greater yield, and stronger response to H_2O_2, with an increase in ascorbic acid and chlorogenic acid, is recommended for further exploration of hormetic treatment effects on potato microplants. The 2 mM hydrogen peroxide dosage appeared optimal, although it was not substantially different from the 4 mM treatment for all parameters (Table 4.1). The overall pattern of response to hormetic stress is unknown, as only one harvest was done, 1-week following the second application of hydrogen peroxide. The peak response may not have been observed, and the duration of this response is unknown. Investigation of a detailed response pattern is recommended for future studies (Chapter 5).

Table 4.1. Effect of H_2O_2 treatment on microtuber fresh mass (FM), total antioxidant capacity (2,2 Diphenyl-1-Picryl Hydrazyl (DPPH), total phenolics (Folin-Ciacalteu), ascorbic acid, chlorogenic acid, and caffeic acid . Values expressed as means ± SE (n=5).

Cultivar	Treatment (mM H_2O_2)	FM* (g)	DPPH (mg AAE/g)	Total phenolics (mg CAE/g)	Ascorbic acid (mg/g)	Chlorogenic acid (mg/g)	Caffeic acid (mg/g)
Goldrush	0	10.232±0.429 ^{a}	9.709±0.334 ^{c}	6.569±0.222 ^{a}	0.174±0.010 ^{b}	0.468±0.008 ^{b}	0.013±0.001 ^{a}
	2	10.250±0.438 ^{a}	13.169±0.199 ^{a}	7.076±0.462 ^{a}	0.269±0.015 ^{a}	0.561±0.010 ^{a}	0.008±0.001 ^{b}
	4	10.262±0.666 ^{a}	12.147±0.369 ^{b}	6.354±0.142 ^{a}	0.264±0.003 ^{a}	0.459±0.012 ^{b}	0.011±0.000 ^{a}
Onaway	0	5.098±0.316 ^{a}	7.622±0.190 ^{b}	4.119±0.149 ^{a}	0.147±0.006 ^{a}	0.258±0.005 ^{b}	0.002±0.000 ^{a}
	2	5.086±0.200 ^{a}	8.629±0.228 ^{a}	4.375±0.263 ^{a}	0.170±0.009 ^{a}	0.304±0.014 ^{a}	0.002±0.000 ^{a}
	4	5.066±0.226 ^{a}	8.827±0.421 ^{a}	3.788±0.179 ^{a}	0.164±0.012 ^{a}	0.264±0.019 ^{ab}	0.001±0.000 ^{b}

Values with same superscript letters within the same column were not significantly different based on Duncan's New Multiple Range Test (P< 0.05). ******* *Mean of 20 containers per treatment. AAE-Ascorbic acid equivalent; CAE-Chlorogenic acid equivalents.*

Table 4.2 Microtuber tuber mean correlations among different factors, total antioxidant capacity (2,2 Diphenyl-1-Picryl Hydrazyl (DPPH)), total phenolics (TP), ascorbic acid (AA), chlorogenic acid (CGA), and caffeic acid (CFA) at both 2 and 4 mM H_2O_2 in cv. Goldrush (Pearson Correlation Coefficient test).

	DPPH	**TP**	**AA**	**CGA**	**CFA**
DPPH	1.00	(0.33) NS	(0.86) ***	(0.58) *	(-0.64) *
TP	(0.33) NS	1.00	(0.30) NS	(0.52) *	(-0.14) NS
AA	(0.86) ***	(0.30) NS	1.00	(0.44) NS	(-0.52) *
CGA	(0.58) *	(0.52) *	(0.44) NS	1.00	(-0.63) *
CFA	(-0.64) *	(-0.14) NS	(-0.52) *	(-0.63) *	1.00

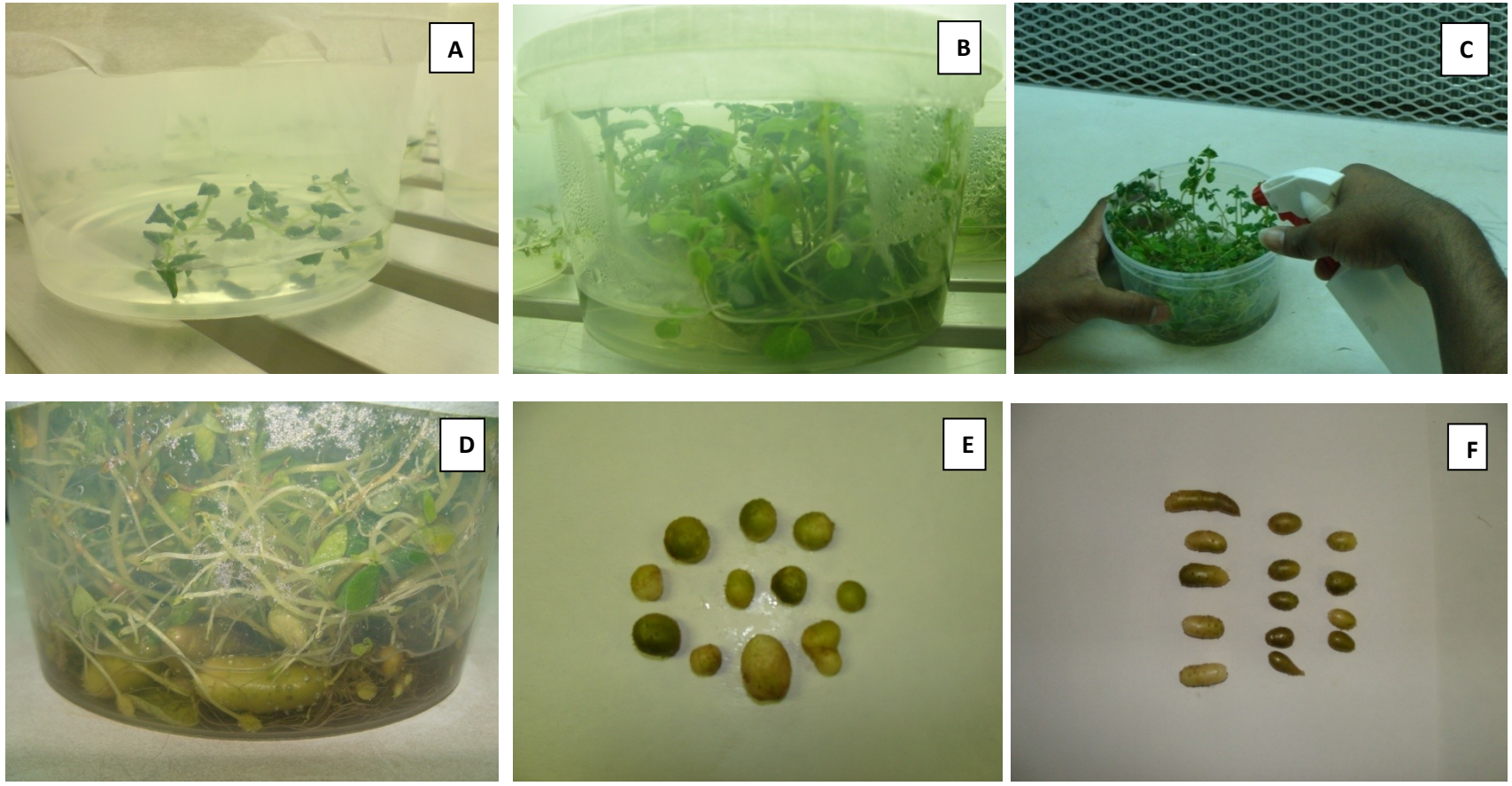
NS – Not significant; * Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level.

Table 4.3 Microtuber tuber mean correlations among different factors, total antioxidant capacity (2,2 Diphenyl-1-Picryl Hydrazyl (DPPH)), total phenolics (TP), ascorbic acid (AA), chlorogenic acid (CGA), and caffeic acid (CFA) at both 2 and 4 mM H_2O_2 in cv. Onaway (Pearson Correlation Coefficient test).

	DPPH	**TP**	**AA**	**CGA**	**CFA**
DPPH	1.00	(-0.23) NS	(0.14) NS	(0.29) NS	(-0.17) NS
TP	(-0.24) NS	1.00	(0.43) NS	(0.58) *	(0.29) NS
AA	(0.14) NS	(0.43) NS	1.00	(0.21) NS	(-0.05) NS
CGA	(0.29) NS	(0.58) *	(0.21) NS	1.00	(0.21) NS
CFA	(-0.17) NS	(0.29) NS	(-0.05) NS	(0.21) NS	1.00

NS – Not significant; * Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level.

Figure 4.1. (A) Root and shoot tip severed microplants layered in 500 ml containers (1 day of Phase-I), (B) Luxuriant vegetative growth of microplants at the end of Phase-I, (C) Microplants treated with H_2O_2 in Phase-II, (D) Microplants with tubers at the end of Phase-II, (E) Microtubers of cv. Onaway, (F) Microtubers of cv. Goldrush.



CHAPTER V

SUMMARY, CONCLUSIONS, AND SUGGESTIONS FOR FUTURE RESEARCH

5.1 General summary and conclusions

The potato, considered one of the most important staple food crops globally, is the world's third and Canada's most important food crop (FAOSTAT, 2008). It is the world's number one non-grain food commodity, with a production of 315 million MT in 2008. Potatoes have been extensively studied for their traits related to yield (Ojala et al., 1990), processing quality (Gordon and Katz, 2005), and many other attributes, with less attention to characterization or improvement of their nutritional composition. However, they have been recently recognized as an important source of high quality proteins, complex carbohydrates, minerals such as calcium, potassium, and phosphorus (Casanas et al., 2002) and antioxidant compounds (Brown, 2005). This thesis was focused on phytonutrient status, including AOC and polyphenolic content of potato.

This thesis was divided into two main studies. In the first study, the phytonutrients and their distribution in tuber tissue layers of 12 Canadian-grown and 5 foreign cultivars were examined (Chapter III). In the second study, the effect of chemical (H_2O_2) hormesis on AOC and polyphenolic content of potato microtubers was studied (Chapter IV). This thesis was part of on-going project in our laboratory, where these twelve Canadian-grown cultivars and others were screened for calcium uptake (Habib, 2004), total soluble protein content (Ortiz-Medina, 2007); ultimately, to identify the best cultivars in terms of human nutrition.

In Chapter III, the antioxidant capacities of 12 Canadian-grown and 5 foreign cultivars using DPPH and FRAP assays are described. Specific antioxidants, vitamins (ascorbic acid), phenolic acids (chlorogenic acid, caffeic acid, ferulic acid) and flavonoids (rutin) were identified using HPLC. The

distribution of these antioxidants and polyphenolics in skin, cortex, and pith was determined by analyzing these tissues separately. The tubers were stored in a walk-in fridge (5±1^{o}C) to determine the effect of 1 and 7 months storage on the AOC of tuber tissues from different cultivars. Inter-cultivar comparison was done between the whole tubers, by converting the concentration data (mg/g DM) of separated tissue layers into whole/virtual tuber data (mg/100 g FM) using conversion factors unique for selected cultivars (Ortiz-Medina et al., 2009).

Cultivar variation was clearly apparent for AOC. AOC was greatest in the six Canadian-grown cvs. Goldrush, Kennebec, Onaway, Red Pontiac, Sebago, and Shepody and the two foreign cvs. Bora Valley and Purple Valley. There was a wide range in the AOC of the cultivars with up to 6-fold and 2-fold variation, in Canadian-grown, and foreign cultivars, respectively (Tables 3.3, 3.4).

Among the five specific compounds analysed using HPLC, ascorbic acid content was greater in the Canadian-grown cultivars (Table 3.10) and chlorogenic acid content was greater among the foreign cultivars (Table 3.11). The AOC and polyphenolic distribution in tissue layers clearly indicated relative greater concentration in skin tissue than the inner cortex and pith both among Canadian-grown and foreign cultivars (Table 3.5). Though the flesh tissues (cortex and pith) were not different in AOC among the foreign cultivars, they varied significantly among the Canadian-grown cultivars with greater AOC in the pith. Except for ascorbic acid content in the skin of the foreign cultivars, skin tissue was greatest in concentration of the other four compounds, both in the Canadian-grown and foreign cultivars (Table 3.8). Though the skin tissue is a relatively negligible portion of the whole tuber (2 %) (Ortiz-Medina et al., 2009), its greater concentration of antioxidant compounds makes it an important source nutritionally when potatoes are consumed as table stock. The majority of potatoes are consumed as processed foods with the peel removed (Al-Weshahy and Venket Rao, 2009), which generates a lot of bioactive sludge, composed largely of peel. Peels are highly concentrated in antioxidant compounds and may make an excellent source for extraction of antioxidants.

The effect of storage on the AOC was evident both in Canadian-grown cultivars and foreign cultivars, with the latter showing a relatively lesser effect of storage. Storage of 7 months caused a dramatic decrease in the AOC of the tubers relative to 1 month storage. A wide range was observed in the % reduction of AOC among the cultivars (Tables 3.1, 3.2, 3.3, 3.4). Cultivars Norland and Yukon Gold showed the least % reduction among Canadian-grown cultivars and cv. Bora Valley showed the least % reduction among the foreign cultivars. However, since cvs. Norland and Yukon Gold showed relatively lower overall AOC, they would be a poor choice for antioxidant benefits as compared with the other cultivars. Among the Canadian-grown cultivars, for the five compounds analyzed (HPLC), chlorogenic acid showed relatively better retention with 60.27 % reduction after 7 months storage (Table 3.10), whereas among the foreign cultivars, ascorbic acid showed relatively better retention with 36.09 % reduction after 7 months storage (Table 3.11).

Findings in this thesis research were valuable in identifying Canadian-grown cvs. including Red Pontiac, Onaway, Kennebec, Shepody, Sebago, and Goldrush, and foreign cultivars Bora Valley and Purple Valley with relatively greater AOC and polyphenolics among the Canadian-grown and foreign cvs analyzed. This can be useful information for Canadian consumers to choose the cultivars with relatively greater health-benefiting antioxidant compounds. This study also helped in identifying the tissues with relatively greater and lesser AOC and polyphenolics which can be useful information for recommending the consumption of potatoes with the peel, which is relatively concentrated in antioxidant compounds.

In Chapter IV, the effect of abiotic chemical hormesis (H_2O_2) is described, on yield and AOC of microtubers of cvs. Goldrush and Onaway. The microtubers were produced using a layering method (Leclerc et al., 1994). The layering method was divided into 2 Phases, of 4 weeks each. Phase I involved vegetative growth and Phase II involved microtuber induction and enlargement. The plantlets in Phase II were sprayed twice, at weekly intervals, (week-2 and week-3), with ~ 6 ml of 0, 2, or 4 mM H_2O_2 solution. The AOC and total phenolics of the

microtubers were analysed using DPPH and Folin-Ciocalteu colorimetric assays, respectively. Specific antioxidants, the vitamin ascorbic acid, phenolic acids (chlorogenic acid, caffeic acid, ferulic acid), and flavonoid (rutin) were identified and quantified using HPLC.

The hormetic H_2O_2 treatment did not affect the yield of microtubers for either cultivar. The results showed a clear enhancement of total AOC (DPPH assay) of the microtubers following H_2O_2 treatment. The H_2O_2 treatment enhanced by 20-26% and 12-14% the AOC of cvs. Goldrush and Onaway, respectively (Table 4.1). There was no effect found in the total phenolics (F-C assay) of microtubers of either cultivar; likely due to increased chlorogenic acid but decreased caffeic acid content. Among the five specific antioxidant compounds analysed, ascorbic acid showed a 34-35 % increase with H_2O_2 treatment in cv. Goldrush, and a positive trend but not a significant increase in cv. Onaway. H_2O_2 treatment also induced a positive effect on chlorogenic acid concentration in the microtubers of both cvs. Goldrush and Onaway, and a negative effect on caffeic acid concentration in cv. Goldrush. Among the treatments applied, 6 ml x 2 sprays of 2 mM H_2O_2 was better than 4 mM H_2O_2 as a hormetic dosage in the microtuber system. The cv. Goldrush, with greater yield overall, showed a relatively greater response to H_2O_2 treatment and greater increase in AOC compared with cv. Onaway.

Findings in Chapter IV contribute to the knowledge of plant hormesis, and provide valuable information on potential use of H_2O_2 as an abiotic hormetic agent. To the best of our knowledge, this is the first report on the usage of H_2O_2 to enhance the phytonutrients (AOC, antioxidant chemicals) of potato microtubers or potatoes, in general. The microtuberization (layering) method appears to be a sensitive and useful model for future experiments on the effect of hormesis on phytonutrient content in potato, as it was performed under controlled conditions, and can be successfully used year-round. Hormetic studies with microtubers will need to be validated through cultural experiments on field-grown potato.

5.2 Suggestions for future research

1. In the present study, 12 Canadian-grown and 5 foreign cultivars were examined for their relative AOC and content of ascorbic acid and polyphenolics. A similar study to screen all of the major Canadian-grown cultivars should be conducted; this would provide valuable information for nutritionists.
2. The qualitative and quantitative distribution of different antioxidant compounds in tissue layers of potato was examined in this study, which showed inconsistencies among the cultivars. Screening efforts suggested above (1.) should also establish the distribution pattern of antioxidants in different tissue layers. This can be useful information for various food industries.
3. The method of extraction and analysis of AOC of samples should be standardized to reflect both lipophilic and hydrophilic antioxidants (e.g., ORAC), and to enable comparison with other crops.
4. Hydrogen peroxide treatment enhanced the AOC of potato microtubers in our study, but more extensive in vitro (and eventually field) studies are necessary to explore the use of H_2O_2 as a hormetic agent; the response pattern after each application should be explored, the enzymes involved, as well as the genes activated, should be examined.
5. The biochemical mechanisms behind the role of H_2O_2 in enhancement of AOC of potato microtubers should be explored to better understand the implications of using H_2O_2 in other crops as a hormetic agent.
6. Use of the microtuber model system to compare other hormetic agents, such as UV-C radiation and abscisic acid is necessary to better understand the elicited responses in plant tissue. Ultimately, the best hormetic agent should be selected to maximize the human health response to “hormetically activated” potato dietary items.

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