RELATIONSHIPS AMONG ANTIOXIDANTS, PHENOLICS, AND SPECIFIC GRAVITY IN POTATO CULTIVARS, AND EVALUATION OF WILD POTATO SPECIES FOR ANTIOXIDANTS, GLYCOALKALOIDS, AND ANTI-CANCER ACTIVITY ON HUMAN PROSTATE AND COLON CANCER CELLS *IN VITRO*

A Dissertation

by

MAGNIFIQUE NDAMBE NZARAMBA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2008

Major Subject: Plant Breeding

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Approved by:

Chair of Committee,	J. Creighton Miller Jr.
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ABSTRACT

Relationships among Antioxidants, Phenolics, and Specific Gravity in Potato Cultivars, and Evaluation of Wild Potato Species for Antioxidants, Glycoalkaloids, and Anti-cancer Activity on Human Prostate and Colon Cancer Cells *In Vitro*. (December 2008)

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Chair of Advisory Committee: Dr. J. Creighton Miller Jr.

Understanding the influence of environment and correlation/relationships among traits is necessary in selection for crop quality improvement. Therefore, correlations among antioxidant activity (AOA), total phenolics (TP), phenolic composition, and specific gravity (SPG) in four potato (*Solanum tuberosum*, L.) cultivars (Atlantic, Red La Soda, Russet Norkotah, and Yukon Gold) grown in nine states (California, Idaho, Michigan, Minnesota, New Jersey, North Carolina, Oregon, Texas, and Wisconsin) for three years, and in 15 advanced selections grown in Texas were investigated. Cultivars within and between locations were significantly different in AOA, TP, and SPG. Significant effects of cultivar, year, location and their interactions on AOA, TP, and SPG were observed. There were significant positive correlations among the four cultivars between AOA and TP, and negative correlations between AOA and SPG, and between TP and SPG. However, correlations between AOA and SPG, and between TP and SPG, in the advanced selections were not significant.

Some tuber-bearing wild potato species were higher in AOA and TP than the commercial cultivars; therefore, they could be used as parental material in breeding for

high AOA and TP. However, use of wild species that might be higher in total glycoalkaloids (TGA) than cultivars could result in progenies with high TGA if the traits are positively correlated. To elucidate the relationships among AOA, TP and TGA, accessions of *Solanum jamesii* and *S. microdontum* from the US Potato Genebank were screened for these traits and their correlations determined. Also, anti-proliferative and cytotoxic effects of 15 *S. jamesii* tuber extracts (5 and 10 μg/ml) on human prostate (LNCaP) and colon (HT-29) cancer cells was determined *in vitro*.

Alpha-solanine and α-chaconine were found in both species, while tomatine and dehydrotomatine were quantified in some *S. microdontum* accessions. Both species were higher in all above traits than the Atlantic, Red La Soda, and Yukon Gold cultivars. More than 90% of *S. jamesii* accessions had TGA levels < 20 mg/100g fresh weight, while only two accessions of *S. microdontum*, P1 500041 and PI 473171, exhibited TGA < 20 mg/100g. Neither AOA nor TP was significantly correlated with TGA in both species. Also, individual phenolics were not correlated with TGA. *Solanum jamesii* accessions significantly reduced proliferation of HT-29 (5 and 10μg/ml) and LNCaP (10μg/ml) cells and were not cytotoxic compared to the control (DMSO). Therefore, since AOA and TP were not found to be correlated with TGA, using wild accessions in breeding for increased health promoting compounds would not necessarily increase glycoalkaloids in newly developed potato cultivars.

DEDICATION

This dissertation is dedicated to my parents the late Rev. Canon Martin Blaise Nzaramba and Espérence Mutamba Nzaramba, and my brothers and sisters who have been the pillar of support throughout my academic endeavor.

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CHAPTER I

INTRODUCTION

Crop plants have long been known as a source of essential nutrients such as proteins, carbohydrates, vitamins, and lipids, which are required for human development, growth, and survival. These nutrients, in addition to producer-oriented traits like pest and disease resistance and drought tolerance, have been the focus of crop improvement initiatives for generations.

In addition to proteins, carbohydrates, vitamins, and lipids, crop plants provide bioactive compounds that play a significant role in disease prevention and health promotion. The bioactive non-nutrients from plant foods, also referred to as phytochemicals, are numerous, and more than 5000 have been identified, but several more are still unknown (Shahidi and Naczk, 1995a).

The bioactive phytochemicals are still referred to as non-nutrients, implying that they are not yet qualified to be in the same category as proteins, carbohydrates, fats and vitamins. Duyff (2002) referred to these compounds as phytonutrients, meaning plant chemicals, and categorized them differently from vitamins and minerals. As more research findings support and confirm their ultimate necessity in the diets of animals and humans, they will be upgraded to the level of essential nutrients.

The format and style of this dissertation follows that of the Journal of the American Society for Horticultural Science.

The prominence of phytochemicals comes from several epidemiological studies that have shown consumption of fruits, vegetables, and grains to be associated with reduced risk of chronic diseases such as cardiovascular disease, cancer, diabetes, Alzheimer's, cataracts, and other age-related ailments (Arai et al., 2000; Joshipura et al., 2001; Liu, 2003). It is established that fruits and vegetables are rich in phytochemicals such as phenolic acids, flavonoids, anthocyanins, and carotenoids.

Currently, research has intensified on investigating the health benefits of phytonutrients. Several research reports have indicated that the benefit of plant foods is due not only to levels of vitamins, proteins, lipids or carbohydrates they provide, but also to activity of the non-nutritive factors found in plants. Many of these plant secondary components are antioxidants (Riedl et al., 2002). With the discovery of health benefits from certain phytochemicals, i.e. antioxidant capabilities, the meaning of a balanced diet is changing from provision of sufficient amounts of carbohydrates, proteins, fats and vitamins, to inclusion of such compounds as carotenoids, anthraquinones, flavonoids etc., that are believed to possess antioxidant activity (Nzaramba, 2004).

Antioxidants are compounds that can quench free radicals (oxidants) thereby delaying or inhibiting oxidation of molecules and protect biological systems against potential harmful effects of free radical (Arnao, 2000; Morello et al., 2002). Oxidative stress induced by free radicals and other external agents can damage DNA and other molecules, and if not repaired may set off a cascade of events such as mutations, DNA strand breakage, and chromosomal breakage and rearrangement resulting in disease risks like cancer.

Humans and animals are exposed to various disease-causing agents, ranging from external agents such as bacteria, fungi, viruses, radiation, chemical agents etc, and internally generated agents like reactive oxygen (ROS) and reactive nitrogen species (NOS) from body metabolic activities. Therefore, protection against these agents is paramount. Reactive oxygen and nitrogen radicals act as oxidants, thereby causing oxidative stress within the body. It is therefore, necessary to keep a balance between oxidants and antioxidants to maintain healthy physiologic conditions. Phytochemicals such as phenolics and carotenoids in plant foods have antioxidant capabilities that help. to protect cellular systems from oxidative damage (Chu et al., 2002; Eberhardt et al., 2000; Liu, 2003).

Several studies have reported that phytochemicals, especially antioxidants from plants, can inhibit metagenesis and carcinogenesis, and reduce cancer risks by scavenging oxidative radicals (Boyle et al., 2000; Giovannelli et al., 2000; Rodriguez et al., 2007; Shahidi, 2002), modulation of detoxifying enzymes, stimulation of the immune system, regulation of cell proliferation and apoptosis (Kern et al., 2007; Kim et al., 2006; Reddivari et al., 2007b), and antiviral and antibacterial effects (Friedman et al., 2006).

Le Marchand et al. (2000) indicated that consumption of quercetin from onions and apples was inversely associated with lung cancer risk in Hawaii. Similarly, Giovannelli et al. (2000) demonstrated that polyphenols from wine significantly decreased DNA oxidative damage in rat colon mucosal cells, and concluded that dietary

polyphenols can modulate *in vivo* oxidative damage in the gastrointestinal tract of rodents.

Other studies have shown a link between intake of dietary phytochemicals and reduced risk of cardiovascular disease. Ridker et al. (2002) stated the inflammation is a critical factor in cardiovascular disease. Inflammation promotes initiation and development of atherosclerosis. Since phenolic compounds exhibit anti-inflammatory activity (Dai et al., 2007), they play a role in cardiovascular disease prevention. Joshipura et al. (2001) reported that high fruit and vegetable intake is associated with decreased risk of coronary artery disease. A study in Japan indicated that intake of flavonoids was inversely correlated with the amount of total cholesterol and low-density lipoprotein (LDL) in plasma (Arai et al., 2000).

The importance of antioxidants in preventing diseases and maintenance of health has raised interest among scientists, food producers/manufacturers, and consumers towards functional foods (Robards et al., 1999; Velioglu et al., 1998). The Food and Nutrition Board of the National Academy of Sciences (FNB/NAS, 1994) defined functional foods as any "food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains". Several authors (Al-Saikhan et al., 1995; Hale, 2004; Kanatt et al., 2005; Kawakami et al., 2000) have suggested that potato is a functional food due to presence of antioxidant compounds in potato tubers.

Significance of the Research

Given the importance of antioxidants in disease prevention, plant breeders need to develop cultivars with substantial amounts of antioxidants to complement medical and

social activities in preventing diseases. However, in developing high antioxidant cultivars, other traits such as high specific gravity have to be maintained if not increased. Therefore, breeders need to known the relationships among traits, information which helps in understanding how selection for one trait would affect others. Ascertaining the effect of other factors – genotype, environment, and genotype x environment, on traits of interest is also helpful.

Potato cultivars and breeding lines exhibit varying amounts of phenolic compounds and antioxidants (Kawakami et al., 2000; Reddivari et al., 2007a). Furthermore, identification of related wild species with desirable nutritional benefits would provide parental material in breeding improved cultivars with enhanced health benefits. Several wild species have been screened for antioxidant activity and some were reported to possess more antioxidant activity than currently grown potato cultivars. Species identified as containing high antioxidant activity were *S. jamesii*, *S. pinnatisectum*, *S. megistacrolobum*, and *S. microdontum* (Hale, 2004; Nzaramba et al., 2007). In the above studies, only a few accessions of each species in the mini-core collection were screened.

Having identified some species as containing more antioxidant activity than cultivated varieties, it was important to screen all populations of these species to identify specific accessions that are the highest in antioxidant activity and phenolic compounds. However, it should be noted that breeding with wild *Solanum* species can result in toxic levels of glycoalkaloids in new progenies (Laurila et al., 2001). Glycoalkaloids are known to be toxic to humans by acting as cholinesterase inhibitors, and also interact

synergistically in destabilizing cell membranes (Smith et al., 2001). Therefore, glycoalkaloid accumulation affects food quality and safety, and the accepted level in tubers is < 20 mg/100g fresh weight (Papathanasiou et al., 1998). Yet, wild potato species are believed to contain amount of glycoalkaloids above this level.

Given that high glycoalkaloids levels, and in some cases very high amounts of antioxidants and phenolics are undesirable, wild potato species need to be evaluated for cytotoxicity before their introduction into breeding programs. Also, tuber extracts from wild potato species may contain other unknown cytotoxic compounds that might be undesirable for human consumption.

Objectives

One of the objectives of the present study was to investigate the relative importance of cultivars, environment, seasons, and their interaction on antioxidant activity, total phenolic content, and specific gravity in potato cultivars grown under widely diverse environmental conditions (nine states) for three years (2005, 2006, and 2007 seasons), and also to determine the correlations among these traits to ascertain how selection for any of the traits would affect others.

In addition, ninety-two wild accessions of *S. jamesii* and eighty-six of *S. microdontum* species in the US Potato Genebank, Sturgeon Bay, WI., were fine-screened for antioxidant activity, total phenolic content, and total glycoalkaloid levels. Also, the linear correlations among these traits were investigated. This information is necessary in selecting accessions to use in introgressing desirable traits into cultivated

potato varieties, while avoiding introducing or increasing levels of undesirable compounds such as glycoalkaloids.

Finally, anti-proliferative activity and cytotoxicity potential of tuber extracts from 15 *S. jamesii* accessions, representing the whole range of glycoalkaloid content in this species, was investigated using human prostate (LNCaP) cells and colon (HT-29) cancer cell lines *in vitro*.

CHAPTER II

LITERATURE REVIEW

Origin of the Potato

The word "potato" commonly refers to the potato of commerce belonging to the species *Solanum tuberosum* L. and other cultivated tuber-bearing species found in South America. These plants belong to the family *Solanaceae*, genus *Solanum*, section *Petota*. Most species in section *Petota* possess underground stolons bearing potato tubers at their tips, but some species lack these characteristic structures. Therefore, section *Petota* was divided into two subsections; subsection *Potatoe* containing both cultivated and wild tuber-bearing species, and subsection *Estolonifera* that contains non-tuber-bearing series (Hawkes, 1992). The tuber is the edible part of the potato, which is a part of the stem that stores food and plays a role in propagation. The tuber is also regarded as an enlarged stolon. Stolons are formed from lateral buds at the bottom of the stem (Beukema and van der Zaag, 1990).

Potatoes originated in many countries of South America: Peru, Ecuador, Chile, Colombia, and Bolivia (Harris, 1978; Hawkes, 1978a). According to Correll (1962) and Hawkes (1992), the potato was cultivated in South America long before the arrival of Europeans. Hawkes (1978a) stated that the cultivated potato was derived from one of the many wild species found in South America, more specifically in the Andes of Peru and Bolivia. He reported that the introduction of the potato into Europe was first into Spain in about 1570, then into England between 1588 and 1593, later spreading to almost

every part of the world. From Spain, it diffused into continental Europe, and from England it spread to Ireland, Scotland, and British overseas colonies, including the US.

The documented number of potato species has been increasing as more plant collection excursions are undertaken. Hawkes (1978b) indicated that there were seven cultivated species and 154 wild species, whereas Horton (1987) stated that eight cultivated and 200 wild species were known. Miller, Jr. (1992) estimated that more than 2,000 species of potato exist, about 200 of which are tuber bearing. According to Spooner and Hijmans (2001), *Solanum* section *Petota* contains about 200 wild species distributed from the southwestern United States to central Argentina and Chile, with a secondary center of diversity in the central Mexican highlands. Spooner et al. (2004) provide a summary of recent morphological and molecular studies on interrelationships among potato species in North and Central America. They recognized twenty-five species and four nothospecies which they assigned to eleven informal species.

According to Huamán and Spooner (2002), all landrace populations of cultivated potatoes are a single species, *S. tuberosum*, with eight cultivar groups. The landrace potato cultivars are highly diverse, containing diploids ($2n = 2x = 24$), triploids ($2n = 3x = 36$), tetraploids ($2n = 4x = 48$), and pentaploids ($2n = 5x = 60$). The tetraploids are the highest yielding and they are the sole cytotype of modern cultivars (Ames and Spooner, 2008).

The taxonomy of cultivated potatoes has been controversial with anywhere from one to 20 species recognized (Huaman and Spooner, 2002). Spooner et al. (2005) reported that all landraces of cultivated potato form a common gene pool and have a

monophyletic origin from Andean and Chilean landrace complex. Using simple sequence repeat (SSR) genotyping in combination with morphological analysis, (2007) suggested classifying the cultivated potatoes into four species; *S. tuberosum*, *S. ajanhuiri*, *S. juzepczukii*, and *S. curtilobum*.

Potatoes are among the most widely-grown crop plants in the world, giving good yield under various soil and weather conditions (Lisinska and Leszcynski, 1989). Potato has been ranked as the fourth important food crop worldwide after wheat, rice and corn, and one of the main vegetables consumed in European diets (Tudela et al., 2002). More recently, potato has been ranked third by the FAO. According to Lachman et al. (2001), annual world-wide production of potatoes is approximately 350 million tons (771,618 million lbs). The US potato production was about 44 billion lbs (0.02 billion tons) in 2006 (USDA, 2007). The world average per capita consumption in 2005 was estimated at 33.7 kg (74.3 lbs) (FAO, 2007), while the US per capita consumption of potatoes is about 57kg (126 lbs) (National Potato Council, 2008). Highest potato consumption is in Europe with a per capita consumption of about 96 kg, followed by North America at 57 kg. Per capita consumption is low in Africa and Latin America, but is increasing (FAO, 2007).

The high consumption rate of potatoes is attributed to both their palatability and high nutritive value (Rytel et al., 2005). Potatoes serve as a major food source, as well as an inexpensive source of energy and good quality protein (Lachman et al., 2001).

Nutritional Value of the Potato

Potato tubers are important sources of vitamins and minerals such as calcium, potassium, and phosphorous, and their value in the human diet is often understated or ignored, particularly as a source of ascorbic acid (Dale et al., 2003). The potato is a very low fat food, and is an important source of vitamins C, B, and A (Dale et al., 2003; Kolasa, 1993; Lachman et al., 2000). According to Kolasa (1993), the potato's contribution of nutrients to diet or its role in human nutrition is actually greater than it appears on the nutrition label, because of the volume of potatoes consumed in the U.S. Therefore, the potato plays a more important role in nutrition than might be expected based on its absolute nutrient values.

Kant and Block (1990) stated that potatoes are the third largest source of vitamin B_6 for adults 19-74 years of age. They also reported that potatoes were the second most important contributor of vitamin B_6 for the elderly, who are especially at risk of chronic disease. Vitamin B_6 is involved in amino acid, nucleic acid, glycogen, and lipid metabolism. It influences hormone modulation, erythrocyte production, and immune and nervous system functions. It is also proposed to play a role in the etiology and /or treatment of various chronic diseases such as sickle cell anemia, asthma, and cancer (Kolasa, 1993).

Potato tubers contain several minerals that are important in diet, including phosphorous, calcium, zinc, potassium, and iron (Andre et al., 2007; Yilmaz et al., 2005). Potatoes are also a good source of high-quality protein such as lysine (Friedman,

2004). They also contain significant levels of functional compounds such as antioxidants and polyphenols (Breithaupt and Bamedi, 2002; Kanatt et al., 2005; Reyes et al., 2005).

Tuber Specific Gravity

Specific gravity is an important quality attribute and is one of the properties of potato that could be used as a basis for nondestructive quality evaluation especially in processing. The relationship between specific gravity and cooking quality of potato is well known (Gould, 1999; Komiyama et al., 2007), and the potato processing industry needs cultivars with high tuber specific gravity and acceptable color of processed products (Haynes, 2001). Processers usually pay less for tubers with low specific gravity. According to Gould (1999) potatoes with higher specific gravity are well formed, smooth, and firm, and every 0.005 increase in specific gravity results in an increase in the number of chips that can be processed from 100 pounds of raw potatoes by one pound.

Specific gravity and solids-content have also been shown to have an effect on fat uptake into french fries. Potatoes with a high specific gravity (>1.090) have been shown to produce a high yield of French fries with a lower fat content than lower specific gravity potatoes (Lulai and Orr, 1979). Hagenimana et al. (1998) reported a linear relationship between dry matter content and fat uptake in thin sliced sweet potato crisps, with fat uptake decreasing as dry matter increased.

Antioxidant Activity

Antioxidants are compounds which, when present in low concentrations compared to oxidizable substrates, can quench free radicals and significantly delay or inhibit oxidation of the substrate and protect biological systems against potential harmful effects of free radicals (Arnao, 2000; Diplock et al., 1998).

Antioxidants are categorized as synthetic or natural. Synthetic antioxidants are compounds with phenolic structures of varying degrees of alkyl substitution, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Their usage is being restricted, as they are suspected to cause negative health effects such as carcinogenicity (Barlow, 1990; Ito et al., 1983), and there is increasing interest in replacing synthetic antioxidants with naturally occurring antioxidants (Chang et al., 2002; Koleva et al., 2002).

Antioxidants can also be categorized as either free radical scavengers (non-enzymatic) that trap or decompose free radicals, or cellular and extracellular enzymes (enzymatic) that inhibit peroxidase reactions involved in the production of free radicals. Free radical scavengers or non-enzymatic antioxidants include ascorbate (vit. C) (Kojo, 2004; Suh et al., 2003), tocopherols (vit. E) (Pryor, 2000), carotenoids (El-Agamey et al., 2004; Mortensen et al., 2001; Niles, 2004), flavonoids and polyphenols (Arts and Hollman, 2005; Aviram et al., 2005; Scalbert et al., 2005), α-lipoic acid (Holmquist et al., 2007; Smith et al., 2004) and glutathione (Giustarini et al., 2008; Jones et al., 2000; Masella et al., 2005). Antioxidant enzymes include glutathione peroxidase, superoxide dismutase, and catalase. Enzymatic antioxidants are important for intracellular defenses,

while non-enzymatic antioxidants are the major defense mechanism against extracellular oxidants.

Natural antioxidants can be phenolic compounds (tocopherols, flavonoids, anthocyanins, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids, as well as vitamins C and E, and phospholipids (Hudson, 1990; Shahidi, 2002). Most of these antioxidant compounds are present in foods as endogenous constituents and are referred to as dietary antioxidants (Siddhuraju et al., 2002). The Food and Nutrition Board of the National Academy of Sciences (National Academy of Science, 1998) defined a dietary antioxidant as a substrate in foods that significantly decreases the adverse effects of free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS), or both on normal physiological function in humans.

Free radicals are molecules or molecular fragments containing one or more unpaired electrons. The presence of unpaired electrons confers a considerable degree of reactivity to free radicals (Valko et al., 2004). Free radicals are ubiquitous in the body and can be generated by normal physiological processes, including aerobic metabolism and inflammatory responses, to eliminate invading pathogenic microorganisms (Hussain et al., 2003). Reactive oxygen species can be produced from endogenous sources such as mitochondria, cytochrome P450 metabolism, peroxisomes, and inflammatory cell activation (Inoue et al., 2003).

Troszyńska et al. (2002) reported that imbalance between ROS/RNS and antioxidant defense systems may lead to chemical modification of biologically relevant

macromolecules like DNA, proteins, carbohydrates or lipids. To avoid such modifications, antioxidants inhibit oxidation of these molecules and prevent initiation of oxidizing chain reactions (Klein and Kurilich, 2000; Velioglu et al., 1998). They scavenge free radicals by donation of an electron or hydrogen atom, or by deactivation of prooxidant metal ions and singlet oxygen (Shahidi, 2002).

Antioxidants exert their effects through different mechanisms and functions; therefore, it is essential to clarify which function is being measured when analyzing samples (Niki and Noguchi, 2000). A wide array of assays has been suggested to measure antioxidant activity. Modes of antioxidant action are grouped into two categories based on the chemical reactions involved: hydrogen atom transfer reaction-based assays and single electron transfer reaction-based assays. Hydrogen atom transfer reaction-based assays include total radical trapping antioxidant parameter (TRAP), oxygen radical absorbance capacity (ORAC), and crocin bleaching assays. Electron transfer reaction-based assays include trolox equivalence antioxidant capacity (TEAC), Cu (II) complex antioxidant potential, ferric ion reducing antioxidant power (FRAP), 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid (ABTS), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assays. Other methods used to measure antioxidant activity are superoxide, hydrogen peroxide, the hydroxyl radical, singlet oxygen and peroxynitrite scavenging capacity assays (Huang et al., 2005). The most commonly used assays are $DPPH^{\cdot}$ and $ABTS^{+\cdot}$ radicals because of their ease, speed, and sensitivity. The DPPH radical is only soluble in organic solvents, but $ABTS^{+\cdot}$ is soluble in both hydrophilic and

lipophilic media and can be used over a wide range of pH (Arnao, 2000; Arnao et al., 1999; Lemanska et al., 2001).

Polyphenols

Polyphenolic compounds constitute one of the most commonly occurring ubiquitous groups of secondary plant metabolites and represent an integral part of the human diet (Rice-Evans et al., 1996). There are more than 8,000 known phenolic structures (Bravo, 1998; Harborne, 1998). Polyphenols range from simple molecules like phenolic acids (Fig. 2.1) to highly polymerized compounds such as tannins. Polyphenols are synthesized through two main pathways in plants: the shikimate pathway and the acetate pathway (Bravo, 1998).

The common structural feature of polyphenolic compounds is the diphenylpropane moiety that consists of two aromatic rings linked through three carbon atoms, where together usually form an oxygenated heterocycle (Rodriguez et al., 2007; Sekher Pannala et al., 2001; Teixeira et al., 2005). Polyphenols usually occur as conjugates with one or more sugars, attached either to the hydroxyl group or to an aromatic carbon atom. The attached sugar can be a mono, di or an oligosaccharide, with glucose as the most common type.

Phenolic compounds are essential for plant growth and reproduction. They act as anti-feedants, anti-pathogens, and also aid in recognition of symbionts (Shahidi and Naczk, 1995b). In live plants, phenolic compounds provide protection against oxidative stress and attack by herbivores, and act as UV filters and healing agents.

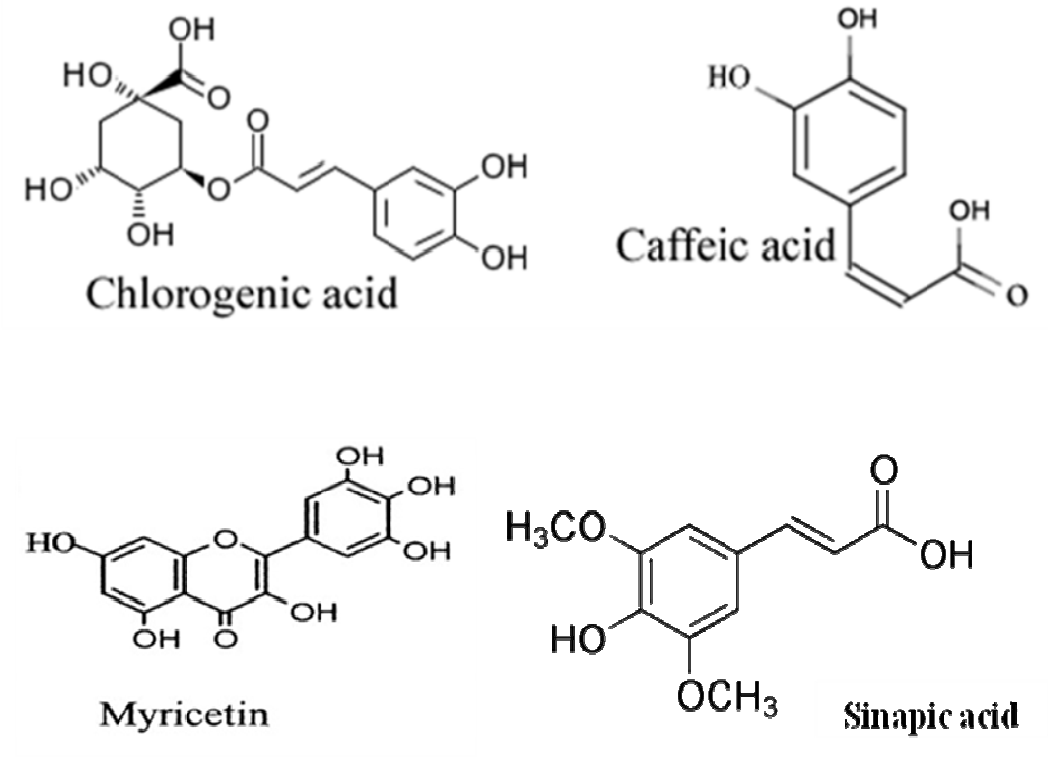


Fig. 2.1. Chemical structure of four important phenolic acids in plants.

Many properties of plant products are associated with the presence and content of polyphenolic compounds. Phenolics and anthocyanins have been reported to possess a very high capacity to quench free radicals (Chu et al., 2000; Kalt et al., 2001). Studies have shown that polyphenols in plants such as flavonols, flavonoids (Comis, 2000; McBride, 1999), anthraquinones (Yen et al., 2000), xanthones and proanthocyanidins (Minami et al., 1994), and zeaxanthin (Stelljes, 2001) act as antioxidants or agents of mechanisms that exhibit cardioprotective or anti-carcinogenic effects.

Phenolic compounds acting as antioxidants may function as terminators of free radicals and as chelators of redox-active metal ions that are capable of catalyzing lipid peroxidation (Schroeter et al., 2002). According to Milde et al. (2007), phenolics together with carotenoids protect low-density lipoproteins (LDL) from oxidation. Oxidation of LDL is believed to lead to development of atherosclerosis and accompanying disorders. Phenolic antioxidants interfere with the oxidation of lipids and other molecules by donation of hydrogen atoms to radicals. The phenoxyl radical intermediates are relatively stable so they do not initiate further radical reactions. The key factors affecting the biological activity of polyphenols are the extent, nature, and position of the substituents and the number of hydroxyl groups (Schroeter et al., 2002). *In vitro* and *in vivo* studies have shown that polyphenols induce responses consistent with the protective effects of diets rich in fruits and vegetables against degenerative conditions like cardiovascular diseases and carcinogenesis (Chung et al., 2003; Manach et al., 2005).

Interest in phenolic compounds has increased recently owing to their antioxidant capacity and their possible beneficial effects on human health. These include the treatment and prevention of cancer, cardiovascular disease, and other pathological disorders (Babich et al., 2007; Damianaki et al., 2000; Polovka et al., 2003; Rice-Evans, 2001; Seeram et al., 2005; Sharma et al., 2007). Regular intake of polyphenols has been linked to lower rates of stomach, pancreatic, lung, and breast cancer (Damianaki et al., 2000).

Kim et al. (2006) reported that these polyphenols induced cell death in SNU-C4 human colon cancer cells in a dose-dependent manner. They observed that polyphenol treatment of cells resulted in the regulation of the expression of apoptotic-regulating genes, decreased expression of the Bcl-2 gene, and increased expression of both the Bax gene and Caspase-3 activity. Friedman et al. (2006) reported that flavonoids in green tea exhibited antimicrobial activities at nanomolar levels and that most compounds were more active than medicinal antibiotics, such as tetracycline or vancomycin, at comparable concentrations.

Antioxidants and Phenolics in Human Health

Antioxidants play a role in balancing the effect of reactive oxygen and nitrogen species and other free radicals to protect biological sites (Valko et al., 2006). Antioxidants quench free radicals, chelate redox metals, and interact with other antioxidants within the antioxidant network, thereby enabling living organisms to overcome the deleterious effects of free radicals while maintaining the beneficial effects of free radicals (Morello et al., 2002; Valko et al., 2007). Enzymatic and non-enzymatic

antioxidants protect living organisms from various oxidative stresses by controlling the redox status and maintaining the redox homeostasis *in vivo* (Droge, 2002).

Antioxidants help maintain or restore cell integrity by preventing reactive oxygen species from damaging cell structures, nucleic acids, lipids, protein, and DNA. Permanent modification of genetic material resulting from oxidative damage represents the initial stages of mutagenesis, carcinogenesis, and ageing (Valko et al., 2007). Several studies have implicated oxidative stress in various pathological conditions, including cardiovascular disease, cancer, neurological disorders, diabetes, and ageing (Dalle-Donne et al., 2006; Makazan et al., 2007; Tappia et al., 2006).

In addition to scavenging deleterious free radicals and maintaining cell integrity, antioxidants modulate cell-signaling pathways (Mates et al., 1999). According to Valko et al. (2007), modulation of cell signaling pathways by antioxidants could help prevent cancer by preserving normal cell cycle regulation, inhibiting proliferation and inducing apoptosis, inhibiting tumor invasion and angiogenesis, suppressing inflammation, and stimulating phase II detoxification enzyme activity.

Enzymatic antioxidants such as L-cysteine, *N*-acetyl cysteine, and non-enzymatic antioxidants such as polyphenols and vitamin E can block activation of nuclear transcription factor κB (NF-κB). The NF-κB regulates several genes involved in cell transformation, proliferation, and angiogenesis (Thannickal and Fanburg, 2000), and its activation has been linked to the carcinogenesis process (Leonard et al., 2004).

Kaneto et al. (1999) reported that antioxidants can help in diabetes prevention. They observed that antioxidant treatment preserved the amounts of insulin content and

insulin mRNA, and also resulted in increased expression of pancreatic and duodenal homeobox factor-1, a β-cell-specific transcription factor. Valko et al. (2007) also reported that antioxidant treatment can exert beneficial effects in diabetes by preserving *in vivo* β-cell function. They noted that antioxidant treatment suppresses apoptosis in β-cells without changing the rate of β-cell proliferation.

Several studies have indicated that consumption of fruits and vegetables helps prevent a wide range of diseases. These observations are attributed to presence of polyphenolic compounds in these fruit and vegetable products. It is believed that the beneficial effects derived from fruits and vegetables include antioxidant nutrients such as vitamins C and E, carotenoids, and phenolics that are thought to be involved in the pathophysiology of many chronic diseases (Stanner et al., 2004).

Epidemiological data have shown that people with a high consumption of fruits and vegetables are at a lower risk of developing several types of cancer (Riboli and Norat, 2003), and cardiovascular disease and stroke (Hu, 2003) than those with low fruit and vegetable consumption. In a study aimed at assessing the relationship between overall mortality in Spanish adults and consumptions of fruit and vegetables, Agudo et al. (2007) reported that a reduction in mortality was associated with increased intake of fresh fruits and vegetables. They also observed that a lower risk of death seemed to be associated with high intakes of vitamin C, provitamin A, carotenoids, and lycopene. They concluded that antioxidant capacity could explain the potential effect of ascorbic acid and provitamin A. Similar results were observed in cohort studies in Greece (Trichopoulou et al., 2003) and in the United States (Steffen et al., 2003).

Hwang and Yen (2008) reported that citrus flavanones, hesperidin, hesperetin, and neohesperidin, which exhibit antioxidant activities have neuroprotective effects against H_2O_2-induced cytotoxicity in the rat pheochromocytoma PC12 cell line. They concluded that these dietary antioxidants are potential candidates for use in intervention for neurodegenerative diseases.

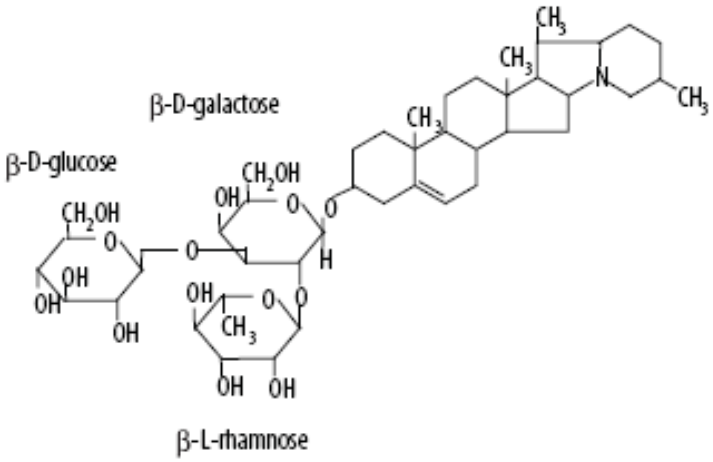
Antioxidants in the diet contribute or exhibit antibacterial, antiviral, anti-inflammatory, and antiallergic actions (Cook and Samman, 1996). Plants vary in composition of phytochemicals with protective functions; therefore, to attain maximum health benefits, sufficient amounts of phytochemicals from a variety of sources such as vegetables, fruits and grains are necessary (Adom and Liu, 2002).

Health benefits of individual phenolic compounds have been investigated. Caffeic acid increased the sensitivity of tumor cells to chemotherapeutic agents *in vitro* (Ahn et al., 1997). Shimizu et al. (1999) and Matsunaga et al. (2002) observed that chlorogenic acid reduced the number of tumors in both colon and stomach at initiation and post initiation stages in F344 rats. Gallic acid from grape seed extract inhibited cell proliferation and induced apoptotic death in DU145 human prostate carcinoma cells. It activated caspase-3 and caspase-9, and cleavage of PARP (Veluri et al., 2006). However, most studies have indicated that complex mixtures of phytochemicals in foods provide better protective benefits than single phytochemicals through additive and/or synergistic effects (Eberhardt et al., 2000).

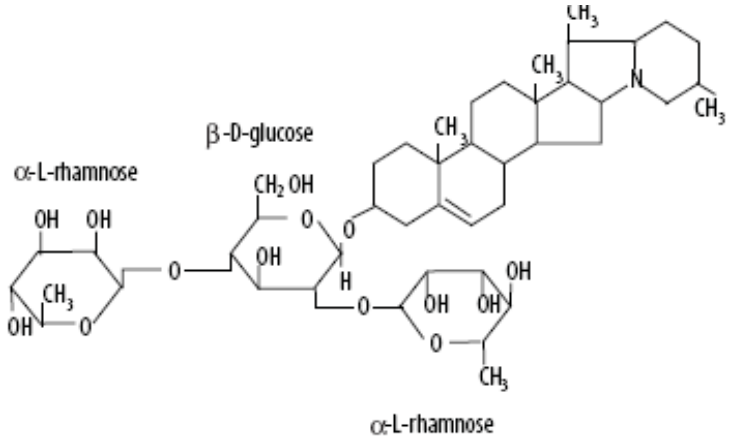
Glycoalkaloids

Glycoalkaloids are steroidal nitrogen–containing metabolites found in potatoes and many *solanaceous* plants (McCue et al., 2007). Steroidal glycoalkaloids are found in almost all parts of the potato, with the highest concentrations associated with tissues that are undergoing high metabolic activity (Jadhav et al., 1973). These include flowers, unripe berries, young leaves, sprouts, peels, and the area around the eyes. Small immature tubers are normally high in glycoalkaloids since they are still metabolically active (Papathanasiou et al., 1998). Glycoalkaloids are concentrated in a 1.5 to 3.0 mm layer immediately under the skin in normal tubers (Pęksa et al., 2006).

The two major glycoalkaloids in potatoes are α-solanine and α-chaconine (Fig. 2.2), which together comprise approximately 95 % of the total glycoalkaloids in the plant (Edwards and Cobb, 1999). The ratio of α-solanine to α-chaconine differs depending on the anatomical part of the potato plant or its variety, and ranges from 1:2 to 1:7 (Bejarano et al., 2000). The other glycoalkaloids found in cultivated potatoes are β- and γ-solanines and chaconines, α- and β-solamarines, demissidine, and 5-β-solanidan-3-a-ol, and in wild potatoes leptines, commersonine, demissine, and tomatine (Lachman et al., 2001).



α-solanine



α-chaconine

Fig. 2.2. Chemical structure of the two major glycoalkaloids in potato tubers.

Various factors influence the concentration of glycoalkaloids in tubers - physical injury due to pest or mechanical injury during harvesting and handling, fungal attack, climate, growing environment, and poor storage conditions. Light exposure during growth, harvesting, and storage is the most important factor influencing the amount of glycoalkaloids in potato tubers (Sengul et al., 2004). Also, breeding with wild *Solanum* species can also result in high glycoalkaloid levels in the new progenies (Laurila et al., 2001).

Steroidal glycoalkaloids are involved in defense against microbial and insect pests (Hollister et al., 2001). However, they are undesirable when present in large amounts in potato tubers. Glycoalkaloids are known to be toxic by acting as cholinesterase inhibitors, causing sporadic out-breaks of poisoning in humans (Smith et al., 1996), and also interact synergistically in destabilizing cell membranes (Smith et al., 2001). Due to their poisonous nature, potato glycoalkaloids have been of major concern and investigated since their discovery in 1820 by the pharmacist Desfosses (Bergers, 1980).

Safety of gylcoalkaloids for humans is still being debated (Friedman et al., 2003; Korpan et al., 2004; Rietjens et al., 2005). Most potato cultivars for human consumption have about 7.5 mg/100g of both α-solanine and α-chaconine (Lachman et al., 2001). Tubers with glycoalkaloid levels greater than 14 mg/100g are bitter in taste, and those with more than 20 mg/100g cause a burning sensation in the throat and mouth. The permitted level of glycoalkaloids in tubers is 20 mg/100g fresh weight (Papathanasiou et

al., 1998). Therefore, new varieties must contain less than 20 mg/100g fresh weight, and varieties containing 2 to 13 mg/100g fresh weight are preferred (Smith et al., 1996).

Wild *Solanum* species are commonly used in potato breeding as a source of valuable germplasm. They are often used to introduce pest and disease resistance into cultivated potato. However, some of these species have high levels of glycoalkaloids such that, together with desirable characteristics, toxic glycoalkaloids may be transferred to potato cultivars (Laurila et al., 2001). Therefore, screening of wild species for glycoalkaloid content is important to determine their suitability as potential parental material in breeding programs.

Cancer and Carcinogenesis

According to the American Cancer Society (2008), cancer refers to a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Abnormal or cancerous cells are caused by both external factors such as chemical toxins, tobacco, radiation, and infectious organisms, and internal factors such as hormones, immune conditions, and mutations from metabolism or inherited mutations. The causal factors may act together or in sequence to initiate and promote carcinogenesis.

Oxidative stress caused by free radicals has been implicated in oncogenic stimulation by inducing cellular redox imbalance. Elevated levels of cellular oxidative stress might result in permanent modification of genetic material (DNA), RNA, proteins, and lipids which normally represent the initial steps involved in mutagenesis and carcinogenesis (Marnett, 2000; Valko et al., 2007).

In addition to causing mutations in cancer-related genes or post-translational modification of proteins, free radicals can also modulate cell growth and tumor promotion by activating signal-transduction pathways that results in the transcriptional induction of proto-oncogenes, including c-FOS, c-JUN, and c-MYC, involved in stimulating growth (Hussain et al., 2003; Vogelstein and Kinzler, 2004). Proteins such as DNA-repair enzymes, those involved in signal transduction, apoptotic modulators, and the p53 protein can be modified both structurally and functionally when exposed to free radicals (Hussain et al., 2003).

Reactive oxygen species can structurally and functionally modify DNA, resulting in single- or double-stranded DNA breaks, purine, pyrimidine, or deoxyribose modifications, arrest or induction of transcription and signal transduction pathways, replication errors, and genomic instability (Marnett, 2000; Poli et al., 2004). Reactive nitrogen species (RNS) such as peroxynitrites and nitrogen oxides have also been implicated in DNA damage. In addition, various redox metals with the ability to generate free radicals, and non-redox metals with the ability to bind to critical thiols, have been implicated in the mechanism of carcinogenesis (Leonard et al., 2004; Roy et al., 2002; Valko et al., 2005; Waalkes et al., 2004). Valko et al (2001) reported that iron-induced stress is considered to be a principal determinant of human colorectal cancer.

Carcinogenesis is a complex multi-sequence/stage process leading a cell from a healthy to a precancerous state and finally to an early stage of cancer (Klaunig and Kamendulis, 2004; Trueba et al., 2004). The process of cancer development involves initiation, promotion, and progression stages occurring in a single cell. The initiation

stage involves non-lethal mutation of DNA that produces an altered cell followed by at least one round of DNA synthesis to fix the damage that occurred during initiation (Loft and Poulsen, 1996). The promotion stage is characterized by clonal expansion of initiated cells by the induction of cell proliferation and/or inhibition of programmed cell death (apoptosis). This stage requires a continuous presence of the tumor promoting stimulus and is therefore reversible by eliminating the stimuli. The progression stage involves cellular and molecular changes that occur from preneoplastic to neoplastic states. This stage is irreversible and involves additional genetic damage, genetic instability, and disruption of chromosome integrity resulting in transition of the cell from benign to malignant. Because tumor promotion may be the only reversible event during cancer development, its suppression is regarded as an effective way to inhibit carcinogenesis (Friedman et al., 2007).

Two mechanisms have been proposed for the induction of cancer. One suggests that an increase in DNA synthesis and mitosis by nongenotoxic carcinogens may induce mutations in dividing cells through misrepair. These mutations may then clonally expand from an initiated preneoplastic cell state to a neoplastic cell state (Ames and Gold, 1990; Guyton and Kensler, 1993). The other mechanism stipulates that a breakdown of equilibrium between cell proliferation and cell death induces cancer. Therefore, carcinogenesis can be described as an imbalance between cell proliferation and cell death shifted towards cell proliferation (Valko et al., 2006).

During cell proliferation, protein p53 plays a primordial role, checking the integrity of DNA (Oren, 2003; Zurer et al., 2004). It triggers mechanisms that eliminate

the oxidized DNA bases that cause mutations. And when cell damage is great, p53 triggers cell death by apoptosis. According to Hussain et al. (2003), uncontrolled apoptosis can be harmful to an organism, leading to destruction of healthy cells. Hence, there is a regulatory system consisting of pro-apoptotic factors such as p53 and anti-apoptotic factors. Most cancers have defects in upstream or downstream genes of p53 function.

Colon Cancer

Colorectal cancer is the third most common cancer in both men and women in the U.S. (American Cancer Society, 2008). It accounted for about 10% of cancer mortality in the United States, and caused about 57,000 deaths in 2004 (Jemal et al., 2004). It is estimated that colon and rectal cancer will account for 9% of all cancer deaths in 2008 (American Cancer Society, 2008).

Colon cancer development is often characterized in an early stage by a hyper-proliferation of the epithelium leading to the formation of adenomas. Colon carcinogenesis is a multi-step process, and early intervention should target inhibition of enhanced cell proliferation in transformed cells by induction of the apoptotic pathway to delete cells carrying mutations (Hawk et al., 2005).

Diet and lifestyle are thought to be major risk factors for developing colorectal cancer (Bray et al., 2002). Other studies have associated the risk of developing colorectal cancer to inflammatory bowel disease (IBD) (Munkholm, 2003; Podolsky, 2002).

Prostate Cancer

The prostate is a small sex accessory gland surrounding the urethra at the base of the bladder and consists of epithelial and stromal cells (Cunha et al., 2004). A normal human prostate is divided into three regions according to their position in relation to the urethra – the transition the transition zone comprising 5%–10%, the central zone comprising approximately 25%, and the peripheral zone which makes the bulk (70%) of the prostate glandular tissue (Dehm and Tindall, 2006). The cells within these zones vary significantly in their contribution to the prevalence of prostate cancer (Che and Grignon, 2002).

The American Cancer Society (2006) reported that prostate cancer is the most frequently diagnosed cancer and the third leading cause of cancer death among men in the US. At the time, it was estimated that 27,350 deaths would occur due to prostate cancer. Current estimates have placed prostate cancer as the second leading cause of cancer death in men, with about 28,660 deaths expected to occur in 2008 (American Cancer Society, 2008).

Prostate cancer initially develops as a high-grade intraepithelial neoplasia (HGPIN) in the peripheral and transition zones of the prostate gland. The HGPIN eventually becomes a latent carcinoma, which may subsequently progress to a large, higher grade, metastasizing carcinoma (Abate-Shen and Shen, 2000; Bosland et al., 1991; Shukla and Gupta, 2005). Promotion and progression stages are controlled by signal transduction molecules triggered by hormones such as androgens (Giovannucci, 1999; Shukla and Gupta, 2005). Androgen receptor (AR) signaling, cell proliferation and

cell death play a critical role in regulating the growth and differentiation of epithelial cells in the normal prostate (Cunha et al., 2004).

Occurrence of prostate cancer is influenced by both genetic and non-genetic factors. About 43% of cancer cases are attributed to genetic factors and these factors are important at younger ages. Aging increases the risk of prostate cancer development (Brothman, 2002).

Prostate cancer is classified as androgen-dependent or androgen-independent (Dehm and Tindall, 2006; Eder et al., 2000; Haag et al., 2005). In androgen-dependent prostate cancers, the cells depend on androgens for their growth and survival and can be treated by either blocking the androgen pathway or using anti-androgens. Androgen ablation initially inhibits androgen receptors and reduces prostate specific antigen (PSA). Androgen-independent type of cancer appears in later stages of cancer reoccurrence and is resistant to hormonal treatment (Roy-Burman et al., 2005).

Cell Proliferation

Cell proliferation is the increase in number of cells as a result of cell growth and division. Cancerous cells are characterized by uncontrolled increase in cell numbers. Cell proliferation is balanced by programmed cell death in normal organs, while mutated cells gain a proliferative advantage resulting in excessive growth (Denmeade et al., 1996; Magi-Galluzzi et al., 1998).

According to Vogelstein and Kinzler (2004) cancer-gene mutations enhance net cell growth or proliferation, and they suggested that there are fewer pathways than genes involved in carcinogenesis. In normal cells, the cell cycle is regulated at two check

points; G1–S and G2–M phases. Most of the cancer genes control transitions from the resting stage (G1) to a replicating phase (S) of the cycle. Some of the products of these genes include proteins such as kinases and cyclins.

Studies in human tumors have shown that some of the molecules often altered in cancer are those involved in the control of the G1–S transition of the cell cycle, particularly the cyclin-dependent kinase (CDK) and CDK inhibitors. These cell cycle regulators have been found to be altered in more than 80% of human neoplasias, either by mutations within the genes encoding these proteins or in their upstream regulators (Ortega et al., 2002).

Mutations in tumor-suppressor genes encoding CDK inhibitors such as p16 (Ortega et al., 2002) and in genes encoding transcription factors such p53 (Oren, 2003) result in enhanced cell proliferation. Expression of the nuclear transcription factor kappa B (NF-κB) has been shown to promote cell proliferation, while inhibition of NF-κB activation blocks cell proliferation. Several studies reported that tumor cells from colon, breast, and pancreas cell lines expressed activated NF-κB (Storz, 2005; Valko et al., 2006).

Several studies have also shown that mitogen-activated protein kinase (MAPK) signaling pathways also play a critical role in both cell proliferation and apoptosis. The three sub-groups of MAPKs in mammalian cells are extracellular signal-regulated kinase (ERK), the c-Jun NH_2-terminal Kinase (JNK), and the p38 MAPK (Kyriakis and Avruch, 2001; Zhao et al., 2006). The extracellular signal-regulated kinase (ERK) pathway is activated by growth factors and JNK by a variety of environmental stressors.

These kinases can induce both survival and apoptotic responses in cells depending on cell type and environment (Lu and Xu, 2006). Valko et al (2007) reported that the balance between ERK and JNK activation is important for cell survival since both a decrease in ERK and an increase in JNK are required for the induction of apoptosis.

There are several assays for measuring cell proliferation *in vitro* by using colorimetric methods such as the tetrazolium salt assay (Lawnicka et al., 2004). The number of cells *in vitro* can be counted using a haemocytometer or coulter counter. Cell proliferation can also be measured *in vivo* by tumor volume (Nakanishi et al., 2003).

Apoptosis

Apoptosis is an evolutionarily conserved form of programmed cell death that requires a specialized mechanism to get rid of excess or potentially dangerous cells (Thornberry and Lazebnik, 1998). Programmed cell death (apoptosis) is required for proper development and to destroy cells that represent a threat to the integrity of the organism. According to Hengartner (2000), apoptosis is as important as cell division and cell migration, since regulated cell death allows the organism to tightly control cell numbers and tissue size, and to protect itself from rogue cells that threaten homeostasis.

Apoptosis is not random but normally occurs in cells with damaged DNA. When a cell becomes mutated and does not repair itself, apoptosis selectively eliminates the altered cells. Programmed cell death results in morphological changes in cells such as shrinkage, development of blebs, chromatin condensation, and biochemical changes such as DNA fragmentation (Chaudhary et al., 1999).

According to Hale et al. (1996), there are three mechanisms by which a cell commits suicide by apoptosis: one triggered by an internal signal (the intrinsic or mitochondrial pathway), another triggered by an external signal (extrinsic or death receptor pathway), and a third by apoptosis inducing factor (AIF). The major component of the apoptotic machinery is a proteolytic system involving a family of cysteine proteases called caspases (Thornberry and Lazebnik, 1998). Caspases are considered the central executioners of the apoptotic pathway and over a dozen of them have been identified in humans (Hengartner, 2000).

Valko et al (2007) stated that the intrinsic or mitochondrial pathway is represented by intracellular damage of the cell causing Bc1-2 protein in the outer membranes of mitochondria to activate Bax that causes cytochrome *c* to release from the mitochondria. This pathway can be caspase-dependent or caspase-independent. In the released caspase-dependent pathway, cytochrome *c* binds to apoptotic protease activating factor-1 (APAF-1) forming apoptosomes. The apoptosome complex binds to and activates caspase-9. Cleaved caspase-9 activates other caspases (3 and 7) leading to digestion of structural proteins in the cytoplasm, degradation of DNA, and phagocytosis of the cell. The caspase-independent pathway involves activation of apoptosis inducing factor (AIF) or endonuclease G through translocation from mitochondria to nucleus (Mohamad et al., 2005)

The transmembrane pathway of apoptosis involves the tumor necrosis factor (TNF) ligand and receptor superfamily members (TNFα, Fas ligand and TNF-related apoptosis-inducing ligand; TRAIL). Apoptosis pathways can start at the plasma

membrane by death receptor ligation (transmembrane or Fas-ligand dependent pathway) or at the mitochondria (mitochondrial or Fas-ligand independent pathway) (Delmas et al., 2003; Fulda and Debatin, 2006; Huang et al., 2006).

CHAPTER III

RELATIONSHIPS AMONG ANTIOXIDANT ACTIVITY, PHENOLICS, AND SPECIFIC GRAVITY IN POTATO (*SOLANUM TUBEROSUM* L.) CULTIVARS GROWN IN DIFFERENT ENVIRONMENTS

Introduction

Antioxidant compounds are present in foods as endogenous constituents or phytochemicals (Siddhuraju et al., 2002) and efforts are underway to extract them from plant sources. Some of the phytochemicals present in plants are polyphenols, and these compounds, such as flavonols, flavonoids (Comis, 2000; McBride, 1999), anthraquinones (Yen et al., 2000), xanthones and proanthocyanidins (Minami et al., 1994) and zeaxanthin (Stelljes, 2001) act as antioxidants and agents of mechanisms that exhibit cardioprotective or anticarcinogenic effects. Phenolics and anthocyanins have been reported to possess a very high capacity to quench free radicals (Chu et al., 2000; Kalt et al., 2001), hence attracting scientists to investigate fruits and vegetables for their antioxidant properties.

Plants vary in composition of phytochemicals with protective functions; therefore, to attain maximum health benefits, sufficient amounts of phytochemicals from a variety of sources such as vegetables, fruits and grains are necessary (Adom and Liu, 2002). Previous studies have also indicated that complex mixtures of phytochemicals in foods provide better protective benefits than single phytochemicals through additive and/or synergistic effects (Eberhardt et al., 2000).

Tuber specific gravity is an important quality factor and is one of the properties of potato tubers used as a measure of tuber quality. Processors usually pay less for tubers with low specific gravity. Environmental factors influence tuber specific gravity (Davenport, 2000; Sterrett et al., 2003). According to Gould (1999) potatoes with higher specific gravity are well formed, smooth, and firm, and every 0.005 increase in specific gravity results in increase of the number of potato chips that can be processed from 100 pounds of raw potatoes by one pound.

Specific gravity and solids-content have also been shown to have an effect on fat uptake into French fries. Potatoes with a high specific gravity (>1.090) have been shown to produce a high yield of French fries with a lower fat content than lower specific gravity potatoes (Lulai and Orr 1979). Hagenimana et al. (1998) reported that there is a linear relationship between dry matter content and fat uptake in thin sliced sweet potato crisps, with fat uptake decreasing as dry matter increased.

Environmental conditions influence crop productivity and quality, including phytonutrient levels. Crops perform differently under different environments, thereby exhibiting genotype-by-environment interaction. Genotype-environment interaction in crops is the differential response of genotypes to changing environmental conditions. Such interactions complicate testing and selection in breeding programs and result in reduced overall genetic gain (Goncalves et al., 2003). Differential performance of genotypes due to genotype-by-environment interaction results in yield and quality parameter instability in crops. Peterson et al. (1992) suggested that for breeders, stability is important in terms of changing ranks of genotypes across environments and affects

selection efficiency. For end-users, such as wheat millers and bakers, consistency in quality characteristics of cultivars is very important regardless of changing cultivar ranks (Rharrabti et al., 2003a).

Becker and Léon (1988) suggested that a stable genotype is one with an unchanged performance in various environments, i.e. static stability concept. According to Rharrabti et al. (2003a) stability of quality parameters in crop products is an important requirement in product development and may result into economic instability for end-users. Economic instability is commonly caused by both environment and genotype-by-environment interaction effects. Grausgruber et al. (2000) stated that the quality of a genotype usually reacts like other quantitative characters to changing environmental conditions. Therefore, a genotype is considered economically stable if its contribution to G x E interaction is low.

Several studies have investigated effects of genotype and environment on yield, nutrients and antioxidant activity in potato (Dale et al., 2003; Nzaramba et al., 2006), oats (Emmons and Peterson, 2001), wheat (del Moral et al., 2003; Grausgruber et al., 2000; Graybosch et al., 2004), barley (Atlin et al., 2000), and maize (Epinat-Le Signor et al., 2001). All these studies aimed at gaining an understanding of genotype x environment interactions. Producers experience local and annual variations in crop yield and quality, yet the industry and consumers demand a constant quality of crop products.

Breeding programs normally select for local adaptation in order to exploit genotype x environment interactions. Before new cultivars are released, they have to be tested at several locations and for many years. Multi-environment trials encounter

problems with genotype x environment interactions, especially differential genotypic responses to environmental condition which limit identification of superior and stable genotypes (Epinat-Le Signor et al., 2001). However, in so doing, stable genotypes for broad adaption and unstable ones for local adaption are identified.

Given the importance of antioxidants in disease prevention, developing potato cultivars with high antioxidant levels would complement physical, medical, and social activities in preventing diseases. Also, to ensure tuber quality, high specific gravity in newly developed varieties should be maintained. However, to accomplish this there is need to understand the relative importance of cultivar, environment, and their interaction on antioxidant activity, phenolic compounds, and specific gravity, and also understand the relationship among these traits. Relationships among traits provide information on how selection for one trait would affect other traits. Therefore, the objective of this study was to investigate the relative importance of cultivar, environment and season, and their interaction on antioxidant activity, total phenolic content, and specific gravity in potato cultivars grown under widely diverse environmental conditions (nine states) for three years (2005, 2006, and 2007 seasons). Also, correlations among antioxidant activity, total phenolics, individual phenolic compounds, and specific gravity were investigated.

Materials and Methods

Plant Materials

Four popular potato cultivars representing different market classes, Yukon Gold (fresh, yellow flesh), Atlantic (chipper), Red La Soda (fresh, red) , and Russet Norkotah (fresh, russet), were selected for this study. These cultivars were grown in nine states

(locations): Springlake, TX., Tulelake, CA., Becker, MN., Plymouth, NC., Aberdeen, ID., Powell Butte, OR., East Lansing, MI., Rhinelander, WI., and Pitstown, NJ. Agronomic practices at all locations were assumed to be typical for potato production in these areas. Potato samples were collected in the 2005, 2006, and 2007 growing seasons. Fifteen advanced selections from the Texas A&M University Potato Improvement Program grown near Springlake, TX were also analyzed.

Chemicals

DPPH (2,2-Diphenyl-1-picrylhydrazyl), Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), potassium persulfate, monobasic sodium phosphate ($NaHPO_4$), dibasic sodium phosphate (Na_2HPO_4), sodium chloride, sodium carbonate, and Folin-Ciocalteu reagent were purchased from Fisher Scientific (Pittsburgh, PA). Methanol and acetonitrile were obtained from VWR International (Suwanee, GA). Pure phenolic compounds (chlorogenic acid, rutin hydrate, caffeic acid, myricetin, and sinapic acid) were obtained from Acros Organics (Pittsburgh, PA).

Specific Gravity Measurement

Specific gravity of each cultivar from every location was measured by weighing ten tubers in air and then when immersed in water. Specific gravity was estimated as the quotient of weight in air and the difference between weight in air and weight in water.

Sample Extraction

Three samples were randomly selected from tubers whose specific gravity had been measured. Each sample contained three tubers. The tubers in each sample were

diced together, and five grams of diced material were placed in Corning centrifuge tubes, and 15 ml of HPLC-grade methanol was added. Samples were homogenized with an IKA Utra-turrax tissuemizer for 3 min. Tuber extract was centrifuged at 31,000 g for 20 min. with a Beckman model J2-21 refrigerated centrifuge. One and one half ml of supernatant was collected into microcentrifuge tubes for antioxidant and total phenolic content analysis. Seven ml of supernatant were collected in glass vials for individual phenolics compound analysis with HPLC. Sample extracts were stored at -20^{o} C until analysis.

Antioxidant Analysis

DPPH assay

Antioxidant activity in tuber extracts was estimated using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method (Brand-Williams et al., 1995). One-hundred-fifty µl of extract was placed in a scintillation vial, 2,850 µl of DPPH methanol solution was added, and the mixture was placed on a shaker for 15 min. The mixture was transferred to UV-cuvettes and absorbance recorded using a Shimadzu BioSpec-1601 spectrophotometer at 515 nm. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a synthetic antioxidant, was used as a standard, and total AOA was expressed as micrograms of trolox equivalents per gram of tuber fresh weight (µg TE/gfw).

ABTS assay

Radical scavenging capacity of potato methanolic extracts was measured against the ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)

radical (Awika et al., 2003). Seventy-five µl of methanol was added to 25 µl of potato extract to make 100 µl of diluted sample extract. Two-thousand-nine-hundred µl of the working solution was added to the diluted sample extract and reacted for 30 min. on a shaker. Absorbance of the solution was measured at 734 nm with a Shimadzu BioSpec-1601 spectrophotometer. The working solution composed of a mixture of 5 ml of mother solution [mixture of equal volumes of 8 mM of ABTS and 3 mM of potassium persulfate solutions] and 145 ml of phosphate buffer solution pH 7.4 [40.5 ml of 0.2 M Na_2HPO_4 (dibasic), 9.5 ml of 0.2 M $NaHPO_4$ (monobasic) and 150 mM NaCl. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a synthetic antioxidant, was used as a standard, and total AOA was expressed as micrograms of trolox equivalents per gram of potato fresh weight (µg TE/gfw).

Total Phenolic Analysis

Total phenolic content was determined following the method of Singleton et al. (1999). One-hundred-fifty µl of sample extract was pipetted into scintillation vials, and 2.4 ml of nanopure water was added. One-hundred-fifty µl of 0.25 N Folin-Ciocalteu reagent was added and, after 3 min of reaction, 0.3 ml of 1N Na_2CO_3 reagent was added and allowed to react for 2 hours. The spectrophotometer (Shimadzu BioSpec-1601) was zeroed with a blank (0.150 ml methanol, 2.4 ml H_2O, 150 µl of 0.25 N Folin, and 0.3 ml 1 N Na_2CO_2) before sample analysis. Absorbance of sample extracts was read at 725 nm. Chlorogenic acid was used as a standard, and total phenolic content expressed as milligrams of chlorogenic acid equivalents per 100 grams of potato fresh weight (mg CGA/100gfw).

Phenolic Composition

Composition of tuber methanolic extracts was determined using a HPLC system. Samples were concentrated before analysis by drying 5 ml of potato extract with a speed vac concentrator, and then re-dissolved in 1 ml of methanol. The concentrated extracts were filtered through 0.45 μm syringe filters. The phenolic acid composition of extracts was analyzed using an Atlantis C-18 reverse-phase column (4.6 x 150 mm, 5 μm) from Waters, Milford, MA, maintained at 40^0 C. The HPLC system consisted of a binary pump system (Waters 515), autoinjector (Waters 717 plus), a photodiode array (PDA) detector (Waters 996), and column heater (SpectraPhysics SP8792). Separation of phenolic acids was done using a linear gradient elution with mobile phase solvents A (acetonitrile) and B (water/acetic acid, pH 2.3). Solvent flow rate was set at 1 ml/min and gradient 15 to 100% in 35 min. Pure phenolic compounds were used as standards for HPLC analysis: chlorogenic acid, rutin hydrate, caffeic acid, myricetin, and sinapic acid. Identification and quantification of phenolic acids present in the potato extracts was done by comparing retention time and area of the peaks in the extracts with that of the standard compounds. Quantities of the phenolic acids were expressed as μg/g of fresh weight.

Statistical Analysis

Data from each location in each year were analyzed separately using analysis of variance (ANOVA). In some locations not all four cultivars were grown in all years. Therefore, cultivar, location, year, and their interaction effects were determined following a factorial design using data from locations in which all cultivars were grown

in all years. A combined analysis of variance for all the factors (cultivars, locations, and years) was performed using the following factorial general linear model (GLM); $Y_{ijkl} = \mu + \alpha_i + \beta_i + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{jk} + (\beta\gamma)_{ik} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl}$, where Y_{ijkl} is the i^{th} value of the l^{th} variety in j^{th} location in the k^{th} year. The four terms, μ, α_i, β_i, and γ_k are the mean and the main effects of cultivars, locations, and years, respectively. The terms, $\alpha\beta_{ij}$, $\alpha\gamma_{jk}$, and $\beta\gamma_{ik}$ are first order interactions, and $\alpha\beta\gamma_{ijk}$ is a second order interaction involving all three factors, and the environmental deviation within locations and years was denoted as ε_{ijkl} (Lentner and Bishop, 1993). The model was a mixed effects model with cultivars and locations considered as fixed effects while years were random.

Mean separation was by least squares analysis. Phenotypic correlations between traits were computed following Pearson's correlation method. Principal component analysis (PCA) was performed on the mean data of all replicates. General linear regression was performed with SAS software version 9.1 (SAS, 2002), while principal component analysis was performed with GGEbiplot software version 5.2 (Yan, 2001).

Results

Location, Year, Cultivar, and Interaction Effects

The combined analysis of variance showed a strong influence of the main effects of cultivar, location, and year on antioxidant activity, total phenolic content, and specific gravity (Table 3.1). Two-way interaction effects of location x year and cultivar x location were significant for all measured traits. Interaction effect between cultivar and year was significant for antioxidant activity (both DPPH and ABTS assays) and specific

gravity but not significant for total phenolic content. The three-way interaction effect was also significant for all parameters.

Growing location accounted for 16% of the total observed variability in antioxidant activity measured by the DPPH assay (Table 3.2). This percentage was equal to variability due to cultivar differences, and also close to that due to seasons (year). More than 50% of the observed variability in antioxidant activity measured by the ABTS assay, total phenolic content, and specific gravity was attributed to the cultivar main effect.

The location main effect accounted for 10.3, 11.8, and 20% of the variability in the ABTS assay, total phenolics, and specific gravity, respectively (Table 3.2). All interaction effects accounted for less than 10% of the variability in ABTS assay, total phenolics, and specific gravity. However, interaction between location and year (Location x Year) was responsible for 29.8% of the observed variability for antioxidant activity measured by the DPPH assay.

According to Rharrabti et al. (2003b), the ratio of variances associated with environmental effects (σ^2_e) to variances associated with genotypes (σ^2_g) shows the relative influences of genotype and environment on traits of interest. In this study, the environmental variance component was considered as that due to location, year and their effects ($\sigma^2_e = \sigma^2_l + \sigma^2_y + \sigma^2_{lxy}$), and the genotype x environment component was the sum of cultivar x location, cultivar x year, and their three-way interaction effects ($\sigma^2_{gxe} = \sigma^2_{gxl} + \sigma^2_{gxy} + \sigma^2_{gxyxl}$).

Table 3.1. Analysis of variance mean squares and significance of cultivar, location, year, and interaction effects for antioxidant activity, phenolic content, and specific gravity of four potato cultivars grown in five locations during the 2005, 2006, and 2007 growing seasons.

		Mean squares			
		Antioxidant activity			
Source of variation	df	DPPH Assay	ABTS Assay	Total phenolics	Specific gravity
Location	4	108097.7**	2319774.1**	2078.0**	0.00148**
Year	2	204787.4**	1596437.3**	577.1**	0.00029**
Location x Year	8	98080.3**	278589.1**	724.9**	0.00016**
Rep (Location x Year)	30	1230.5	86266.3	53.8	0.00001
Cultivar	3	143602.1**	18053028.2**	14588.4**	0.00519**
Cultivar x Year	6	19758.5**	476911.0**	88.5	0.00014**
Cultivar x Location	12	9441.9**	207621.9**	158.9**	0.00015**
Cultivar x Year x Location	24	6375.8**	230659.7**	119.0**	0.00008**
Residual	90	1675.2	85260.2	48.4	0.00001

*significance at p-value <0.05
**significant at p-value <0.01

Table 3.2. Percentage of total observed variability in antioxidant activity, total phenolics, and specific gravity contributed by each variance component- cultivar, location, year, and interactions.

	Antioxidant activity			
Source of variation	DPPH Assay	ABTS Assay	Total phenolics	Specific gravity
Location	16.5	10.3	11.8	20.0
Year	15.6	3.5	1.6	1.9
Cultivar	16.4	60.2	62.3	52.8
Location x Year	29.8	2.5	8.2	4.4
Cultivar x Year	4.5	3.2	0.8	2.8
Cultivar x Location	4.3	2.8	2.7	6.0
Cultivar x Year x Location	5.8	6.2	4.1	6.4

The ratio σ^2_e / σ^2_g was greater than 1 for the DPPH assay (Table 3.3), which implies that environmental effects were greater than genetic effects. Ratios less than 1 were observed for the ABTS assay, total phenolics, and specific gravity, indicating more genetic influence than environmental effects on these traits. All traits, antioxidant activity (both DPPH and ABTS assays), total phenolic content, and specific gravity exhibited a ratio $\sigma^2_g / \sigma^2_{gxe}$ greater than 1 (Table 3.3). This demonstrates that genetic effects (cultivar differences) were greater than variability due to interaction of genotype and environment.

Table 3.4 shows actual values of cultivar performances in each location. Russet Norkotah exhibited the highest amount of antioxidants (DPPH and ABTS assays) and total phenolics, whereas Atlantic had the lowest antioxidants and phenolics in all locations. Rankings of Red La Soda and Yukon Gold for antioxidants and phenolic content changed over locations, with one performing better than the other in some states and worse in other states. As for specific gravity, Atlantic consistently exhibited highest specific gravity, while Russet Norkotah, Red La Soda, and Yukon Gold switched rankings from state to state.

Table 3.3. Ratios of environmental (σ^2_e) to genetic (cultivar) (σ^2_g) variance components and genetic to genotype-by environment (σ^2_{gxe}) interaction effects for antioxidant activity, total phenolics, and specific gravity of four potato cultivars grown in five states for three seasons.

	Antioxidant activity			
Ratio	DPPH Assay	ABTS Assay	Total phenolics	Specific gravity
σ^2_e / σ^2_g	2.9	0.2	0.2	0.4
$\sigma^2_g / \sigma^2_{gxe}$	4.0	19.7	39.8	14.3

Table 3.4. Mean values of antioxidant activity (DPPH and ABTS), total phenolics, and specific gravity over three years for four potato cultivars grown at nine locations (States).

		Locations									
Parameter	Cultivar	CA	ID	MI	MN	NJ	NC	OR	TX	WI	*LSD*[a]
DPPH	Russet Norkotah	315	379	303	144	217	257	171	241	254	*201*
	Red La Soda	274	332				193	171	148	242	*168*
	Yukon Gold	226	264	170	153	171	212	149	239	236	*100*
	Atlantic	168	177	155	90	90	129	82	133	130	*111*
	LSD[b]	*92*	*154*	*199*	*70*	*53*	*79*	*42*	*77*	*105*	
ABTS	Russet Norkotah	2875	2587	3009	2656	2023	2501	2087	2410	2565	*544*
	Red La Soda	2605	2033				1811	1911	1651	2157	*638*
	Yukon Gold	2454	1748	2157	2199	1509	1887	1676	1816	1971	*606*
	Atlantic	1214	929	1046	944	898	1010	1028	799	1033	*369*
	LSD[b]	*408*	*402*	*577*	*353*	*180*	*768*	*439*	*342*	*289*	
Total phenolics	Russet Norkotah	91	90	91	77	81	92	71	80	78	*21*
	Yukon Gold	83	69	80	67	64	63	53	70	62	*15*
	Red La Soda	80	70				56	61	60	70	*21*
	Atlantic	46	40	44	34	35	40	34	38	37	*10*
	LSD[b]	*10*	*10*	*11*	*14*	*8*	*34*	*8*	*15*	*6*	
Specific gravity	Atlantic	1.107	1.082	1.089	1.071	1.086	1.089	1.088	1.089	1.084	*0.013*
	Yukon Gold	1.088	1.083	1.059	1.061	1.079	1.077	1.083	1.077	1.074	*0.011*
	Red La Soda	1.078	1.065				1.066	1.068	1.067	1.057	*0.008*
	Russet Norkotah	1.074	1.064	1.066	1.047	1.075	1.079	1.073	1.073	1.063	*0.013*
	LSD[b]	*0.005*	*0.009*	*0.014*	*0.035*	*0.005*	*0.009*	*0.006*	*0.005*	*0.005*	

[a] LSD among location means of a cultivar trait; [b]LSD among cultivars within a location

Relationships among Antioxidant Activity, Phenolic Content, and Specific Gravity

Regression analysis revealed significant relationships among antioxidant activity, total phenolics, and specific gravity (Fig. 3.1). Correlation coefficient between antioxidant activity (AOA) measured by the DPPH assay and AOA measured by the ABTS assay was significant (p-value <0.01) with a value of r = 0.508. Antioxidant activity (measured by both DPPH and ABTS) and total phenolic content were significantly correlated with correlation coefficients (r) = 0.579 and 0.876, respectively. Similar results showing significant correlation between AOA and total phenolic content in the potato (Reddivari et al 2007) and sweet potatoes (Huang et al., 2004) were reported. Negative relationships between antioxidant activity (DPPH and ABTS assays) and specific gravity, and between total phenolic content and specific gravity were observed (Fig. 3.1). The correlation coefficient between AOA (DPPH assay) and specific gravity was -0.232 and significant at p-value = 0.01. Relationship between the ABTS assay and specific gravity was also significant (p-value = 0.01) with a correlation coefficient of -0.494. Likewise, total phenolic content was negatively correlated (r = -0.452) with specific gravity. These results indicate that breeding for high antioxidants and phenolic content may result in reduced specific gravity of the tubers.

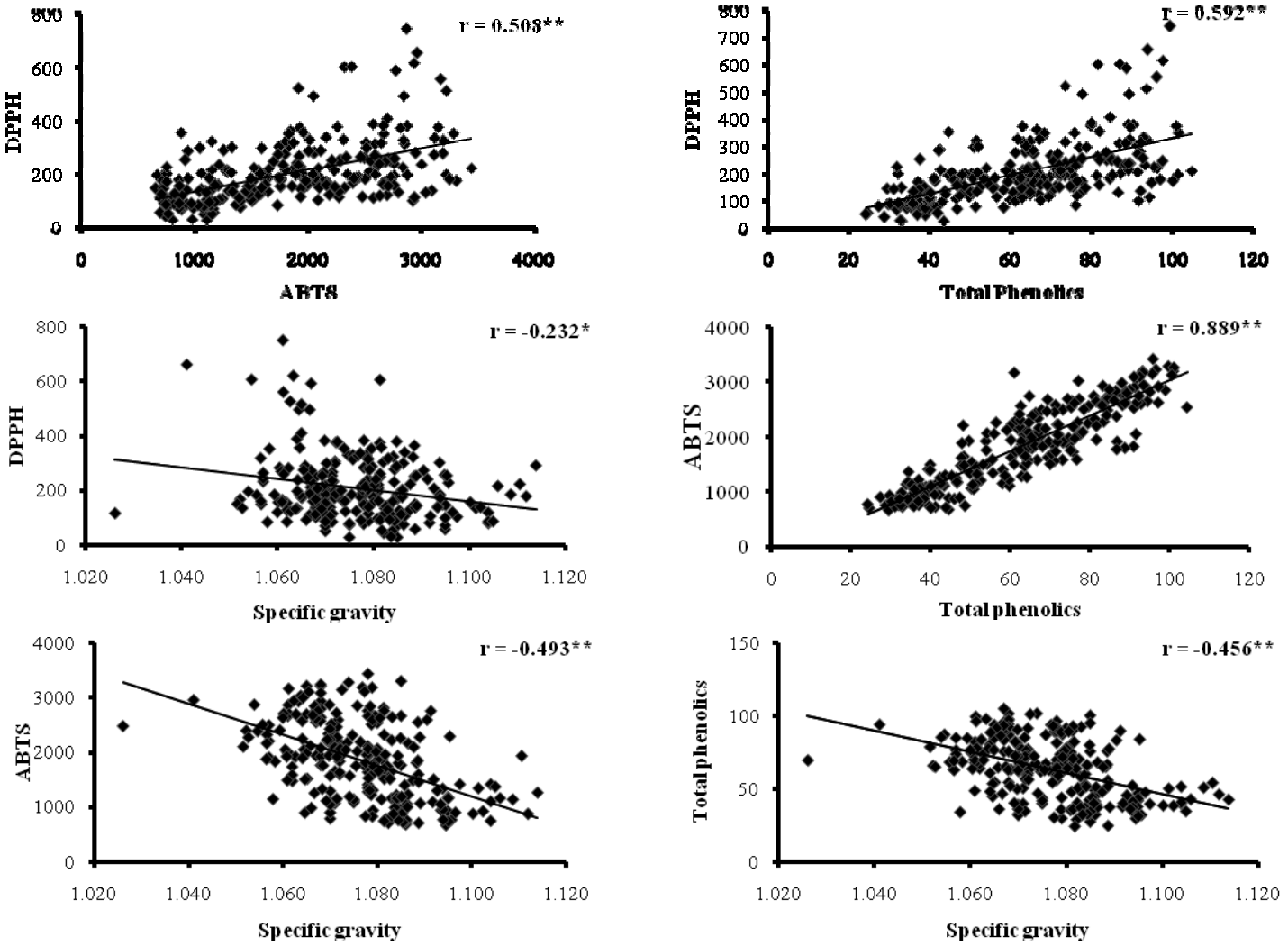


Fig. 3.1. Regression analysis and correlation coefficients among antioxidant activity (DPPH and ABTS assays), phenolic content, and specific gravity of four potato cultivars grown over three years at nine locations.

This study used cultivars that are commonly grown in the United States or grown by most potato breeders as standard checks. However, given the small sample of cultivars used, correlations of antioxidant activity and phenolic content with specific gravity may have been biased due to sampling size. Also, the cultivar Atlantic was consistently the lowest in antioxidant activity and total phenolic content, and the highest in specific gravity, thereby behaving as an influential outlier. Therefore, several advanced selections from the Texas A&M University Potato Improvement Program, College Station, were used to confirm the observed correlation analysis results above. Tuber specific gravity, antioxidant activity, total phenolic content, and individual phenolic compounds in the breeding lines are shown in Table 3.5. Phenolic compounds quantified with HPLC analysis were chlorogenic acid, rutin hydrate, caffeic acid, myricetin, and sinapic acid. Results showed no significant linear relationship between antioxidant activity and specificity gravity in potato breeding lines. Also there was no significant correlation between total phenolic content and specific gravity, or between all individual phenolic compounds and specific gravity (Table 3.6). .

Table 3.5. Mean values of antioxidant activity, phenolic content, specific gravity, and individual phenolic compounds of potato advanced selections grown near Spring Lake, TX in the 2005 growing season.

	Antioxidant activity				Phenolic composition				
Genotype	DPPH	ABTS	Total phenolics	Specific gravity	Sinapic	Rutin	Myricetin	Caffeic	Chlorogenic
A833501-9R	59.7	2273.3	65.6	1.066	3.1	14.6	12.0	29.8	60.9
Atlantic	190.7	713.4	44.8	1.086	3.4	7.7	10.4	29.6	109.2
ATTX961014-1AR/Y	96.4	2339.9	66.6	1.062	7.9	6.5	10.9	29.6	123.4
ATTX98444-16R/Y	225.7	2620.3	89.0	1.074	2.6	25.4	11.2	36.2	276.5
ATTX98462-9R/Y	169.5	2478.8	71.9	1.071	11.3	7.1	10.7	30.0	215.7
ATTX98468-3R/Y	296.2	3448.2	92.5	1.070	4.2	5.8	11.2	33.8	344.4
ATTX98493-2P/P	422.1	3037.5	96.4	1.067	10.3	14.3	11.8	31.8	441.0
ATTX98510-1R/Y	336.7	3999.0	104.6	1.063	2.9	7.2	10.4	32.9	363.0
COTX01403-4R/Y	204.2	2602.8	80.5	1.061	7.4	8.6	10.7	31.7	250.2
NDTX4271-5R	122.5	2389.2	68.8	1.058	2.8	4.1	10.8	30.8	104.8
NDTX4304-1R	138.6	2478.4	69.6	1.058	5.0	8.9	11.2	29.2	61.3
NDTX4756-1R/Y	359.2	3684.7	101.6	1.062	8.4	7.1	11.4	31.9	315.2
NDTX731-1R	113.0	2133.1	66.2	1.058	4.1	9.7	10.9	30.1	142.9
POTX03PG19-1Pu/YR	394.2	2731.9	92.1	1.082	5.5	7.3	10.7	31.8	542.7
PORTX03PG25-2R/P	471.1	3558.7	106.1	1.057	22.7	22.8	11.5	42.3	659.1
Russet Norkotah	201.9	2103.1	86.5	1.068	6.0	10.6	11.3	31.6	231.6
TX1674-W/Y	196.8	2884.0	86.3	1.080	4.2	5.2	11.1	30.0	179.3
Yukon Gold	268.3	1682.6	76.0	1.072	10.1	6.8	11.7	30.1	47.4
LSD	*86.3*	*816.5*	*13.2*	*0.005*	*4.5*	*12.5*	*1.2*	*4.5*	*134.2*

Table 3.6. Correlation analysis among antioxidant activity (DPPH and ABTS assays), total phenolics (TP), specific gravity, and individual phenolic compounds of potato advanced selections grown near Spring Lake, TX in the 2005 growing season.

	ABTS	TP	Sinapic	Rutin hydrate	Myricetin	Caffeic	Chlorogenic	Specific gravity
DPPH	0.58*	0.80**	0.55*	0.27	0.14	0.65**	0.88**	0.09
ABTS		0.88**	0.26	0.14	0.11	0.52*	0.65**	-0.39
TP			0.38	0.29	0.23	0.65**	0.79**	-0.18
Sinapic				0.36	0.32	0.60**	0.54*	-0.28
Rutin hydrate					0.43	0.71**	0.40	-0.11
Myricetin						0.18	0.02	-0.20
Caffeic							0.77**	-0.20
Chlorogenic								0.00

*significance at *p*-value <0.05
**significant at *p*-value <0.01

Despite the lack of a significant relationship between specific gravity and any of the measured traits, principal component analysis was done to further elucidate the particularity of the relationships (Yan and Hunt, 2001). The first two principal components (PC1 and PC2) were used to construct a biplot consisting of the measured traits and the potato genotypes (Fig. 3.2). The two principal components accounted for 66.8% of the total variance: 50.6 and 16.2% for PC1 and PC2, respectively. The first PC axis (PC1) separated specific gravity from all other traits. Specific gravity was placed on the negative direction of the PC1 axis, whereas antioxidant activity, total phenolic content, and individual phenolic compounds were all on the positive side of the axis (Fig. 3.2). This clearly demonstrates that there is no positive relationship between specific gravity and any of these traits. The biplot (Fig. 3.2) also shows that, among the individual phenolic compounds quantified, chlorogenic acid was the most closely associated with antioxidant activity and total phenolic content followed by caffeic acid.

Discussion

This study demonstrated the influence of genotype and environment on antioxidant activity, total phenolic content, and specific gravity, and also elucidated the relationships among these traits in potato tubers. Results from this investigation showed that antioxidant activity measured by the ABTS assay, total phenolic content, and specific gravity are mostly governed by genotype. This is so because more than 50% of the observed variability in the ABTS assay, total phenolic content, and specific gravity was attributed to the cultivar main effect (Table 3.2). However, for DPPH assay, location, season and cultivar main effects were equally influential to antioxidant activity.

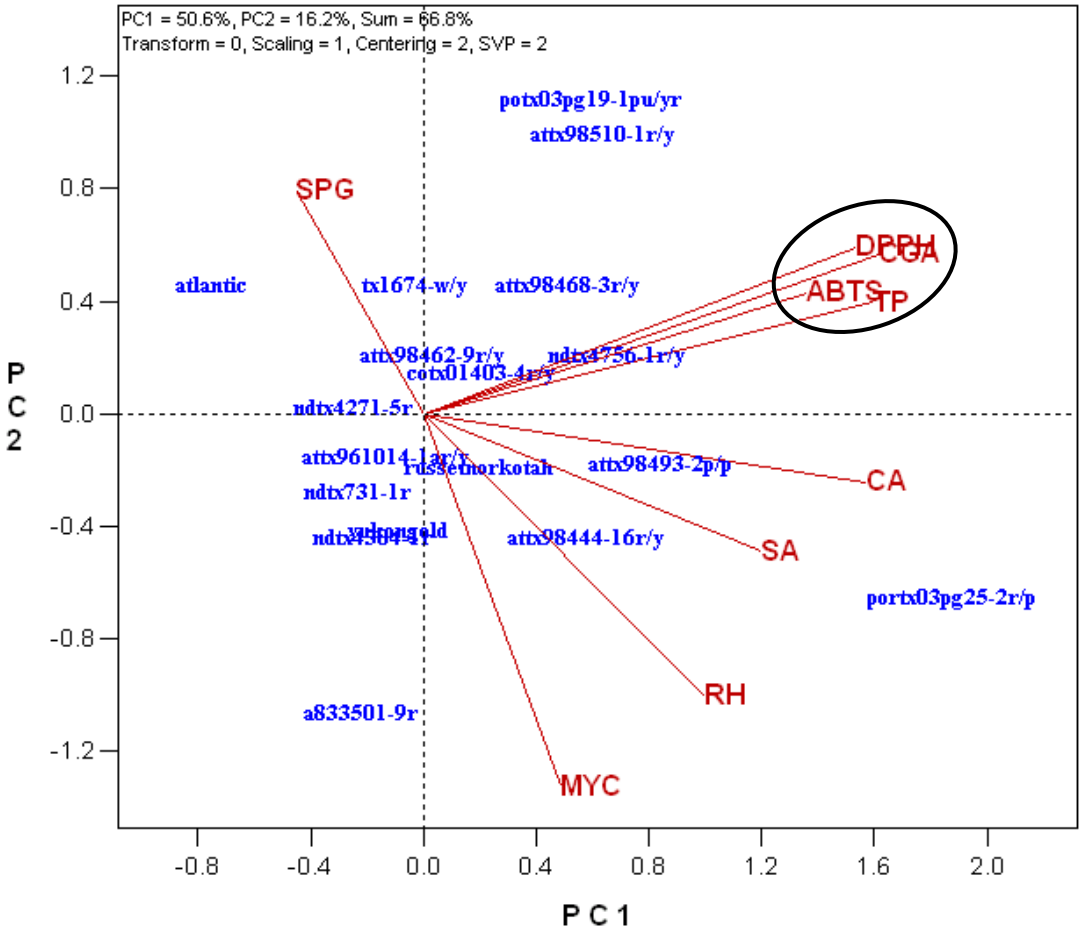


Fig. 3.2. A biplot of genotypes-by-trait in potato advanced selections grown near Springlake, TX, in the 2005 growing season. Traits are in red and upper case and accessions are in blue and lower case. Traits are abbreviated as SPG- specific gravity, DPPH and ABTS- antioxidant activity, TP- total phenolics, CGA- chlorogenic acid, CA- caffeic acid, SA- sinapic acid, RH- rutin hydrate, and MYC myricetin.

Relationships among traits are of interest in plant breeding as they influence strategies employed by breeders in improving crops. A significant positive correlation between desirable traits makes breeding for one or both traits easier, while a negative correlation poses challenges, as increasing one trait results in reduced value of the other. However, if one trait is desirable and the other undesirable, a negative correlation makes breeding easier, while a positive correlation makes trait improvement difficult.

Observations from this study indicated that antioxidant activity and phenolic content in potato tubers have a significant positive linear relationship. These results are in agreement with previous studies in potato tubers (Kanatt et al., 2005; Reddivari et al., 2007a), bamboo extracts (Kweon et al., 2001), fruits (Kim et al., 2003), and vegetables (Troszynska et al., 2002). Therefore, breeding for high antioxidant activity in potatoes can be achieved by increasing the amount of phenolic compounds available in potato tubers.

Specific gravity is the solids content of potato tubers and is an important quality factor for processing. It is one of the properties of potato tubers that could be used as a basis for nondestructive quality evaluation (Chen et al., 2005). Correlation analysis of data from the four common cultivars showed a significant negative relationship between antioxidant activity (DPPH and ABTS assays) and specific gravity, and between total phenolic content and specific gravity. However, correlation analysis of data from advanced selections indicated no significant relationship between antioxidant and specific gravity or between total phenolic content and specific gravity.

The difference in observed results from the two analyses was due to sample sizes. Only cultivars, which could be obtained from breeding programs in the nine states, were used. Therefore, the small sample, in addition to cultivar Atlantic being consistently the lowest in antioxidant activity and total phenolic content and the highest in high specific gravity, could have resulted in biased correlation results. With a larger sample (15 advanced selections and 3 cultivars) with distributed trait values, there was no significant relationship/correlation between antioxidant activity and specific gravity or between total phenolic content and specific gravity. Also, none of the individual phenolic compounds, caffeic acid, chlorogenic acid, myricetin, rutin hydrate and sinapic acid were significantly correlated with specific gravity.

The important finding of the investigation is that there is no significant relationship between antioxidant activity and specific gravity. And also there were no relationships observed between total phenolic content and specific gravity, and between individual phenolic compounds and specific gravity. Therefore, breeding for high antioxidants and phenolic compounds in potato tubers would increase their nutritional value without compromising tuber quality in terms of specific gravity. However, significant genotype-by-environment interactions may hinder rapid progress.

CHAPTER IV

TOTAL GLYCOALKALOIDS, ANTIOXIDANT ACTIVITY, AND PHENOLIC LEVELS IN *SOLANUM MICRODONTUM* AND *SOLANUM JAMESII* ACCESSIONS

Introduction

Research has intensified on investigating the health benefits of phytonutrients (Duyff, 2002), and several research reports have indicated that the benefit of plant foods is due not only to levels of vitamins or other nutritive compounds they provide, but also to activity of the non-nutritive factors they contain. Many of these plant secondary components are antioxidants (Riedl et al., 2002). Food-derived antioxidants, such as vitamins and phytochemicals, are receiving much attention for their function as chemopreventive agents against oxidative damage (Hwang and Yen, 2008).

The importance of antioxidants in preventing diseases and maintenance of health has raised interest among scientists, food producers/manufacturers, and consumers, as the trend of the future is towards functional foods (Robards et al., 1999; Velioglu et al., 1998). Many authors (Al-Saikhan et al., 1995; Hale, 2004; Kanatt et al., 2005; Kawakami et al., 2000) have reported presence of antioxidant compounds in potatoes.

In order to further improve the nutritional value of the potato, plant breeders need to develop cultivars with substantial amounts of antioxidants. Kolasa (1993) proposed analyzing the nutrient value of potatoes not commonly grown in the U.S. to determine if there are significant quantities of antioxidants, like vitamin E and beta-carotene, or other cancer preventing phytochemicals, such as butyric acid, and suggested enhancing the

contribution of potato to human nutrition through education, marketing, breeding, field management, and preparation for consumption. Also, related wild species with desirable nutritional benefits can be used as parental material in developing improved varieties with enhanced health benefits. Several wild species were analyzed for antioxidant activity and total phenolic content by the Potato Improvement Program at Texas A&M University, and many were reported to possess more antioxidant activity and phenolic content than currently grown cultivars. Some of the species identified as containing high antioxidant activity were *Solanum jamesii*, *S. pinnatisectum*, *S. megistacrolobum*, and *S. microdontum* (Hale, 2004; Nzaramba et al., 2007). However, in the above studies, only a few accessions of each species from a "mini-core" collection were analyzed. The mini-core collection was assembled by Dr. John Bamberg, Curator, US Potato Genebank to represent the gene bank's diversity as well as facilitate pioneering research and preliminary evaluation of the germplasm for various traits, given the large number of populations and species held in the gene bank.

Having identified some species as containing more antioxidant activity than cultivated varieties, it was decided to screen all populations of these species to identify specific accessions that are the highest in antioxidant activity and phenolic content. However, many wild potato species are reported to contain high levels (>20 mg/100g) of glycoalkaloids which are toxic to humans.

Therefore, the objective of this study was to screen all accessions of *S. jamesii* and *S. microdontum* species in the US Potato GeneBank for antioxidant activity, total phenolic content, and total glycoalkaloid levels. Also, linear correlations among

glycoalkaloid (TGA) levels, antioxidant activity, and total phenolic content were investigated. *Solanum jamesii* and *S. microdontum* species were selected for this study because they are known to be efficient in tuber calcium accumulation. Also, *S. microdontum* is easily crossable to varieties of tetraploid *S. tuberosum*, while *S. jamesii* is native to southern US and northern Mexico therefore would easily be adaptable to the North American climate. The information obtained from this study would be helpful in selecting accessions to use in introgressing desirable traits into cultivated potato varieties, while avoiding introducing or increasing levels of undesirable compounds such as glycoalkaloids.

Materials and Methods

Plant Material

Ninety-two accessions of *S. jamesii* and 86 accessions of *S. microdontum* were obtained from the US Potato Genebank, Sturgeon Bay, WI. Tubers analyzed were obtained from seedlings transplanted into 10 cm - 400ml pots filled with commercial soilless potting medium in the greenhouse. The seedlings were watered as needed with 1 g/L 20-20-20 fertilizer with micronutrients. Supplementary lighting was provided with 400 W alternating sodium and metal halide lamps 2.5 m apart and 1.5 m above the benchtops for 16 h days. Temperatures were maintained at 22^{o} C day and 13^{o} C night until harvest. Triplicates of five grams of fresh tubers from each accession were washed with water, diced and placed in 50 ml falcon tubes. Samples were store at -20^{o} C until extraction of phytochemicals.

Chemicals

DPPH (2,2-Diphenyl-1-picrylhydrazyl), Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), potassium persulfate, dibasic sodium phosphate, monobasic sodium phosphate, sodium chloride, Folin-Ciocalteu reagent, ammonium phosphate, and sodium carbonate were purchased from Fisher Scientific (Pittsburgh, PA). Methanol, acetonitrile, acetone, and chloroform were obtained from VWR International (Suwanee, GA). Chlorogenic acid, rutin hydrate, caffeic acid, myricetin, α-chaconine and ammonium hydroxide were purchased from Sigma-Adrich (St. Louis, MO). Alpha-solanine and tomatine were obtained from MP Biomedicals (Solon, OH).

Sample Extraction

Five grams of diced tubers were extracted with 20 ml of HPLC-grade methanol. The samples were homogenized with an IKA Utra-turrax tissuemizer for 3 min. The extract was centrifuged at 31,000 g for 20 min. with a Beckman model J2-21 refrigerated centrifuge. Two ml of supernatant was collected into microcentrifuge tubes for AOA determination and total phenolic content analysis, and 5 ml of supernatant was collected in glass vials for individual phenolic compound analysis with HPLC. Sample extracts were stored at -20^{o} C until analysis.

Antioxidant Analysis

Antioxidant activity was measured using two assays, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-Williams et al., 1995) and the 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay (Awika et al.,

2003; Miller and Rice-Evans, 1997). Intense sample color interferes with the DPPH assay estimates; therefore the ABTS assay was used to confirm the results.

DPPH assay:

Antioxidant activity in extracts was estimated using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method (Brand-Williams et al., 1995). The DPPH method is commonly used to determine antioxidant activity of pure compounds as well as natural plant extracts. $DPPH^{\cdot}$ is a stable free radical that absorbs at 515 nm, and when reduced by an antioxidant its absorbance is lost and the change in absorbance is determined spectrophotometrically (Brand-Williams et al., 1995; Fukumoto and Mazza, 2000; Mahinda and Shahidi, 2000). The method is commonly used due to its good repeatability but has little relevance to biological systems. Samples that contain anthocyanins may lead to color interference of $DPPH^{\cdot}$, resulting in underestimation of antioxidant activity (Arnao, 2000). A 150 µl aliquot was placed into a scintillation vial, 2,850 µl of DPPH methanol solution was added, and the mixture was placed on a shaker for 15 min. The mixture was transferred to UV-cuvettes and its absorbance recorded using a Shimadzu BioSpec-1601 spectrophotometer at 515 nm. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a synthetic antioxidant, was used as a standard to generate a standard curve, and antioxidant activity was expressed as micrograms of trolox equivalents per gram of tuber fresh weight (µg TE/gfw).

ABTS assay:

The ABTS assay measures the relative ability of antioxidants to scavenge the $ABTS^{\cdot+}$ radicals generated in an aqueous phase, as compared to a trolox standard which

is a water soluble vitamin E analogue. The $ABTS^{\cdot+}$ is generated by reacting a strong oxidizing agent (potassium permanganate or potassium persulfate) with ABTS salt. The reduction of the blue-green $ABTS^{\cdot+}$ radical by a hydrogen-donating antioxidant is measured by the suppression of its characteristic long-wave absorption spectrum (Miller and Rice-Evans, 1997). It is a rapid method and can be used over a wide range of pH values (Arnao et al., 1999; Lemanska et al., 2001) in both aqueous and organic solvent systems. The assay has good repeatability and is simple to perform. Radical scavenging capacities of potato methanolic extracts were measured against the ABTS radical (Awika et al., 2003). Seventy-five µl of methanol was added to 25 µl of potato extract to make 100 µl of diluted sample extract. Two-thousand-nine hundred µl of the working solution was added to the diluted sample extract and reacted for 30 min. on a shaker. The working solution was composed of a mixture of 5 ml of mother solution [mixture of equal volumes of 8 mM of ABTS and 3 mM of potassium persulfate solutions] and 145 ml of phosphate buffer solution pH 7.4 [40.5 ml of 0.2 M Na_2HPO_4 dibasic, 9.5 ml of 0.2 M $NaHPO_4$ monobasic and 150 mM NaCl]. Absorbance of the solution was measured at 734 nm with a Shimadzu BioSpec-1601 spectrophotometer. Trolox, a synthetic antioxidant, was used as a standard, and total antioxidant activity was expressed as micrograms of trolox equivalents per gram of potato tuber fresh weight (µg TE/gfw).

Total Phenolic Analysis

Phenolic content was determined following the method of Singleton et al. (1999). One-hundred-fifty µl of tuber extract were pipetted into scintillation vials, and 2.4 ml of

nanopure water was added. One-hundred-fifty µl of 0.25 N Folin-Ciocalteu reagent was added, and after 3 min of reaction 0.3 ml of 1N Na_2CO_3 reagent was added and allowed to react for 2 hours. The spectrophotometer (Shimadzu BioSpec-1601) was zeroed with a blank (0.150 ml methanol, 2.4 ml H_2O, 150 µl of 0.25 N Folin-Ciocalteu, and 0.3 ml 1 N Na_2CO_2) before sample analysis. Absorbance of tuber extracts was read at 725 nm. Chlorogenic acid was used as a standard, and total phenolic content was expressed as milligrams of chlorogenic acid equivalents per 100 grams of potato tuber fresh weight (mg CGA/100gfw).

Phenolic Composition

Five ml of tuber extract was concentrated before analysis by drying the extract with a SpeedVac concentrator, and re-dissolved in 1 ml of aqueous methanol (50:50 v/v). The concentrated extracts were filtered through 0.45 µm syringe filters and injected into the HPLC system. The HPLC system consisted of a binary pump system (Waters 515), an auto-injector (Waters 717 plus), a photodiode array (PDA) detector (Waters 996), and a column heater (SpectraPhysics SP8792). An Atlantis C-18 reverse-phase column (4.6 x 150 mm, 5 µm) (Waters, Milford, MA.) maintained at 40^0 C was used to separate phenolic acids in the sample extracts. Twenty µl of extract was injected into the system, and the mobile phase used consisted of two solvents: solvent A acetonitrile and B nano-pure water adjusted to pH 2.3 with acetic acid. Solvent flow rate was set at 1 ml/min with a gradient of 0/85, 6-35/85-0, 36-45/85 (min/%A). Pure phenolic compounds - chlorogenic acid, rutin hydrate, caffeic acid and myricetin, previously reported in potato tubers were used as standards to identify and quantify some of the

phenolic compounds present in the sample extracts. Identification of phenolic acids present in the potato extracts was done by comparing retention time and spectra of peaks detected at 280 nm in the extracts with retention times and spectra of peaks of the standard compounds. Quantification of individual phenolic acids in the sample extract was done by comparing peak area of a known concentration of standards, and results were expressed as µg/g of tuber fresh weight (µg/gfw).

Total Glycoalkaloid Extraction

Extraction of glycoalkaloids followed the method of (Rodriguez-Saona et al., 1999). Five g of fresh tubers was homogenized with 10 ml of acetone to a uniform consistency. The extract was centrifuged at 13,000 g for 15 min., and the clear supernatant collected into a falcon tube. The residue was re-extracted with 10 ml of aqueous acetone (acetone:water 30:70 v/v). The extract was centrifuged and the supernatant combined with the first extract. Chloroform was added to the acetone extract (2 volumes of chloroform for each volume of acetone extract), thoroughly mixed by shaking the tubes and stored overnight at 1^{o} C. The top aqueous portion was collected into glass vials and concentrated in a rotovapor SpeedVac at 40^{o} C until all residue acetone was evaporated. The extract was brought to a known volume with nano-pure water and analyzed for glycoalkaloids.

Glycoalkaloid Analysis

The sample extracts and solvents were filtered through 0.45 µm filters. Glycoalkaloid analysis with a high performance liquid chromatography (HPLC) system followed the method of Sotelo and Serrano (2000) with some modifications. A Waters

HPLC with an Atlantis C-18 reverse-phase column (4.6 x 150 mm, 5 µm) from Waters (Milford, MA.) maintained at 35^0 C was used. The mobile phase used for glycoalkaloid elution was (35:65 v/v) acetonitrile : 0.05 M monobasic ammonium phosphate buffer ($(NH_4)H_2PO_4$), adjusted with NH_4OH to pH 6.5. The solvent flow was isocratic at a rate of 1 ml/min and the UV absorbance detector set at 200 nm with 5% AUFS sensitivity. Amount of sample extract injected was 20 µl. Different concentrations of commercially obtained α-solanine, α-chaconine, and tomatine were injected into the HPLC system and their peaks used to identify glycoalkaloids in the tuber extracts by comparing peak retention times and spectra detected at 200 nm in the extracts with retention times and spectra of peaks of the commercial standard compounds. Also, standard curves prepared by regressing known concentrations to their corresponding peak areas were used to quantify amounts of glycoalkaloids in the extracts.

Statistical Analysis

Analysis of variance (ANOVA) was performed using SAS version 9.1 software (SAS, 2002) to determine the variability of the measured parameters in the potato accessions. Mean separation was by least squares analysis. Phenotypic correlations between traits were computed following Pearson's correlation method and principal component analysis was performed by GGEBiplot software version 5.2 (Yan, 2001).

Results

Antioxidant Activity

Antioxidant activity values determined with the DPPH and ABTS assays were widely variable in both *S. jamesii* (Table 4.1) and *S. microdontum* (Table 4.2) accessions. Antioxidant activity values measured by ABTS were greater than those measured by DPPH. This may be due to differences in the absorption maxima of the two radicals. The DPPH maximum absorption wavelength (515 nm) is in the visible region, and the interference due to sample color is much more pronounced in this region as compared to the ABTS maximum absorption wavelength (725 nm), which is not in the visible region (Kanatt et al., 2005). However, consistency in relative ranking is probably more important than consistency in absolute numerical scores.

The DPPH values in *S. jamesii* ranged from 173 (PI 592408) to 961 µg TE/gfw (PI 620875), while values from the ABTS assay ranged from 1,383 (PI 592408) to 3,513 (PI 275172) µg TE/gfw. Analysis of variance also showed significant differences (*p-value* <0.01) in AOA (DPPH and the ABTS assays) among *S. jamesii* accessions (Table 4.1). The DPPH values in *S. microdontum* ranged from 202 (PI 558097) to 1,535 (PI 498127) µg TE/gfw, while the ABTS values ranged from 1,084 (PI 558097) to 6,288 (PI 498127) µg TE/gfw (Table 4.2), and analysis of variance showed significant differences among accessions.

Total Phenolic Content

Significant differences among *S. jamesii* and *S. microdontum* accessions in total phenolic content were revealed by analysis of variance. Wide variation in TP was also

Table 4.1. Mean values of antioxidant activity (DPPH and ABTS assays), total phenolic content (TP), α-solanine (SOL), α-chaconine (CHA), total glycoalkaloids (TGA), chlorogenic acid (CGA), caffeic acid (CA), rutin hydrate (RH), myricetin (MY), and ratio of solanine to chaconine (S:C) in *S. jamesii* accessions (ACCESS).

ACCESS	DPPH	ABTS	TP	SOL	CHA	TGA	CGA	CA	RH	MY	S:C
PI 275172	784	3513	151	2.2	4.0	6.3	432	282	24	2	0.6
PI 564047	476	1502	87	4.2	5.6	9.8	197	32	12	1	0.8
PI 564048	618	2412	111	4.6	4.5	9.2	282	133	22	2	1.0
PI 564049	647	2618	128	9.3	8.3	17.5	270	132	29	3	1.1
PI 564051	626	1920	128	6.7	7.4	14.2	344	72	51	3	0.9
PI 564052	653	1939	128	6.3	7.4	13.7	344	159	53	2	0.8
PI 564053	711	2145	129	5.1	7.2	12.3	346	138	63	2	0.7
PI 564054	744	2530	140	5.8	6.5	12.3	521	311	54	3	0.9
PI 564055	572	2139	130	6.7	6.6	13.2	397	122	58	2	1.0
PI 564056	820	2279	139	7.9	6.3	14.2	409	223	30	4	1.2
PI 564057	695	2770	131	7.4	8.7	16.0	250	235	27	3	0.8
PI 578236	656	2075	121	5.6	7.5	13.1	308	132	54	5	0.7
PI 578237	636	2245	123	6.5	5.2	11.7	344	121	51	2	1.3
PI 578238	465	1769	92	7.7	6.6	14.3	207	80	28	2	1.2
PI 585116	827	2467	137	20.0	16.8	36.8	292	101	33	3	1.2
PI 585118	397	1474	97	6.2	6.8	13.0	146	123	25	4	0.9
PI 585119	627	1884	125	10.0	6.1	16.1	381	186	34	4	1.6
PI 592397	321	1987	88	12.5	8.6	21.1	178	247	15	4	1.5
PI 592398	589	2051	118	12.9	6.5	19.4	222	254	24	5	2.0
PI 592407	925	2897	145	6.7	9.1	15.8	217	180	34	4	0.7
PI 592408	173	1383	50	9.6	7.2	16.8	123	76	9	2	1.3
PI 592410	682	2449	122	12.7	7.1	19.8	239	129	23	4	1.8
PI 592411	607	2121	124	5.5	6.0	11.4	339	76	44	6	0.9
PI 592413	503	2136	113	8.0	13.6	21.5	213	156	50	5	0.6
PI 592414	289	2122	81	5.5	5.7	11.2	133	124	7	4	1.0
PI 592416	598	2074	107	4.3	6.0	10.3	280	231	41	4	0.7
PI 592417	709	2281	124	11.6	7.4	18.9	309	273	28	8	1.6
PI 592418	458	2074	96	7.0	5.8	12.9	172	231	27	6	1.2
PI 592419	608	2222	119	4.2	6.4	10.6	341	172	60	6	0.7
PI 592422	752	2515	139	5.9	5.9	11.8	393	159	72	3	1.0
PI 592423	846	2372	141	8.5	5.4	13.8	504	339	36	4	1.6
PI 595775	879	2607	161	5.0	6.4	11.3	368	136	71	15	0.8
PI 595777	719	2001	126	8.5	4.3	12.8	440	110	21	4	2.0
PI 595778	829	2497	140	13.1	7.5	20.6	403	167	48	12	1.8
PI 595780	731	2449	129	9.0	5.7	14.7	330	131	31	6	1.6

Table 4.1. Continued..

ACCESS	DPPH	ABTS	TP	SOL	CHA	TGA	CGA	CA	RH	MY	S:C
PI 595782	556	2273	113	11.9	5.8	17.6	227	148	39	9	2.1
PI 595783	644	2647	125	9.8	4.8	14.7	320	186	27	2	2.0
PI 595784	789	2641	137	11.0	5.6	16.7	503	117	40	3	2.0
PI 595785	540	2320	113	9.4	4.6	14.0	319	133	35	3	2.1
PI 595786	588	2064	114	9.1	4.6	13.7	342	63	33	2	2.0
PI 595787	793	3037	140	5.1	6.6	11.7	468	347	42	3	0.8
PI 595788	531	1927	109	6.0	7.4	13.4	226	191	43	3	0.8
PI 596519	707	2444	114	5.1	7.0	12.1	318	156	14	4	0.7
PI 603051	758	2904	129	4.9	6.5	11.4	282	209	32	5	0.8
PI 603052	672	2146	121	2.9	4.6	7.5	289	205	29	4	0.6
PI 603053	572	2401	154	5.3	10.9	16.2	440	314	65	3	0.5
PI 603054	783	2206	140	13.6	6.4	20.0	466	205	31	3	2.1
PI 603055	684	1981	119	6.3	6.3	12.6	349	73	26	2	1.0
PI 603056	599	2021	110	7.1	6.9	14.0	277	113	24	3	1.0
PI 603057	620	2276	123	7.0	6.1	13.1	322	265	31	2	1.2
PI 603058	525	1802	102	6.0	5.9	12.0	270	102	21	3	1.0
PI 605357	875	2573	144	11.5	6.2	17.8	585	140	55	3	1.9
PI 605358	703	2078	126	10.0	5.1	15.1	309	327	32	4	1.9
PI 605359	735	2172	128	8.4	6.1	14.6	353	359	33	4	1.4
PI 605360	624	1858	114	7.0	3.7	10.8	334	202	23	5	1.9
PI 605361	813	2406	132	7.2	4.5	11.7	323	242	40	7	1.6
PI 605362	783	2678	130	3.7	3.2	6.9	350	267	39	6	1.1
PI 605363	622	2502	115	6.5	3.5	10.0	266	221	25	3	1.8
PI 605364	958	2947	140	4.1	4.5	8.6	413	154	42	3	0.9
PI 605365	667	2399	108	2.8	3.4	6.1	189	142	18	4	0.8
PI 605366	703	2161	120	4.9	3.8	8.7	260	114	26	3	1.3
PI 605367	564	1922	102	2.4	3.6	5.9	223	119	24	4	0.7
PI 605368	560	1857	99	4.8	5.1	9.9	217	157	23	3	1.0
PI 605369	750	2163	124	4.2	4.4	8.7	348	278	18	4	0.9
PI 605370	714	2201	118	8.5	4.9	13.5	263	123	34	3	1.7
PI 605371	610	2965	145	7.7	12.2	19.9	297	297	62	6	0.6
PI 605372	687	2687	128	4.3	5.1	9.4	363	254	34	3	0.8
PI 612450	648	2237	112	8.9	6.2	15.0	306	213	21	5	1.4
PI 612451	685	2257	120	6.4	6.6	13.0	356	127	29	3	1.0
PI 612452	531	1807	113	5.9	6.1	12.0	242	242	35	3	1.0
PI 612453	814	1998	127	4.1	4.6	8.7	370	211	31	3	0.9
PI 612454	620	2429	126	3.1	5.0	8.2	359	218	34	5	0.6
PI 612455	662	2461	118	7.0	6.5	13.4	325	173	26	4	1.1

Table 4.1. Continued..

ACCESS	DPPH	ABTS	TP	SOL	CHA	TGA	CGA	CA	RH	MY	S:C
PI 612456	585	2312	112	4.4	4.9	9.3	238	197	15	4	0.9
PI 620869	604	2098	109	3.4	4.3	7.7	248	168	14	4	0.8
PI 620870	895	2727	141	13.3	6.6	19.9	351	221	36	7	2.0
PI 620872	832	2628	125	4.9	5.9	10.8	342	41	33	3	0.8
PI 620875	961	2786	136	5.0	6.3	11.3	347	261	27	3	0.8
PI 620876	652	2000	104	6.3	5.9	12.2	254	106	26	3	1.1
PI 620877	833	2420	124	9.4	5.6	15.0	301	354	30	5	1.7
PI 620878	631	2293	124	10.5	6.0	16.4	372	309	39	7	1.8
PI 632322	713	2555	125	7.1	6.4	13.5	311	108	42	2	1.1
PI 632323	313	2737	87	6.8	9.0	15.8	234	36	25	3	0.7
PI 632324	797	2537	126	6.7	6.7	13.4	363	91	27	3	1.0
PI 632325	416	1906	92	4.1	4.7	8.9	164	44	16	2	0.9
PI 632326	659	2088	115	5.1	6.1	11.2	289	82	20	3	0.8
PI 632329	738	3034	124	3.2	4.9	8.0	205	83	21	4	0.7
PI 632331	627	2534	113	5.8	6.6	12.3	163	48	13	5	0.9
PI 634361	752	2017	112	8.7	9.8	18.5	308	187	27	2	0.9
PI 634362	787	2684	121	6.3	7.5	13.8	372	55	29	2	0.8
PI 634363	847	2843	137	7.7	7.7	15.5	457	272	41	4	1.0
PI 634364	680	2679	118	5.7	7.0	12.7	296	268	25	6	0.8
Mean	**665**	**2311**	**121**	**7.1**	**6.3**	**13.4**	**313**	**175**	**33**	**4**	
LSD	**53**	**212**	**3**	**1.2**	**0.9**	**1.6**	**37**	**29**	**5**	**1**	

observed in accessions of both species. Total phenolic content in *S. jamesii* accessions ranged from 50 (PI 592408) to 161 (PI 595775) mg CGA/100gfw. The range of TP values in *S. microdontum* was from 51 (PI 558097) to 269 (PI 498127) mg CGA/100gfw. Generally, values from *S. microdontum* were higher than those of *S. jamesii* accessions (Tables 4.1 and 4.2).

Phenolic Composition

Identification of individual phenolics present in the sample extract was accomplished by comparing retention times and spectra of peaks in tuber sample extracts detected at 280 nm with retention times and peaks' spectra of the standard compounds. Quantification was done by comparing peak area of a known concentration of standard with peak areas of the sample extract. Four compounds (chlorogenic acid, caffeic acid, rutin hydrate, and myricetin) were observed in all accessions of *S. jamesii* and *S. microdontum* (Tables 4.1 and 4.2). The most abundant phenolic compounds in all accessions were chlorogenic and caffeic acids.

Relative amounts of these acids varied from one accession to another, with some accessions containing more chlorogenic than caffeic acid, and others containing more caffeic than chlorogenic acid. Estimates of chlorogenic acid in *S. jamesii* ranged from 123 (PI 592408) to 585 (PI 605357) µg/gfw, caffeic acid values ranged from 32 (PI 564047) to 359 (PI 605359) µg/gfw, rutin hydrate from 7 (592414) to 72 (PI 592422) µg/gfw, and myricetin ranged from 1 (PI 564047) to 15 (PI 595775) µg/gfw. Similarly, amounts of these phenolic compounds were widely variable in *S. microdontum*. Chlorogenic acid values ranged from 21 (PI 473362) to 147 (PI 545902) µg/gfw and

Table 4.2. Mean values of antioxidant activity (DPPH and ABTS assays), total phenolic content (TP), α-solanine (SOL), α-chaconine (CHA), dehydrotomatine (DTO), tomatine (TOM), total glycoalkaloids (TGA), chlorogenic acid (CGA), caffeic acid (CA), rutin hydrate (RH), myricetin (MY), and ratio of solanine to chaconine (S:C) in *S. microdontum* accessions (ACCESS).

ACCESS	DPPH	ABTS	TP	SOL	CHA	DTO	TOM	TGA	CGA	CA	RH	MY	S:C
PI 195185	543	2340	117	65.5	52.1	3.9	2.7	121.9	115	96	2.7	6.3	1.3
PI 208866	738	2536	133	43.3	38.0			81.2	74	164	3.8	11.3	1.1
PI 218222	968	3145	155	72.0	77.6	12.0	4.3	155.0	79	136	3.2	6.6	0.9
PI 218223	798	2944	134	73.9	71.3	6.2	3.3	148.3	76	108	2.3	5.2	1.0
PI 218226	999	3174	151	57.5	57.6	1.2	3.9	118.5	62	109	2.8	6.5	1.0
PI 265575	683	2792	120	66.4	58.9	15.3	4.8	138.7	52	124	3.1	4.8	1.1
PI 265881	980	3289	157	91.6	63.4			155.1	104	85	5.2	3.3	1.4
PI 275150	357	2078	86	52.3	41.9	9.0	5.4	105.1	69	77	6.2	2.7	1.2
PI 310979	884	3028	150	109.8	114.3	24.7	19.4	268.0	51	140	6.9	3.9	1.0
PI 320304	572	2181	106	38.2	44.3	1.4	3.6	84.1	69	35	1.1	3.0	0.9
PI 320305	255	1225	60	37.8	41.1	0.8	0.7	79.4	52	57	1.3	2.4	0.9
PI 320306	1004	3809	151	81.5	82.1	69.9	27.4	260.8	79	70	2.3	4.5	1.0
PI 320307	408	1988	91	99.8	85.1	15.6	3.2	203.8	60	76	1.7	2.6	1.2
PI 320309	735	2859	132	74.8	71.3			146.1	72	47	2.3	4.4	1.0
PI 320310	569	2290	114	52.2	44.2	5.6		98.2	38	100	2.1	4.4	1.2
PI 320311	982	3001	150	100.8	114.0	27.5	13.7	256.0	47	138	1.2	2.3	0.9
PI 320312	985	2850	160	90.1	91.6	6.6	3.2	191.6	53	51	2.3	3.2	1.0
PI 320313	422	1463	81	34.1	39.9	0.2	0.4	74.1	46	65	2.4	4.5	0.9
PI 320315	545	2345	110	81.6	75.8	2.9	4.5	160.8	58	99	1.1	4.0	1.1
PI 320316	384	1819	86	52.8	50.7	7.0	4.7	109.8	29	29	1.3	2.6	1.0
PI 320319	1201	3789	197	79.7	61.6	5.9	2.8	146.2	122	176	6.9	4.7	1.3
PI 320320	268	1872	70	80.2	54.4	2.9		136.5	54	57	2.8	3.7	1.5
PI 458353	738	2524	126	57.4	84.3	32.5	50.5	224.6	28	192	2.3	5.9	0.7
PI 458354	1047	3698	173	104.1	98.6	4.4	1.6	206.7	119	147	5.0	6.6	1.1
PI 458355	1499	4099	237	93.1	84.6	16.8	7.7	185.9	111	70	1.4	12.0	1.1
PI 458356	1419	3622	219	97.9	61.6	4.0		162.2	71	127	4.0	7.0	1.6
PI 458357	639	2206	119	83.8	55.1		20.6	145.8	97	49	2.1	4.2	1.5
PI 458358	257	2009	77	24.3	14.5	46.9	19.0	104.8	49	50	2.8	3.6	1.7
PI 473166	866	2367	139	49.9	67.4	11.9	5.4	134.5	75	41	3.0	6.9	0.7
PI 473167	939	2863	150	122.7	98.3	28.5	6.1	232.6	93	96	1.6	5.3	1.2
PI 473168	309	1741	73	11.5	8.8	83.2	9.8	113.4	79	65	3.6	3.9	1.3
PI 473169	682	2341	118	77.0	63.9	6.3	1.6	143.5	81	49	2.3	6.5	1.2
PI 473170	329	1874	81	33.6	32.0			65.6	48	30	2.8	3.2	1.0
PI 473171	498	2038	94	8.0	5.2			13.2	67	50	2.3	3.7	1.5

Table 4.2. Continued…

ACCESS	DPPH	ABTS	TP	SOL	CHA	DTO	TOM	TGA	CGA	CA	RH	MY	S:C
PI 473172	505	2227	100	60.0	72.1			132.2	75	57	2.7	5.3	0.8
PI 473173	383	2037	98	52.4	54.0			106.4	58	95	2.3	3.9	1.0
PI 473174	643	2540	128	64.8	66.2			131.0	68	104	1.7	8.3	1.0
PI 473175	425	2152	91	84.1	92.8	9.3	4.3	186.0	78	61	2.2	5.4	0.9
PI 473176	759	2365	123	52.2	53.8			106.0	64	70	1.9	3.5	1.0
PI 473177	465	2436	104	92.3	86.3		7.5	181.0	84	69	2.9	3.9	1.1
PI 473178	987	2821	154	82.4	108.1	3.7	2.4	193.3	62	106	1.8	3.2	0.8
PI 473179	637	2420	122	131.5	144.2		13.4	280.1	131	76	3.8	10.5	0.9
PI 473180	993	2997	170	72.3	66.3	0.2	2.0	139.3	79	106	5.2	7.9	1.1
PI 473362	984	4843	181	151.0	148.0		67.7	321.6	21	65	3.2	7.8	1.0
PI 473363	505	1995	107	140.7	139.4		42.5	294.2	35	60	2.6	6.5	1.0
PI 473525	291	1567	68	108.3	105.6			214.0	47	57	1.6	3.2	1.0
PI 498121	978	3478	173	121.7	109.6	36.8	12.0	280.0	69	141	3.1	6.5	1.1
PI 498123	1171	4236	208	120.8	125.1	206.0	110.2	562.2	36	29	2.1	8.2	1.0
PI 498124	1398	3175	223	190.0	134.3	27.4	8.8	360.5	72	106	2.6	8.1	1.4
PI 498125	1005	4767	175	130.6	115.6	16.6	7.5	264.8	79	76	7.2	13.2	1.1
PI 498126	995	4738	176	115.1	89.3	1.3	1.0	205.6	100	131	19.8	12.8	1.3
PI 498127	1535	6288	269	176.2	191.0		1.6	367.8	91	127	4.3	5.8	0.9
PI 498128	995	3773	170	85.9	72.4		12.7	162.6	79	130	9.5	12.0	1.2
PI 500032	426	2171	84	8.2	7.9	33.9	13.7	63.8	69	56	6.8	7.5	1.0
PI 500033	602	2599	108	62.3	61.5	15.7	5.1	137.7	80	57	4.1	9.0	1.0
PI 500034	523	2008	101	39.0	34.7			73.7	87	97	2.8	7.6	1.1
PI 500035	773	2324	123	43.1	32.1	454.5	196.8	726.6	78	53	1.4	8.5	1.3
PI 500036	321	1783	77	38.5	36.9	1.2	5.1	81.6	53	35	1.4	3.4	1.0
PI 500037	545	2155	102	51.3	45.5			96.7	81	125	1.4	9.3	1.1
PI 500038	379	1845	83	4.8	5.5	136.6	62.4	209.4	77	70	2.4	7.3	0.9
PI 500039	358	1972	84	18.3	18.8	402.3	334.4	773.7	88	63	3.4	7.8	1.0
PI 500040	478	2207	94	15.8	17.7	19.9	14.9	68.2	53	39	1.5	5.9	0.9
PI 500041	770	3026	130	9.3	6.5			15.9	89	51	5.9	10.2	1.4
PI 500044	371	2430	83	27.8	35.7	40.5	18.2	96.5	55	37	2.1	9.1	0.8
PI 500064	413	1472	82	92.6	87.1		5.7	181.5	73	28	1.0	9.9	1.1
PI 545901	763	2919	130	64.6	62.0	8.0	4.5	133.4	121	109	7.2	6.7	1.0
PI 545902	995	4639	176	109.4	95.4	69.6	16.9	291.2	147	153	7.7	11.7	1.1
PI 545904	803	2013	133	109.9	129.5		10.9	243.0	35	32	1.4	6.6	0.8
PI 545905	977	2967	161	115.6	108.0	53.2	21.3	298.0	62	120	4.3	7.6	1.1
PI 558097	202	1084	51	67.6	57.9	16.0	7.9	133.5	59	45	1.1	5.2	1.2
PI 558098	263	1501	62	46.2	39.9			86.1	101	43	1.2	5.2	1.2
PI 558099	1000	3712	172	69.3	71.0	5.1	4.2	143.4	63	32	1.9	10.0	1.0

Table 4.2. Continued…

ACCESS	DPPH	ABTS	TP	SOL	CHA	DTO	TOM	TGA	CGA	CA	RH	MY	S:C
PI 558100	770	2969	144	133.0	104.5	9.3	15.1	250.7	97	73	2.5	5.7	1.3
PI 558101	566	2322	109	33.8	26.1			59.9	82	78	1.2	5.5	1.3
PI 558218	432	2710	101	22.4	24.4			46.8	75	68	4.0	8.4	0.9
PI 565075	331	1696	69	8.3	6.8	304.8	228.1	548.1	57	82	3.3	3.4	1.2
PI 595505	1003	3477	171	89.1	63.9	43.7	18.4	215.0	26	89	2.2	4.2	1.4
PI 595506	722	2337	139	105.3	98.7		10.6	207.5	49	41	1.0	4.8	1.1
PI 595508	416	1843	93	90.9	72.2	13.7	9.4	186.1	88	76	2.0	14.1	1.3
PI 595509	319	1255	72	44.4	34.9			79.3	91	29	1.8	8.1	1.3
PI 595510	445	1684	98	115.4	126.0	99.5	41.8	382.6	55	30	1.9	12.6	0.9
PI 595511	941	2789	157	100.2	95.2	447.9	164.1	807.3	29	45	2.6	5.2	1.1
PI 597756	829	2290	143	135.9	131.3	33.5	10.6	311.4	42	35	1.5	6.4	1.0
PI 597757	652	2187	135	200.3	180.9		19.7	387.7	70	63	1.9	11.0	1.1
PI 631211	862	2641	137	45.9	38.4			84.2	59	55	2.2	3.8	1.2
Mean	**699**	**2636**	**127**	**75.9**	**71.0**	**52.0**	**27.1**	**198.6**	**71**	**79**	**3.1**	**6.4**	
LSD	**22**	**194**	**4**	**9.4**	**8.1**			**19.5**	**14**	**16**	**0.8**	**1.2**	

caffeic acid ranged from 28 (PI 500064) to 192 (PI 458353) µg/gfw. Rutin hydrate ranged from 1 (PI 500064) to 20 (PI 498126) µg/gfw, and myricetin ranged from 2 (PI 320311) to 14 (PI 595508) µg/gfw. The analysis of variance for these compounds showed significant differences (*p-value* <0.01) among accessions of both *S. jamesii* (Table 4.1) and *S. microdontum* (Table 4.2).

Glycoalkaloid Composition

The main glycoalkaloids in potato tubers are α-solanine and α-chaconine, and they comprise more than 95 % of all glycoalkaloids in the potato plant. Pure compounds of α-solanine, α-chaconine, and tomatine were used as standards in HPLC analysis of glycoalkaloids present in the tuber extracts. Figure 4.1 shows examples of typical chromatographs obtained from HPLC glycoalkaloid analysis of *S. jamesii* accession PI 593408 and *S. microdontum* PI 498123, respectively. High amounts of α-solanine and α-chaconine were found in all *S. jamesii* and *S. microdontum* accessions. Tomatine and dehydrotomatine were identified and quantified in several *S. microdontum* accessions but not in *S. jamesii* (Table 4.2). Total glycoalkaloid values for *S. microdontum* accessions reported in Table 4.2 are sums of all glycoalkaloids quantified, including tomatine and dehydrotomatine for the accessions that exhibited it.

Generally, the amount of glycoalkaloids in *S. microdontum* was higher than the levels in *S. jamesii* accessions. The amount of α-solanine was significantly different (p-value <0.01) in *S. jamesii*, with values ranging from 2.3 (PI 275172) to 20 (PI 585116) mg/100g fresh weight. Alpha-solanine in *S. microdontum* ranged from 4.8 (PI 500038) to 200.3 (PI 597757) mg/100gfw. Also, α-chaconine was appreciably variable in both

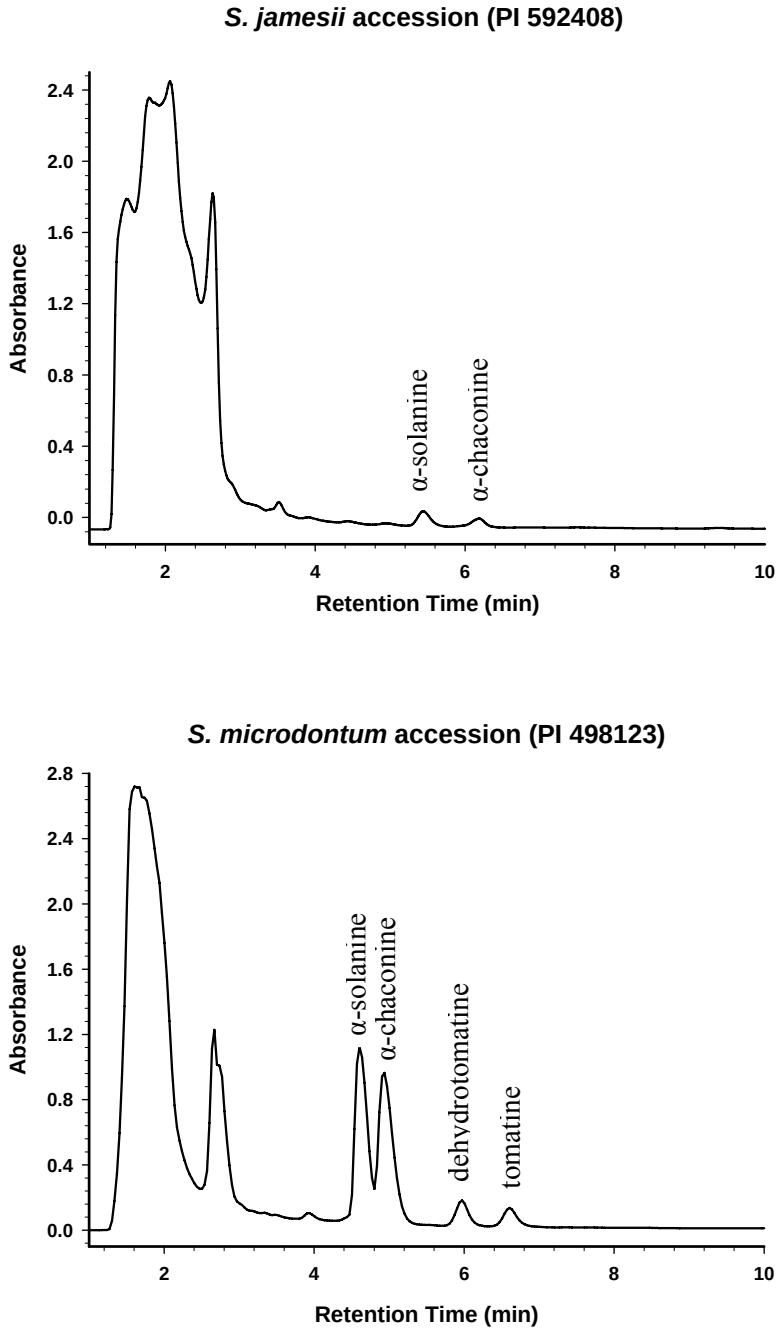


Fig. 4.1. Typical chromatographs from HPLC analysis of glycoalkaloids in *S. jamesii* and *S. microdontum* tuber extracts.

species, ranging from 3.2 (PI 605362) to 16.8 (PI 585116) mg/100gfw and from 5.2 (PI 473171) to 191 (PI 498127) mg/100gfw in *S. jamesii* and *S. microdontum*, respectively. Ratios between α-solanine and α-chaconine varied among accessions, with values ranging from 0.5 to 2.1 in *S. jamesii* (Table 4.1) and from 0.7 to 1.7 in *S. microdontum* (Table 4.2). Several accessions of *S. microdontum* exhibited high amounts of tomatine and dehydrotomatine. Some accessions such as PIs 500039, 565075, 500035, 595511, 498123, 500038, 500032, and 473168 exhibited more dehydrotomatine and tomatine than α-solanine and α-chaconine (Table 4.2).

The reported safety level of potato tuber total glycoalkaloids (TGA) for human consumption is 20 mg/100gfw (Friedman et al., 2003). Therefore, any variety to be released must contain less than 20 mg/100gfw of total glycoalkaloids.

To avoid high levels of glycoalkaloids in progenies, accessions to be used in any breeding programs should contain less than 20 mg/10gfw TGA. Results from this study show that only eight of the 92 *S. jamesii* accessions screened (PI 585116, PI 592413, PI 592397, PI 595778, PI 605371, PI 620870, PI 592410, and PI 603054) contain total glycoalkaloid levels close to or greater than the safety limit (20 mg/100gfw). Only two accessions (PI 473171 and PI 500041) of *S. microdontum* exhibited total glycoalkaloid levels less than 20 mg/100gfw. Therefore, most *S. jamesii* accessions and the two accessions of *S. microdontum* can potentially be used in breeding for traits of interest without increasing amounts of glycoalkaloids in the progenies, since they contain low levels.

Comparison of Wild Accessions with Common Cultivars

Antioxidant activity, total phenolics and glycoalkaloids in *S. jamesii* and *S. microdontum* accessions and in three cultivars, Atlantic, Yukon Gold, and Red La Soda were compared (Table 4.3). This was done to determine whether the two wild species contain some accessions that are lower in total glycoalkaloids and higher in antioxidant activity than popular commercial cultivars. Such accessions would be potential candidates as parental material for breeding for high antioxidant activity and phenolic compounds in new cultivars. Results in Table 4.3 show that the common cultivars are at the lower end distribution of the traits of interest in wild species. This implies that most accessions of both species exhibit higher levels of these traits than the cultivars. Therefore, those accessions with higher values of the desirable traits can be used as parents in breeding of new cultivars.

Relationships among Antioxidant Activity, Phenolics, and Glycoalkaloid Content

Accessions screened contained high levels of both desirable (antioxidants and phenolics) and undesirable (glycoalkaloids) compounds. Hence, there may be a risk of increasing glycoalkaloids in progenies if these wild species are used as parental material for breeding. This is of much concern in instances where there are linear positive relationships between antioxidant activity and total glycoalkaoids, and between phenolics and glycoalkaloids, implying that increasing antioxidant and/or phenolics might result in increased levels of glycoalkaloids.

Table 4.3. Range of antioxidant activity (AOA), total phenolic content (TP), α-solanine (SOL), α-chaconine (CHA), and total gylcoalkaloids (TGA) in *S. jamesii* and *S. microdontum*, and means of three commercial cultivars, Atlantic, Red La Soda, and Yukon Gold.

	Wild species		Cultivars		
	S. jamesii	*S. microdontum*	Atlantic	Red La Soda	Yukon Gold
AOA (DPPH)	173 - 961	202 - 1535	48	96	133
AOA (ABTS)	1383 - 3513	1084 - 6288	819	1405	1674
TP	50 - 161	51 - 269	27	39	52
SOL	2.2 – 20.0	4.8 – 200.3	3.1	2.3	1.3
CHA	3.2 – 16.8	5.2 – 191.0	7.8	6.4	4.6
TGA	5.9 – 36.8	13.2 – 807.3	10.9	8.7	5.9

Principal component analysis (PCA) was used to investigate relationships among antioxidants, phenolics, and glycoalkaloids (Yan and Hunt, 2001). PCA is a technique used to reduce multidimensional data sets to lower dimensions for analysis. It is mostly used as a tool in exploratory data analysis and for making predictive models. Results from PCA are shown as plots of primary principal component PC1 versus PC2 (Figs 4.2 and 4.3). On the plots, accessions are in blue and lower case, traits are in red and upper case, and the two principal components explained at least 60% of the variations in traits in both *S. jamesii* accessions (Fig. 4.2) and *S. microdontum* accessions (Fig. 4.3). These plots show relationships among traits and the performance of each accession. Relationships among traits are indicated by the angle between trait vectors. These angles show the extent of the correlations among traits, acute angles indicate positive correlation, obtuse angles indicate negative correlation, and a right angle means that there is no correlation between the traits of interest. Strongly correlated traits are normally grouped together in the biplot.

Figures 4.2 and 4.3 show that the traits are grouped in two; one group consists of glycoalkaloids and the other contains phenolic compounds and antioxidant activity. The observation that glycoalkaloids are grouped separately from antioxidant activity and phenolics suggests that there is no linear relationship between glycoalkaloids and antioxidant activity, or between glycoalkaloids and phenolic content.

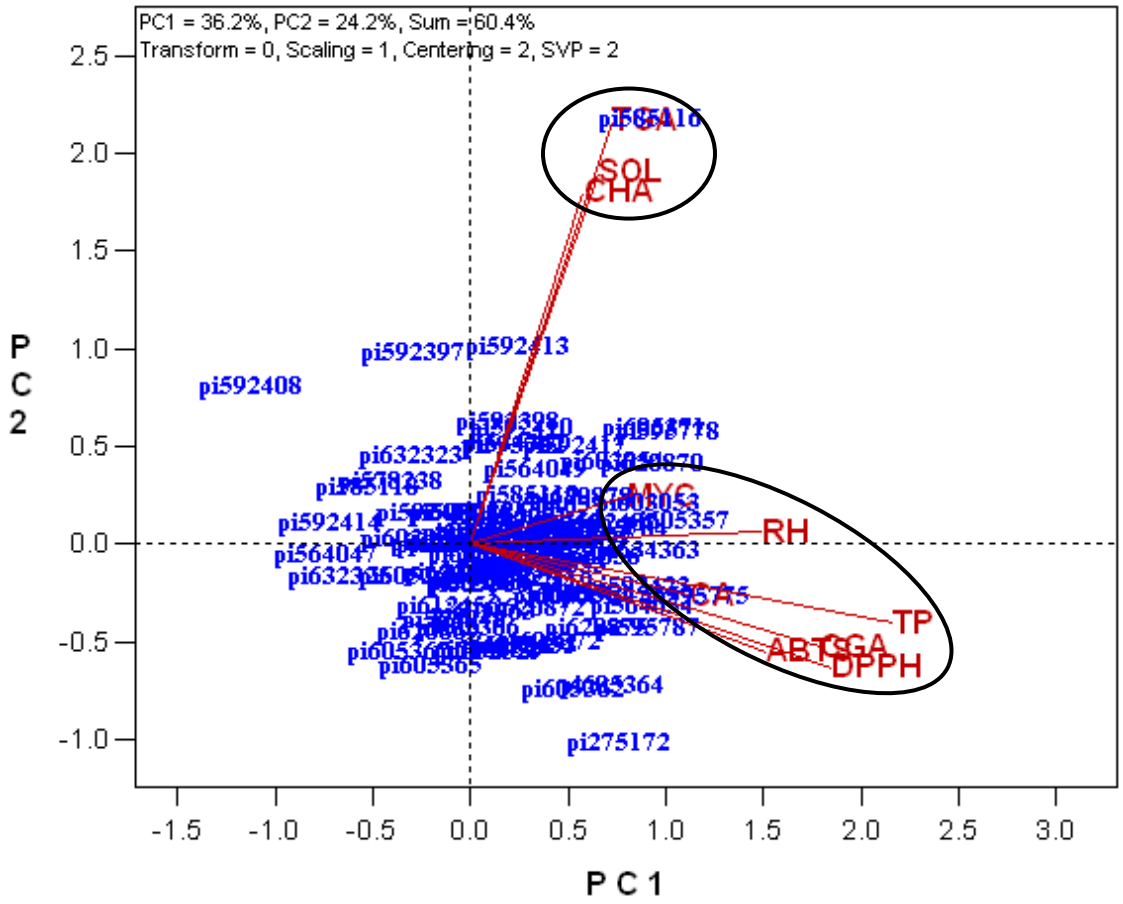


Fig. 4.2. A biplot of principal component 1 (PC1) vs. principal component 2 (PC2) demonstrating interrelationships among traits; antioxidant activity (ABTS & DPPH assays), total phenolic content (TP), chlorogenic acid (CGA), caffeic acid (CA), rutin hydrate (RH), myricetin (MYC), α-solanine (SOL), α-chaconine (CHA), and total glycoalkaloids (TGA) in *S. jamesii* accessions. Traits are in red and upper case while accessions are in blue and lower case.

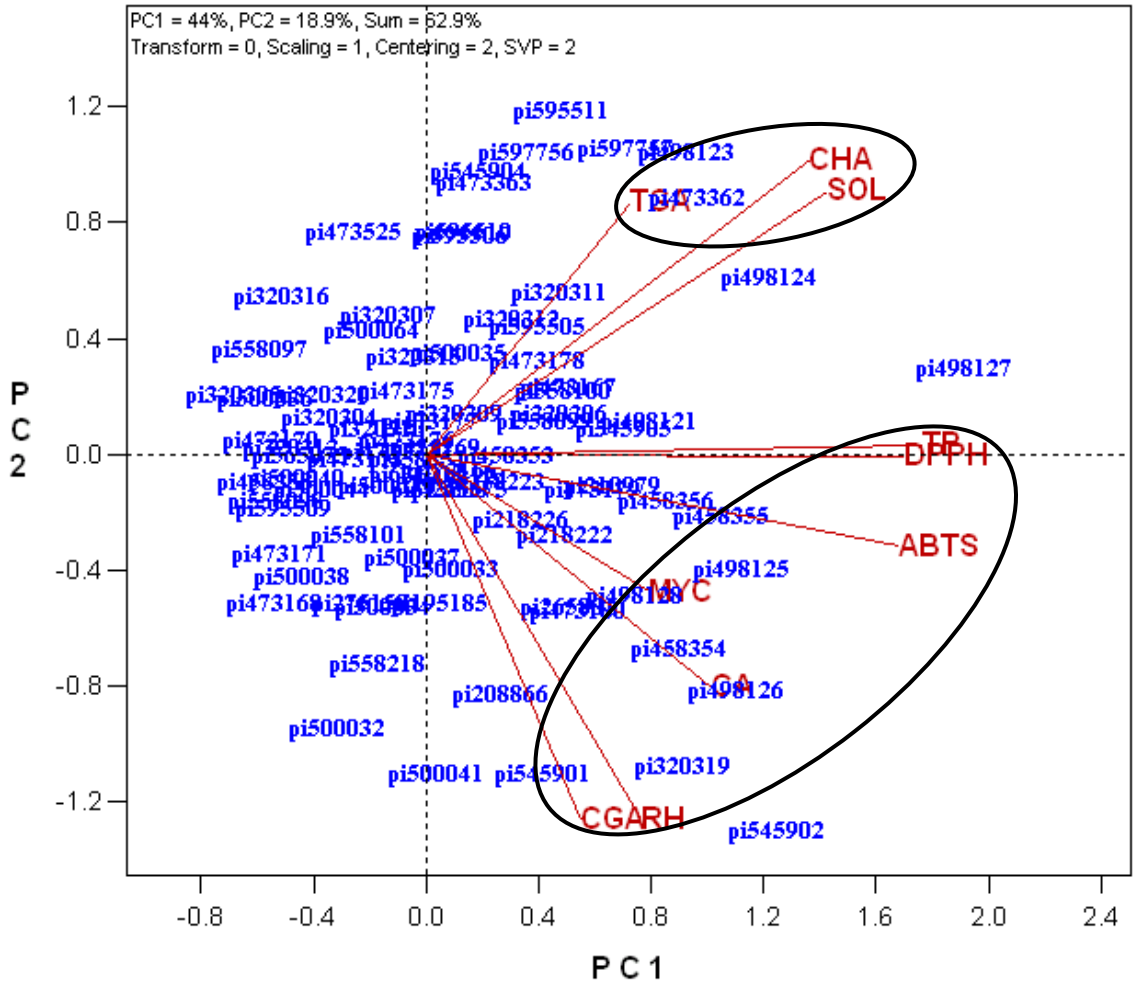


Fig. 4.3. A biplot of principal component 1 (PC1) vs. principal component 2 (PC2) demonstrating interrelationships among traits; antioxidant activity (ABTS & DPPH assays), total phenolic content (TP), chlorogenic acid (CGA), caffeic acid (CA), rutin hydrate (RH), myricetin (MYC), α-solanine (SOL), α-chaconine (CHA), and total glycoalkaloids (TGA) in *S. microdontum* accessions. Traits are in red and upper case while accessions are in blue and lower case.

The significance of the relationships observed from PCA results were confirmed by correlation analysis shown in Table 4.4. Results show that there were significant correlations (*p-value* <0.01) between antioxidant activity (DPPH) and total phenolics, with correlation coefficients (r) = 0.83 and 0.98 in *S. jamesii* and *S. microdontum*, respectively. Also, correlations between antioxidant activity (ABTS) and total phenolics were highly significant with values of r = 0.64 and 0.89 in *S. jamesii* and *S. microdontum*, respectively. Similar results showing significant correlation between antioxidant activity and total phenolic content were previously reported in potatoes (Reddivari et al., 2007a; Reyes et al., 2005) and sweet potatoes (Huang et al., 2004). Glycoalkaloids were not significantly correlated to antioxidant activity or total phenolic content in *S. jamesii*. However, in *S. microdontum*, α-solanine and α-chaconine were significantly correlated with antioxidant activity and total phenolic content, but there was no significant correlation between total glycoalkaloids and antioxidant activity, or between total glycoalkaloids and total phenolic content (r = 0.27). Individual phenolic compounds analyzed with HPLC showed no significant correlation with glycoalkaloids in either *S. jamesii* or *S. microdontum* accessions (Table 4.5).

Results from this study indicate that antioxidant activity and total phenolic content are not correlated with total glycoalkaloids. Also, there was no significant correlation between individual phenolic compounds and glycoalkaloids. Therefore, using wild accessions in breeding for high antioxidant activity and total phenolics would not necessarily increase glycoalkaloids in the developed potato progenies.

Table 4.4. Correlation analysis of antioxidant activity (AOA), total phenolic content (TP), α-solanine (SOL), α-chaconine (CHA), and total glycoalkaloids (TGA) in *S. jamesii* and *S. microdontum* accessions.

S. jamesii					
	AOA (ABTS)	TP	SOL	CHA	TGA
AOA (DPPH)	0.599**	0.832**	0.069	-0.047	0.026
AOA (ABTS)		0.642**	-0.048	0.086	0.007
TP			0.105	0.124	0.131
SOL				0.462**	0.909**
CHA					0.789**
S. microdontum					
	AOA (ABTS)	TP	SOL	CHA	TGA
AOA (DPPH)	0.847**	0.982**	0.553**	0.517**	0.260
AOA (ABTS)		0.888**	0.491**	0.469**	0.212
TP			0.610**	0.561**	0.279
SOL				0.951**	0.396**
CHA					0.404**

** refers to significant values at *p-value* < 0.01

Table 4.5. Correlation analysis of individual phenolic compounds [chlorogenic acid (CGA), caffeic acid (CA), rutin hydrate (RH) and myricetin (MYC)], total phenolic content (TP), antioxidant activity (DPPH and ABTS), individual glycoalkaloids [α-solanine (SOL) and α-chaconine (CHA)], and total glycoalkaloids (TGA) in *S. jamesii* and *S. microdontum* accessions.

S. jamesii									
	CA	RH	MYC	DPPH	ABTS	TP	SOL	CHA	TGA
CGA	0.328**	0.526**	0.016	0.652**	0.391**	0.749**	0.115	-0.057	0.055
CA		0.177	0.239*	0.283**	0.275**	0.401**	0.069	-0.006	0.046
RH			0.285**	0.315**	0.213*	0.612**	0.043	0.274*	0.158
MYC				0.187	0.138	0.269**	0.184	0.032	0.139
S. microdontum									
	CA	RH	MYC	DPPH	ABTS	TP	SOL	CHA	TGA
CGA	0.288**	0.423**	0.389**	0.185	0.246*	0.188	0.033	-0.051	-0.102
CA		0.418**	0.108	0.462**	0.462**	0.456**	0.197	0.173	-0.006
RH			0.272*	0.246*	0.459**	0.271*	0.031	-0.003	0.016
MYC				0.238*	0.313**	0.279**	0.239*	0.207	0.154

* refers to significant values at *p-value* < 0.05
** refers to significant values at *p-value* < 0.01

Discussion

Glycoalkaloids have several roles both in plant and in humans. They provide a defense mechanism for potato plants against different pests and pathogens, such as insects, viruses, bacteria, and fungi (Friedman, 2006; Lachman et al., 2001). However, glycoalkaloids are known to be toxic to humans by acting as cholinesterase inhibitors and cause sporadic out-breaks of poisoning (Mensinga et al., 2005; Smith et al., 1996), and may also affect reproduction in animals (Wang et al., 2005). Despite their toxic nature to humans and animals, recent studies have suggested that glycoalkaloids do have health promoting effects in humans. Studies by Friedman et al (2005), Yang et al. (2006), and Reddivari et al. (2007b) reported that glycoalkaloids exhibit anticancer properties by inhibiting proliferation of various cancer cell lines through induction of apoptosis.

The multi-effect of glycoalkaloids makes determining their necessity in plants and humans difficult. Hence, their safety to humans is still being debated (Korpan et al., 2004; Rietjens et al., 2005). Since the beginning of the 20th century, the acceptable level of glycoalkaloids in the potato of commerce has been 20 mg/100g tuber weight (Papathanasiou et al., 1998; Smith et al., 1996). This threshold value has largely remained unchanged to date. Therefore, care is always taken in breeding new varieties to ensure that they do not contain glycoalkaloid levels above 20 mg/100g fresh weight. Most potato varieties released contain less than 10 mg/100gfw of both α-solanine and α-chaconine (Lachman et al., 2001).

Wild *Solanum* species are commonly used in potato breeding as a source of valuable germplasm. They are often used to introduce pest and disease resistance into cultivated potato. Some of the wild species contain more health-benefiting phytochemicals such as antioxidants and phenolics than currently grown popular cultivars (Hale, 2004; Nzaramba et al., 2007). Therefore, not only could wild species provide genes for pest and disease resistance, but also genes for high antioxidants and phenolic compounds. However, some of these species have high levels of glycoalkaloids such that, together with desirable characteristics, toxic glycoalkaloids might be transferred to potato cultivars.

Much as there is need to develop new potato varieties resistant to pests and diseases, and which can provide more health-benefiting phytochemicals like antioxidants, prior screening of wild species for glycoalkaloid content is important to ascertain their suitability as potential parental material in breeding programs. In this study, accessions from two wild species (*S. jamesii* and *S. microdontum*) previously observed to contain high levels of antioxidants were fine-screened for antioxidant activity, total phenolics, and total glycoalkaloid content.

Results from this study showed that most accessions of both species exhibited higher levels of both desirable (antioxidants and phenolics) and undesirable (glycoalkaloids) traits than three important common cultivars (Table 4.3). Most (95%) of the *S. jamesii* accessions exhibited glycoalkaloid levels less than 20 mg/100gfw compared to only two accessions of *S. microdontum* that were below this value.

Therefore, accessions with low glycoalkaloid values and higher antioxidants could be used as parents in breeding for high antioxidant and phenolic content.

If there is a relationship between antioxidant activity and glycoalkaloids or between phenolic content and glycoalkaloids, the use of wild species in breeding becomes challenging depending on the nature of the correlations among the traits. According to Falconer and Mackay (1996), different traits can be correlated if the same loci affect the traits (pleiotropy), different loci affect the traits but these loci are linked together - linkage in coupling causing positive correlation and in repulsion negative correlation, or the environment may affect the traits in the same way creating correlation. To elucidate the relationships among these traits principal component analysis was carried out on their values.

Principal component analysis results illustrating genetic phenotypic correlations among traits in both species are shown in Figs 4.2 and 4.3. The figures show two groupings of traits, one consisting of glycoalkaloids and the other phenolic compounds and antioxidant activity. This suggests that no linear relationship exist between glycoalkaloids antioxidant activity or between glycoalkaloids and phenolic content. Therefore, there is no correlation between either glycoalkaloids and antioxidant activity or glycoalkaloids and phenolics. Therefore using wild accessions in breeding for high antioxidant activity and total phenolics would not necessarily increase glycoalkaloids in the developed potato progenies, if the selected parental materials (accessions) are low in glycoalkaloids.

CHAPTER V

ANTI-PROLIFERATIVE ACTIVITY AND CYTOTOXICITY OF *SOLANUM JAMESII* TUBER EXTRACTS TO HUMAN COLON AND PROSTATE CANCER CELLS *IN VITRO*

Introduction

According to the American Cancer Society (2008), colorectal cancer is the third most common cancer in both men and women in the United States. It accounted for about 10% of cancer mortality in the US, and caused about 57,000 deaths in 2004 (Jemal et al., 2004). It is estimated that about 49,960 deaths from colon and rectal cancer will occur in 2008, accounting for 9% of all cancer deaths.

Prostate cancer is the most frequently diagnosed cancer and is the leading cause of cancer death in men. The American Cancer Society (2006) reported that prostate cancer is the third leading cause of cancer death among men in the US. At the time, it was estimated that 27,350 deaths would occur due to prostate cancer. Current estimates (American Cancer Society, 2008) of about 28,660 deaths expected to occur in 2008, has placed prostate cancer as the second leading cause of cancer death in men.

Cancer development or carcinogenesis is a complex, multi-sequence/stage process that leads a normal cell into a precancerous state and finally to an early stage of cancer (Klaunig and Kamendulis, 2004; Trueba et al., 2004). Carcinogenesis involves initiation, promotion, and progression stages. According to Friedman et al. (2007) tumor promotion is the only reversible event during cancer development. Therefore, Hawk et

al. (2005) and Friedman et al. (2007) suggested that early intervention should target inhibition of cell proliferation and induction of apoptotic pathways in cancerous cells.

Cancer is characterized by uncontrolled growth of abnormal cells. The abnormal or cancerous cells are caused by both external factors such as chemical toxins, tobacco, radiation, and infectious organisms, and internal factors such as inherited mutations or mutations from metabolism, hormones, and immune conditions. The causal factors may act together or in sequence to initiate and promote carcinogenesis (American Cancer Society, 2008).

Endogenous cellular processes such as metabolism as well as external factors like chemical toxins and radiation generate free radicals known as reactive oxygen (ROS) and reactive nitrogen (RNS) species (Inoue et al., 2003). The free radicals create an oxidative or nitrosative stress within cells that may result in oxidative damage of DNA, lipids, and proteins (Chu et al., 2002; Hussain et al., 2003; Kovacic and Jacintho, 2001).

High levels of cellular oxidative stress might result in permanent modification of genetic material which normally represents the initial steps involved in mutagenesis, carcinogenesis, and aging. Elevated levels of oxidative DNA lesions have been noted in various tumors, strongly implicating such damage in the etiology of cancer (Valko et al., 2007). Several studies have implicated oxidative stress in initiation of various diseases like cardiovascular disease, cancer, neurological disorders, diabetes, and aging (Dalle-Donne et al., 2006; Makazan et al., 2007; Tappia et al., 2006). Cellular oxidative stress is assuaged by enzymatic and non-enzymatic antioxidants that maintain cellular

homeostasis. A chronic shift in the maintenance of cellular homeostasis can lead to permanent changes associated with carcinogenesis (Droge, 2002; Hussain et al., 2003).

Antioxidants derived from fruits and vegetables complement enzymatic antioxidants in reducing oxidative stress, and thereby help boost defensive mechanisms against the risk of chronic diseases. Some of the beneficial phytochemicals from fruits and vegetables are vitamins C and E, carotenoids, and phenolics, and are thought to be involved in the pathophysiology of many chronic diseases (Stanner et al., 2004; Steffen et al., 2003; Trichopoulou et al., 2003).

Onset of colon cancer is characterized by hyperproliferation of the epithelial cells resulting in formation of adenomas (Hawk et al., 2005). Prostate cancer initially develops as a high-grade intraepithelial neoplasia (HGPIN) in the peripheral and transition zones of the prostate gland. The HGPIN eventually becomes a latent carcinoma, which may subsequently progress to a large, higher grade, metastasizing carcinoma (Abate-Shen and Shen, 2000; Bosland et al., 1991; Shukla and Gupta, 2005). Promotion and progression stages are controlled by signal transduction molecules which are triggered by hormones such as androgens (Giovannucci, 1999; Shukla and Gupta, 2005; Thompson, 1990). Androgen receptor (AR) signaling, cell proliferation and cell death play a critical role in regulating the growth and differentiation of epithelial cells in the normal prostate (Cunha et al., 2004).

Several studies have reported that phytochemicals present in fruits and vegetables are important in prevention of chronic diseases, such as cancer, cardiovascular diseases, and diabetes, (Chu et al., 2002; Hu, 2003; Liu, 2004; Riboli and

Norat, 2003). Benefits derived from phytochemicals in regard to disease prevention are attributed to their antioxidant capabilities (Agudo et al., 2007; Stanner et al., 2004). Several anti-inflammatory, anti-necrotic, and neuroprotective drugs have an antioxidant and/or radical scavenging mechanism as part of their activity (Liu et al., 2004; Perry et al., 1999; Repetto and Llesuy, 2002).

Juan et al. (2006) reported that olive fruit extract inhibited proliferation of HT-29 human colon cancer cells by inducing apoptosis. Crude extracts from sweet potato (*Ipomoea batatas*) inhibited proliferation of the human leukemia NB4 cell line *in vitro* (Huang et al., 2004). Romero et al. (2002) observed that polyphenols in red wine inhibit proliferation and induce apoptosis in prostate LNCaP cancer cells. Several studies have demonstrated that tea extracts exhibit anticancer properties on breast (MCF-7), liver (HepG2), colon (HT-29) (Friedman et al., 2007), lung (Yang et al., 2005), stomach (Mu et al., 2005), prostate (PC-3) (Bettuzzi et al., 2006; Friedman et al., 2007), and skin (Camouse et al., 2005).

Potato tuber extracts have also been tested on several cancer types. Chu et al. (2002) observed minor anti-proliferative activity of potato tuber extract to $HepG_2$ human liver cancer cells *in vitro*. Reddivari et al. (2007b) reported that whole potato tuber extract and anthocyanin fractions inhibited proliferation and induced apoptosis in both LNCaP and PC-3 prostate cancer cells. Glycoalkaloids from commercial potato cultivars have also been reported to inhibit growth of human colon (HT-29), liver (HepG2), cervical (HeLa), lymphoma (U937), stomach (AGS and KATO III) (Friedman et al.,

2005; Lee et al., 2004), and both LNCaP and PC-3 human prostate (Reddivari et al., 2007b) cancer cell lines.

Most recent research is focusing on increasing the amount of beneficial phytochemicals in food crops and also increasing the variety of plant products consumed by introducing exotic and wild fruits and vegetables (Nzaramba et al., 2006), spices and herbs (Ko et al., 2007; Siddhuraju et al., 2002). Some of the wild and exotic products contain natural toxicants at levels that might cause health problems. Development of pest resistant varieties or changes in methods of cultivation, storage, and preparation can change the balance between beneficial and toxic compounds in staple foods, with significant consequences to human health (Phillips et al., 1996). This has been especially observed in the potato of commerce as regards glycoalkaoids (Abreu et al., 2007; Griffiths and Dale, 2001; Laurila et al., 2001; Pęksa et al., 2002; Rytel et al., 2005).

Plant products contain a mixture of complex compounds with both toxic and beneficial effects. Also, compounds that are toxic at high concentrations may be beneficial at lower concentrations; therefore, it is appropriate to test whole plant products. *In vitro* techniques such as mammalian cell culture were developed to screen complex materials like plant extracts for inhibition of diseased cell proliferation and toxicity to normal cells and organs (Hostanska et al., 2007; Phillips, 1996).

Several *in vitro* assays have been developed and vary in sensitivity to different types of toxins. Potato extracts were reported to be more toxic in the LDH (Lactate dehydrogenase) release assay than in the protein synthesis assay (Phillips, 1996). Despite

having little value *per se*, *in vitro* techniques of measuring cytotoxicity are useful in initial screening of exotic plant materials.

Wild potato species have been reported to contain higher amounts of antioxidants and polyphenolic compounds (Nzaramba et al., 2006) and glycoalkaloids (Laurila et al., 2001) than the potato of commerce. Given that high levels of glycoalkaloids in tubers are undesirable, wild potato species should be screened for cytotoxicity before their introduction in breeding programs. Tuber extracts from wild potato species may also contain other unknown compounds that may be toxic to humans.

Therefore, the objective of this study was to investigate anti-proliferative activity and cytotoxicity potential of tuber extracts from *Solanum jamesii* accessions with different antioxidant, phenolic, and glycoalkaloid contents on human prostate (LNCaP) and colon (HT-29) cancer cell lines *in vitro*. Also, the study sought to determine correlations among antioxidant activity, anti-proliferative activity and cytotoxicity, and among glycoalkaloids, anti-proliferative activity and cytotoxicity.

Materials and Methods

Plant Material

Ninety-two accessions of *S. jamesii* were obtained from the US Potato Genebank, Sturgeon Bay, WI. Fifteen accessions, representing the range of total glycoalkaloids in the 92 accessions, were selected for evaluation of anti-proliferative effects and cytotoxicity on human prostate (HT-29) and colon (LNCaP) cancer cells lines *in vitro.*

Chemicals

The DPPH (2,2-Diphenyl-1-picrylhydrazyl), Folin-Ciocalteu reagent, Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), potassium persulfate, Na_2HPO_4 (dibasic), $NaHPO_4$ (monobasic), NaCl, ammonium phosphate, and Na_2CO_3 were purchased from Fisher Scientific (Pittsburgh, PA). Methanol, dimethly sulfoxide (DMSO), and acetonitrile were obtained from VWR International (Suwanee, GA). Alpha-chaconine and ammonium hydroxide were purchased from Sigma-Adrich (St. Louis, MO). Alpha-solanine and tomatine were obtained from MP Biomedicals (Solon, OH). The cell proliferation reagent WST-1 [4-(3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio)-1,3-benzene disulfonate] and cytotoxicity detection kit were obtained from Roche Applied Sciences (Indianapolis, IN).

Cell Lines

Human prostate cancer LNCaP (androgen-dependent) cells and HT-29 colon cancer cells were obtained from the American Type Culture Collection (Manassas, VA). The cells were maintained at 37^0 C in 5% CO_2 jacketed incubator in RPMI 1640 (Sigma; St. Louis, MO) supplemented with 2.38 g/L HEPES 2.0 g/L sodium bicarbonate, 0.11 g/L sodium pyruvate, 4.5 g/L glucose, 100 ml/L FBS, and 10 mL/L antibiotic antimycotic solution (Sigma).

Sample Extraction

Five grams of diced tubers were extracted with 20 ml of HPLC-grade methanol. The samples were homogenized with an IKA Utra-turrax tissuemizer for 3 min. The

tuber extract was centrifuged at 31,000 g for 20 min. with a Beckman model J2-21 refrigerated centrifuge. Five ml of supernatant was collected in glass vials, and the methanol evaporated using a Speed Vac. The dried extract was re-dissolved in DMSO and filtered through 0.45 μm syringe filters. Sample extracts were stored at -20^{o} C until analysis of antioxidant activity (AOA), phenolics and glycoalkaloids.

Antioxidant Activity Analysis

Total antioxidant activity was estimated using both the DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay (Brand-Williams et al., 1995) and ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] assay (Miller and Rice-Evans, 1997).

DPPH assay:

A 150 μl aliquot was placed into scintillation vials, 2,850 μl of DPPH methanol solution was added, and the mixture was placed on a shaker for 15 min. The mixture was transferred to UV-cuvettes and its absorbance recorded using a Shimadzu BioSpec-1601 spectrophotometer at 515 nm. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a synthetic antioxidant, was used as a standard to generate a standard curve (Figure 1), and total AOA in tuber extracts was expressed as micrograms of Trolox equivalents per gram of potato tuber fresh weight (μg TE/gfw).

ABTS assay:

The $ABTS^{\cdot+}$ radical was generated by reacting potassium persulfate with ABTS salt [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt]. A working solution composed of a mixture of 5 ml of mother solution and 145 ml of

phosphate buffer was prepared. The mother solution contained equal volumes of 8 mM of ABTS and 3 mM of potassium persulfate solutions, and the phosphate buffer solution pH 7.4 was composed of 40.5 ml of 0.2 M Na_2HPO_4 dibasic, 9.5 ml of 0.2 M $NaHPO_4$ monobasic and 150 mM NaCl. One-hundred µl of tuber extract were used for analysis. Two-thousand-nine hundred µl of the working solution was added to tuber extracts and reacted for 30 min on a shaker. Absorbance of the solution was measured at 734 nm with a Shimadzu BioSpec-1601 spectrophotometer. Trolox was used as a standard, and total AOA was expressed as micrograms of Trolox equivalents per gram of tuber fresh weight (µg TE/gfw).

Total Phenolic Analysis

Total phenolic content was determined following the method of Singleton et al. (1999). One-hundred-fifty µl of tuber extract was pipetted into scintillation vials, and 2.4 ml of nanopure water was added. One-hundred-fifty µl of 0.25 N Folin-Ciocalteu reagent was added, and after 3 min of reaction 0.3 ml of 1 N Na_2CO_3 reagent was added and allowed to react for two hours. The spectrophotometer (Shimadzu BioSpec-1601) was zeroed with a blank (150 µl methanol, 2.4 ml H_2O, 150 µl of 0.25 N Folin-Ciocalteu, and 0.3 ml 1 N Na_2CO_2) before sample analysis. Absorbance of tuber extracts was read at 725 nm. Chlorogenic acid was used as a standard, and total phenolic content was expressed as milligrams of chlorogenic acid equivalents per 100 grams of potato tuber fresh weight (mg CGAequ/100gfw).

Determination of Glycoalkaloids

Glycoalkaloids were analyzed with a high performance liquid chromatography (HPLC) system following the method of Sotelo and Serrano (2000). An HPLC system (Waters, Milford, MA) and Atlantis C-18 reverse-phase columns (4.6 x 150 mm, 5 μm) were used for glycoalkaloid analysis. The mobile phase used for separating glycoalkaloids was (35:65 v/v) acetonitrile:0.05 M monobasic ammonium phosphate buffer ($(NH_4)H_2PO_4$), adjusted to pH 6.5 with NH_4OH. The solvent flow was isocratic at a rate of 1 ml/min, with the UV absorbance detector set at 200 nm with 5% AUFS sensitivity. The amount of extract sample injected was 20 μl. Different concentrations of pure α-solanine, α-chaconine, and tomatine standards were used to identify glycoalkaloids in the tuber extracts by comparing peak retention times and spectra detected at 280 nm. Standard curves were prepared by regressing known concentrations of glycoalkaloid standards to their corresponding peak areas, and these curves were used to quantify amounts of glycoalkaloids in the tuber extracts.

Cell Proliferation

Cells were plated at a density of $1x10^4$ /well in 96 well plates. They were allowed to attach to the plate for 24 h. After 24 h, media was replaced with DMEM F-12 media containing 2.5% charcoal-stripped serum and tuber extracts. Two concentrations (5 and 10 μl/ml) of tuber extract were tested. After every 24 h, cell proliferation was measured using the WST assay. The assay required pre-incubation of cells in media with the tetrazolium salt WST-1 (4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) (10 μl/well) for 4 h, followed by measuring absorbance at 450 nm

with the ELISA plate reader. The cell proliferation assay was repeated at 48 and 72 h of incubation with potato extracts. Percent cell proliferation due to each tuber extract treatment was calculated based on control (DMSO) absorbance (100%) after each incubation period. All extracts were tested in triplicate.

Cytotoxicity Analysis

Cytotoxicity of tuber extracts to cancer cells was determined by measuring the amount of lactate dehydrogenase (LDH) enzyme leaked from the cytosol of damaged cells into the medium (Phillips, 1996) after exposure of the cells to the extracts for 24 h. The LDH release represents necrosis as opposed to apoptosis. Lactate dehydrogenase in the supernatant was measured using the Cytotoxicity Detection Kit (LDH) (Roche Applied Science, Mannheim, Germany) following the manufacturer's protocol. One-hundred µl of the supernatant from the cells was placed in a 96, well plate and 100 µl of LDH assay solution [mixture of catalyst lyophilisate (catalyst, diaphoraase/NAD^+, lyophilizate) and dye solution (iodotetrazolium chloride and sodium lactate)] were added to each well and incubated for 30 min in the dark. Absorbance of the mixture was read with an ELISA plate reader at 490 nm. Extract cytotoxicity was calculated as a percent of the control (DMSO) absorbance (100%). All samples were analyzed in triplicate.

Statistical Analysis

Results for each treatment were expressed as means ± standard error. Analysis of variance (ANOVA) was performed to determine the variability of anti-proliferative activity and cytotoxicity of the accessions' tuber extracts. Mean separation procedure (LSD) was done to compare accessions for each measured variable. Correlations among

antioxidant activity, total phenolics, and glycoalkaloid content were computed following Pearson's correlation method. All statistical analyses were done using SAS Version 9.1 software (SAS, 2002).

Results

Effect of Tuber Extract on Cell Proliferation

Analysis of variance results for anti-proliferative activity exhibited by 15 tuber extracts from *S. jamesii* accessions on human colon (HT-29) and prostate (LNCaP) cancer cells *in vitro* are presented in Figures 5.1 and 5.2, respectively. The tuber extracts decreased proliferation of HT-29 colon cancer and LNCaP prostate cancer cells in a dose and time-dependent manner. Proliferation of both HT-29 colon cancer cells and LNCaP prostate cancer cells decreased with increased time of incubation with extracts. Cell proliferation was least after 72 h of incubation. Also, accessions exhibited varying degrees of cell proliferation inhibition at each incubation period, and all accessions showed more anti-proliferative activity with longer times of incubation.

A significant reduction in proliferation of HT-29 colon cancer cells by all extracts was observed. All accession extracts at concentrations of 5 and 10 μg/ml significantly reduced proliferation of HT-29 cells compared to the DMSO control (Fig. 5.1). Cell proliferation was less than 60 % of the control (DMSO) after 24 h of cell incubation with tuber extracts of either 5 or 10 μg/ml concentration (Fig. 5.1A and D).

After 48 and 72 h of incubation with any of the extracts (5 and 10 µg/ml), HT-29 cell proliferation was less than 40% of the DMSO control (Fig. 5.1B, E, C and F).

Prostate (LNCaP) cancer cells were not as responsive to tuber extract treatment as were the HT-29 colon cancer cells. At the 5 µg/ml extract concentration, only three accessions (PI 595784, PI 592411, and PI 620870) significantly reduced LNCaP cell proliferation more than the control (DMSO) after 24 and 48 h of incubation (Fig 5.2A and B). However, after 72 h of incubation, seven accessions in the following order- PI 620870 > PI 595784 > PI 592411 > PI 603054 > PI 605372 > PI 592398 > PI 564049 significantly inhibited LNCaP cell proliferation compared to the DMSO control (Fig. 5.2C). With a higher extract concentration (10 µg/ml) all accessions exhibited significant inhibition of LNCaP cell proliferation compared to the DMSO control after 24, 48, and 72 h of incubation (Fig. 5.2D, E and F). After 72 h of incubation with 10 µg/ml extracts, all accessions reduced cell proliferation by about 60% of the DMSO control (Fig. 5.2F).

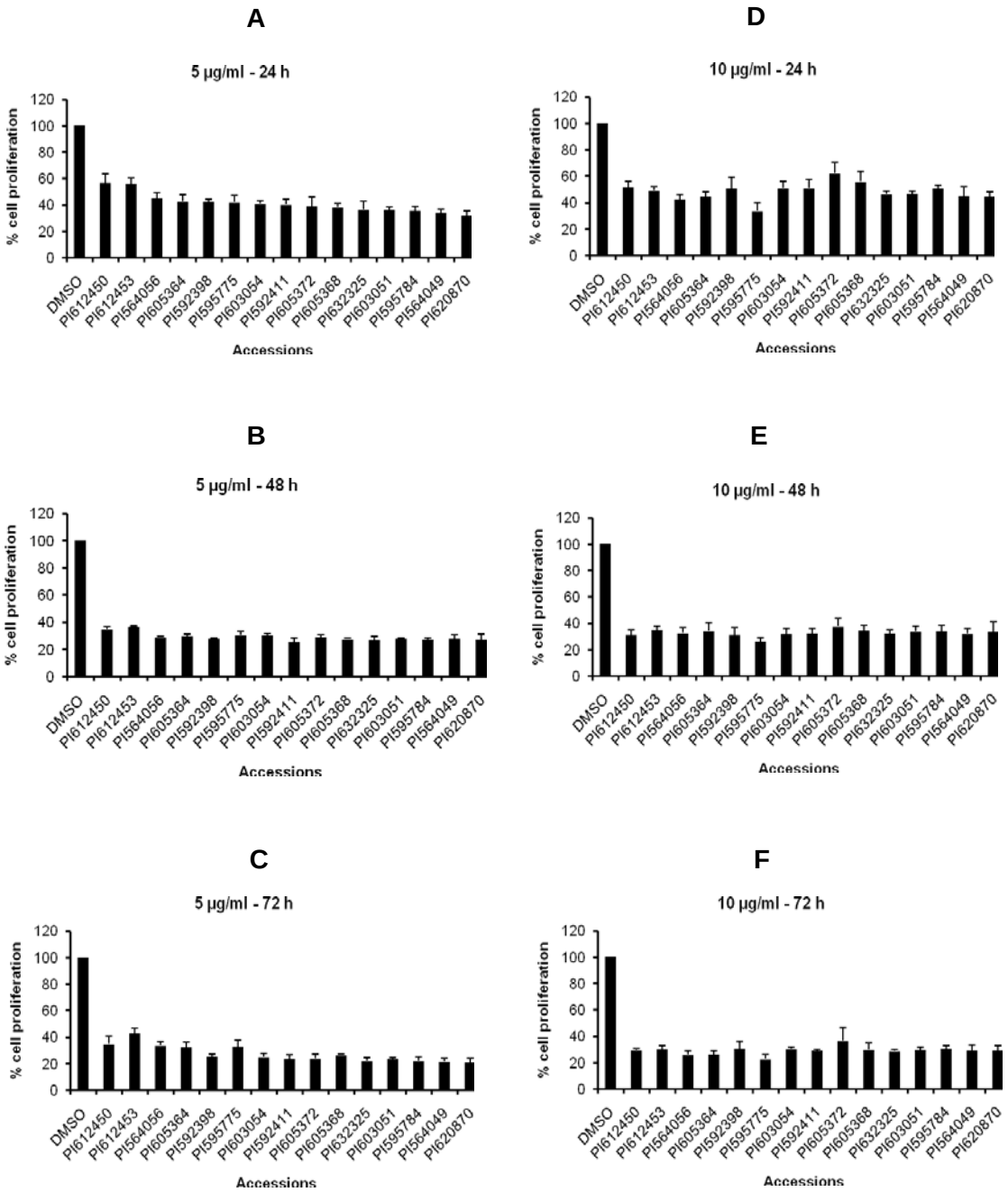


Fig. 5.1. Cell proliferation of HT-29 colon cancer cells measured after 24, 48, and 72 h of incubation with 5 and 10 µg/ml of tuber extracts from 15 *S. jamesii* accessions. Results are presented as means ± SE of three experiments.

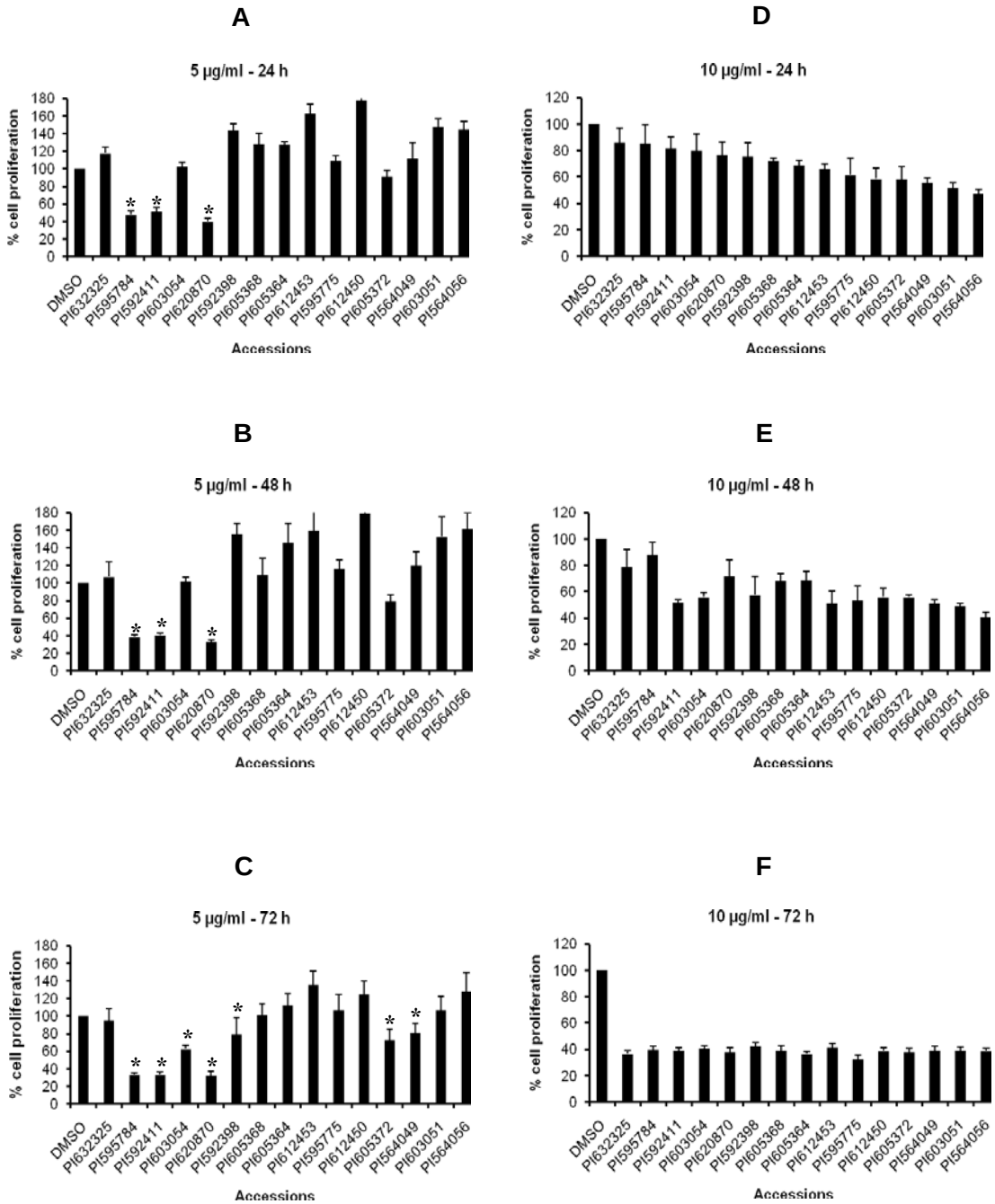


Fig. 5.2. Cell proliferation of LNCaP prostate cancer cells evaluated after 24, 48, and 72 h of incubation with 5 and 10 µg/ml of tuber extracts from 15 *S. jamesii* accessions. Results are presented as means ± SE of three experiments. Significantly lower values than the DMSO control (LSD at $p < 0.05$) are indicated by an asterisk.

Cytotoxicity of tuber extracts to cancer cells *in vitro* due to necrosis was determined by measuring the amount of lactate dehydrogenase (LDH) enzyme leaked from the damaged cells into the medium (supernatant) after exposure of the cells to the extracts for 24 h. Two concentrations (5 and 10 μg/ml) of the extracts were used and the amount of LDH released was expressed as percent of the control (DMSO). Results are reported as mean ± standard error of three replicated analyses. Accessions PI 595784 and PI 620870 at a concentration of 5 μg/ml caused slightly more but not significant LDH leakage from HT-29 cells than the DMSO control. Only PI 592398, PI 592411, PI 603051, PI 603054, and PI 632325 caused significantly less LDH leakage than the control (Fig . 5.3A). At a higher concentration of extract (10 μg/ml), nine accessions- PI 564049, PI 592411, PI 595784, PI 603051, PI 603054, PI 605364, PI 605368, PI 605372, and PI 612453 were significantly less toxic than the control (Fig. 5.3B). All other accessions were not significantly different from the control in cytotoxicity to HT-29 cells.

Lactate dehydrogenase released by LNCaP prostate cells after treatment with 5 μg/ml of tuber extracts of PI 564056, PI 595775, PI 595784, PI 605368, and PI 605372 accessions was significantly lower than that of the control (DMSO) (Fig. 5.4A). Five μg/ml of tuber extracts from PI 592398, PI 612450, and PI 620870 caused more LDH leakage than the control, but they were not significantly different. At twice the concentration (10μg/ml), PI 612453 caused significantly less LDH leakage from LNCaP prostate cells than the DMSO control. Two accessions (PI 595784 and PI 620870) at 10μg/ml concentration caused significantly higher LDH leakage than the control (Fig.

5.4B). It appears that at high concentrations, the two accessions, PI 595784 and PI 620870, might be toxic to LNCaP cells. The other accessions tested were not significantly different from the DMSO control.

In general, all accessions tested exhibited as much as or significantly lower LDH leakage than the control in both HT-29 (Fig. 5.3) and LNCaP cells (Fig. 5.4). Only two accessions (PI 595784 and PI 620870) at a high concentration (10µg/ml) showed significantly higher LDH leakage than the control in LNCaP cells.

The accessions of *S. jamesii* tested for cytotoxicity were not necessarily toxic to HT-29 colon cancer and LNCaP cancer cell lines, since the amount of LDH released after cell incubation with tuber extracts was not significantly different from cells incubated without extracts (only DMSO). Therefore, the observed reduction in proliferation of HT-29 and LNCaP cancer cells after incubation with tuber extracts of *S. jamesii* accessions was not due to necrosis but rather to enhanced apoptosis. A previous study (Reddivari et al., 2007b) reported that tuber extracts from speciality potato cultivars contain phytochemicals that can inhibit LNCaP and PC-3 cell growth and induce apoptosis.

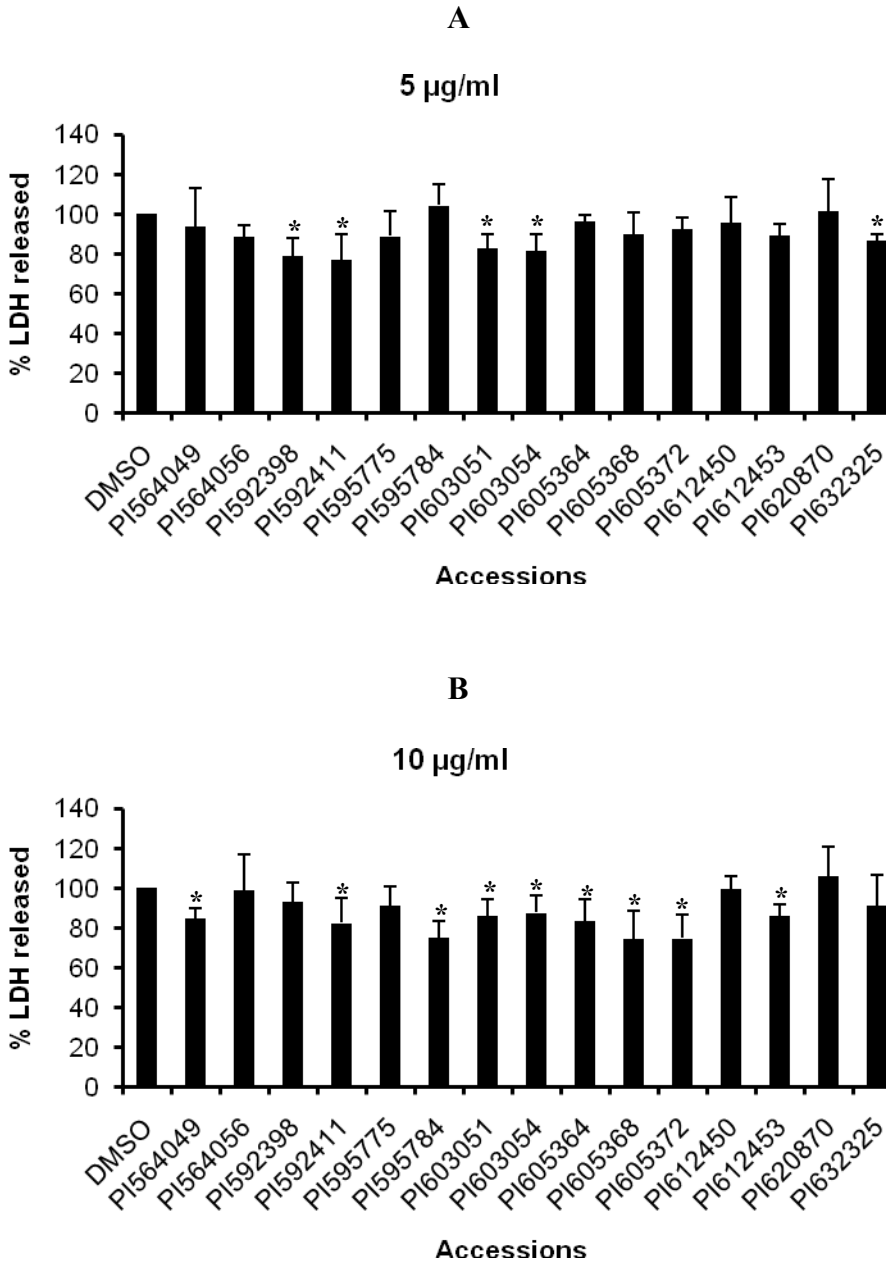


Fig. 5.3. Cytotoxicity of tuber extracts from 15 *S. jamesii* accessions (5 and 10 µg/ml) to HT-29 human colon cancer cells expressed as percentage of lactate dehydrogenase enzyme (LDH) released from the cells after 24 hours of incubation. Results are presented as means ± SE of three experiments. Significantly lower values than the DMSO control (LSD at $p < 0.05$) are indicated by an asterisk.

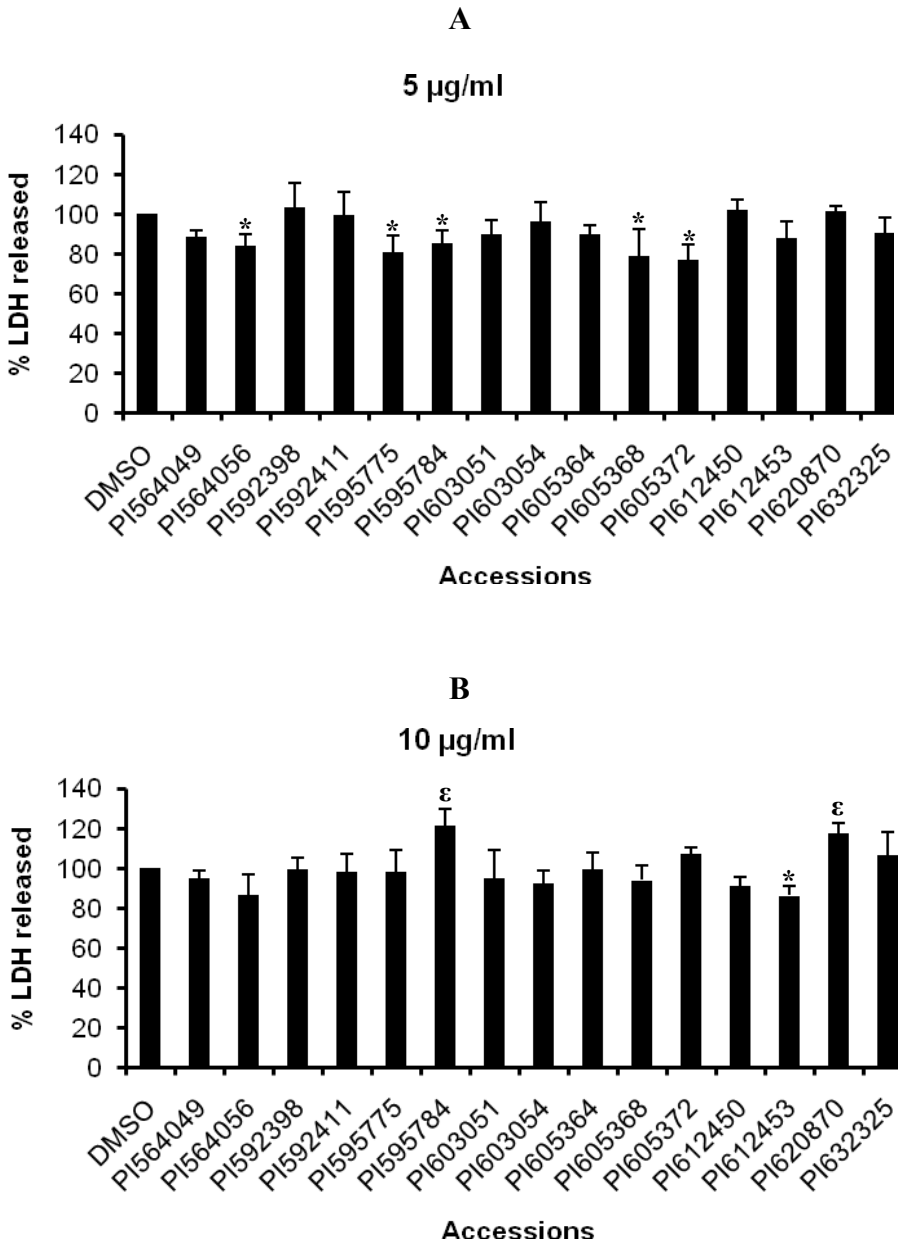


Fig. 5.4. Cytotoxicity of tuber extracts from 15 *S. jamesii* accessions (5 and 10 µg/ml) to LNCaP human prostate cancer cells expressed as percentage of lactate dehydrogenase enzyme (LDH) released from the cells after 24 hours of incubation. Results are presented as means ± SE of three experiments. Significantly lower values than the DMSO are indicated by an asterisk, and values significantly higher (LSD at $p < 0.05$) than the DMSO control are indicated by a symbol ε.

Correlations among Antioxidants, Phenolics, Glycoalkaloids and Anti-proliferative Activity

Several studies have associated consumption of foods rich in antioxidants and polyphenols with decrease in prevalence of degenerative diseases such as cancer (Lui et al., 2002). Other studies have investigated polyphenols extracted from plants for their potential effect in curing colon (Juan et al., 2008; Kim et al., 2006; McCann et al., 2007) and prostate cancers (Bettuzzi et al., 2006; Reddivari et al., 2007b; Romero et al., 2002). Glycoalkaloids have also been reported to play a role in reducing cancer cell proliferation (Friedman et al., 2005; Lee et al., 2004; Reddivari et al., 2007b) by up-regulating apoptosis in these cells. Therefore, relationships among antioxidant activity, phenolic and glycoalkaloid content in tuber extracts, and their anti-proliferative activity in HT-29 colon and LNCaP prostate cancer cell lines were also investigated.

Results from correlation analysis among antioxidant activity, total phenolics, glycoalkaloids, and anti-proliferation activity on HT-29 colon cancer cells show inconsistent relationships (Table 5.1). At 5 μg/ml of tuber extract concentration, the correlation between inhibition of HT-29 cell proliferation after 24 h of incubation and antioxidant activity measured by the DPPH and ABTS assays was positive with correlation coefficients $r = 0.749$ and $r = 0.389$, respectively. Also, correlation between inhibition of HT-29 cell proliferation and total phenolics after 24 h of incubation with 5 μg/ml of tuber extract was significant ($r = 0.81$). These results suggest that cell proliferation was inhibited by increasing the amount of antioxidants and phenolics after 24 h of incubation. However, after 48 and 72 h of incubation, there were no significant

Table 5.1. Correlation analysis of antioxidant activity (DPPH and ABTS), total phenolics (TP), α-solanine (SOL), α-chaconine (CHA), and total glycoalkaloids (TGA) in *Solanum jamesii* accessions, and inhibition of HT-29 colon cancer cell proliferation.

						Inhibition of cell proliferation					
						5 µg/ml			10 µg/ml		
	ABTS	TP	SOL	CHA	TGA	24 h	48 h	72 h	24 h	48 h	72 h
DPPH	0.647**	0.887**	0.144	-0.047	0.109	0.749**	-0.072	0.090	0.407**	0.579**	0.089
ABTS		0.657**	-0.025	0.142	0.013	0.398*	-0.104	0.044	0.184	0.496**	-0.269
TP			0.338*	0.225	0.337*	0.810**	0.092	0.128	0.378*	0.675**	-0.081
SOL				0.600**	0.981**	0.255	0.346*	0.344*	0.278	0.009	-0.184
CHA					0.744**	-0.058	0.253	0.423	0.142	0.009	-0.274
TGA						0.198	0.350*	0.389*	0.267	0.009	-0.220

* refers to significant values at *p-value* < 0.05
** refers to significant values at *p-value* < 0.01

correlations between inhibition of cell proliferation and antioxidant activity or total phenolic content (Table 5.1).

Individual glycoalkaloids and total glycoalkaloids showed no significant correlation with inhibition of HT-29 cell proliferation after 24 h. But after 48 and 72 h α-solanine showed a significant correlation with cell proliferation inhibition, $r = 0.346$ and $r = 0.344$, respectively. Similarly, total glycoalkaloid was significantly correlated with inhibition of cell proliferation after 48 h ($r = 0.35$) and 72 h ($r = 0.389$) of incubation. Actual proliferation values (Fig 5.1) show that proliferation of HT-29 cells was significantly inhibited by treatment with tuber extracts even after 24 h of incubation. Therefore, correlation results, together with cell proliferation data, suggest that not a single but a combination of compounds (acting together) are responsible for inhibiting cell proliferation.

With a higher concentration of tuber extract (10 μg/ml), antioxidant activity (DPPH) and inhibition of cell proliferation were significantly correlated after 24 h ($r = 0.407$) and 48 h ($r = 0.579$) of incubation. The ABTS assay was correlated with cell proliferation inhibition only after 48 h ($r = 0.496$) of incubation, and total phenolic content was significantly correlated with inhibition of cell proliferation after 24 h ($r = 0.378$) and 48 h ($r = 0.675$) of incubation. There were no significant correlations between either antioxidant activity (DPPH and ABTS assays) and inhibition of HT-29 cell proliferation or total phenolic content and inhibition of HT-29 cell proliferation after 72 h of incubation. Also α-solinine, α-chaconine, and total glycoalkaloids showed no

significant correlation with inhibition of HT-29 cell proliferation after 24, 48, and 72 h of incubation.

Generally, correlation analysis indicated that LNCaP prostate cancer cell proliferation was significantly reduced with increasing amounts of antioxidants, total phenolics, and glycoalkaloids in tuber extracts. After 24 h of incubation with 5 μg/ml of tuber extract, total phenolic content and α-solanine exhibited a significant negative correlation with inhibition of LNCaP cell proliferation with correlation coefficients (*r*) of -0.301 and -0.293 (*p*-value <0.05), respectively (Table 5.2). At 72 h of incubation, α-solanine (*r* = -0.411) and total glycoalkaloid (*r* = -0.386) were negatively and significantly correlated with inhibition of LNCaP prostate cancer cell proliferation.

Antioxidant activity measured by ABTS was negatively and significantly correlated with inhibition of LNCaP cell proliferation after 24 h (*r* = -0.358) and 72 h (*r* = -0.343) of incubation with 10 μg/ml of tuber extract. Also, after 72 h of incubation with 10 μg/ml of extract, α-solanine showed a significantly negative correlation with inhibition of LNCaP prostate cancer cell proliferation, while total glycoalkaloid were positively correlated with inhibition of LNCaP prostate cancer cell proliferation (Table 5.2).

Table 5.2. Correlation analysis of antioxidant activity (DPPH and ABTS), total phenolics (TP), α-solanine (SOL), α-chaconine (CHA), and total glycoalkaloids (TGA) in *Solanum jamesii* accessions, and inhibition of LNCaP prostate cancer cell proliferation.

						Inhibition of cell proliferation					
						5 µg/ml			10 µg/ml		
	ABTS	TP	SOL	CHA	TGA	24 h	48 h	72 h	24 h	48 h	72 h
DPPH	0.647**	0.887**	0.144	-0.047	0.109	-0.113	-0.019	-0.033	-0.259	-0.121	-0.083
ABTS		0.657**	-0.025	0.142	0.013	-0.236	-0.151	-0.238	-0.358*	-0.031	-0.343*
TP			0.338*	0.225	0.337*	-0.301*	-0.173	-0.276	-0.226	-0.216	0.018
SOL				0.600**	0.981**	-0.293*	-0.205	-0.411**	0.245	0.147	-0.346*
CHA					0.744**	-0.093	-0.011	-0.173	-0.189	-0.232	0.178
TGA						-0.268	-0.174	-0.386*	0.159	0.067	0.332*

* refers to significant values at *p-value* < 0.05
** refers to significant values at *p-value* < 0.01

Correlations among Antioxidants, Phenolics, Glycoalkaloids and Cytotoxicity

Induction of cell death in cancerous cells may be due to induction of apoptosis (programmed cell death) or necrosis. Necrosis can be determined by measuring the amount of lactate dehydrogenase (LDH) enzyme released from cells into the culture medium. Results of cytotoxicity analysis using the LDH assay exhibited significant positive correlation between antioxidant activity measured with the DPPH assay and the amount of LDH released (r = 0.381), and between the ABTS assay and amount of LDH released (r = 0.471) from HT-29 colon cancer cells (Table 5.3) after incubation with 5 μg/ml of tuber extract. Total phenolics and glycoalkaloids showed no significant correlation with LDH released from HT-29 colon cancer cells at the 5 μg/ml concentration. At 10 μg/ml of extract, there was no significant correlation between LDH released from HT-29 cells and antioxidant activity, total phenolics or glycoalkaloids.

Lactate dehydrogenase released from LNCaP prostate cancer cells was positively correlated with α-solanine (r = 0.369) and total glycoalkaloids (r = 0.356) after incubation with 5 μg/ml tuber extract (Table 5.3). However, no significant correlation was observed between antioxidant activity and LDH, between total phenolics and LDH, and between glycoalkaloids and LDH in LNCaP prostate cancer cells after incubation with 10 μg/ml tuber extract (Table 5.3).

Table 5.3. Correlation analysis of antioxidant activity (DPPH and ABTS), total phenolics (TP), α-solanine (SOL), α-chaconine (CHA), and total glycoalkaloids (TGA) in *Solanum jamesii* accessions, and cytotoxicity to HT-29 colon cancer and LNCaP prostate cancer cell lines.

						%LDH			
						HT-29		LNCaP	
	ABTS	TP	SOL	CHA	TGA	5 µg/ml	10 µg/ml	5 µg/ml	10 µg/ml
DPPH	0.647**	0.887**	0.144	-0.047	0.109	0.381*	0.084	0.054	0.012
ABTS		0.657**	-0.025	0.142	0.013	0.471**	0.012	-0.002	0.269
TP			0.338*	0.225	0.337*	0.247	0.052	0.106	0.074
SOL				0.600**	0.981**	-0.023	0.246	0.369*	0.264
CHA					0.744**	-0.136	0.263	0.195	0.003
TGA						-0.052	0.269	0.356*	0.222

Discussion

Wild potato accessions contain higher amounts of beneficial phytochemicals, such as antioxidants and polyphenols, than the potato of commerce, and are therefore potential sources of parental material in breeding for these phytochemicals. However, wild species are also known to contain higher amounts of toxic compounds such as glycoalkaloids that are considered a health hazard for human consumption.

Solanum jamesii accessions significantly inhibited HT-29 and LNCaP cell proliferation. However, the important finding of this study is that the cytotoxicity of many of these accessions is not necessarily due to necrosis. Therefore, these accessions might not pose a health problem if used as parental material in improving the nutritional value of potato cultivars.

Tuber extracts from 15 accessions of *S. jamesii*, representing the range of total glycoalkaloids in *S. jamesii*, inhibited proliferation of colon (HT-29) and prostate (LNCaP) cancer cell lines. The amount of LDH released from cells incubated with tuber extracts was not significantly different from amounts of LDH released from cells incubated without tuber extracts (DMSO as a control). This implies that the cytotoxic effects of the tuber extracts were not due to necrosis, but probably to induction of apoptosis.

Colon and prostate cancer cells responded differently to tuber extract treatments. Colon (HT-29) cancer cell lines seemed more responsive to tuber extract treatment than prostate (LNCaP) cancer cell lines. Proliferation of colon (HT-29) cancer cells was significantly reduced by all extracts at 5µg/ml (Fig. 5.1), yet a higher concentration

(10μg/ml) of extract was required for all accessions to inhibit proliferation of LNCaP prostate cancer cells (Fig. 5.2). These results agree with Kim et al. (2006) who reported that polyphenol concentrations required for anti-cancer effects depend on the type of cancer cell line. Friedman et al. (2005) and Lee et al. (2004) came to a similar conclusion while investigating anti-carcinogenic effects of glycoalkaloids against cervical, liver, lymphoma, and stomach cancer cells.

Correlation analysis results from this study suggest that compounds, other than those evaluated in this investigation, may also be contributing to anti-proliferative effects of potato tuber extracts. Antioxidants, phenolics, and glycoalkaloids, together with other compounds present in tuber extracts may be acting competitively, additively, and/or antagonistically to inhibit proliferation of colon and prostate cancer cells. This may be the reason why correlations between anti-proliferation and levels of antioxidants, phenolics, and glycoalkaloids in the tuber extracts were not consistent. Chu et al. (2002) reported that correlations between phytochemical contents of five vegetables and anti-proliferative activity of $HepG_2$ human liver cells were not significant. They observed that inhibition of human liver cancer cells by vegetables does not solely depend on their phenolic content, but that other chemicals in the vegetables were also responsible for anti-proliferative activities. The above observations support the idea proposed by Liu (2004), Cirico and Omaye (2006), and Milde et al. (2007) that combinations of different phytochemicals synergistically confer more health benefits than individual chemicals.

It has been reported that antioxidants or phytochemicals with antioxidant capacity can become pro-oxidants depending on their concentration and the environment

in which they act (Mortensen et al., 2001). Therefore, a network of phytochemicals is necessary in promoting health. This may further explain the inconsistencies observed in correlation analysis between single chemicals in tuber extracts and inhibition of cell proliferation. These results suggest that not only concentration of phytochemicals is important in inhibiting cell proliferation, but also moderate combination of diverse phytochemicals. In fact, very high levels of phenolic compounds and certainly glycoalkaloids are toxic for human consumption.

Other studies have explained why no single antioxidant can replace the combination of natural phytochemicals in fruits and vegetables in achieving greater health benefits. This was based on observations that combinations of extracts from different fruits resulted in greater antioxidant activity that was additive and synergistic (Eberhardt et al., 2000; Liu, 2003; 2004; Sun et al., 2002) than individual extracts. Friedman et al. (2005) demonstrated that certain combinations of the two major potato glycoalkaloids (α-solanine and α-chaconine) act synergistically in inhibiting cell proliferations of several human cancer cell lines. Likewise, Rayburn et al. (1995) and Smith et al. (2001) reported that combinations of α-solanine and α-chaconine acted synergistically to cause cytotoxicity and disruption of cell membranes.

Phytochemicals in foods differ in molecular size, polarity, and solubility, and these differences may affect the bioavailability and distribution of each phytochemical in different macromolecules, organelles, cells, organs, and tissues (Liu, 2003). This implies that biological effects of phytochemical mixtures are greater than the expected additive effects of individual compounds. Currently it is not clear how single nutrients and

combinations of nutrients affect one's risk of specific cancers. Many questions remain unanswered until more is known about the specific components of diet that influence cancer risk. Presently the best advice is to consume wholesome foods in a balanced diet (American Cancer Society, 2008).

The complexities of cancer research make it difficult to translate *in vitro* cell assay results to *in vivo* applications. But since previous studies have shown that several plant extracts inhibit cancer cell proliferation, more *in vivo*, i.e. animal experiments, are necessary to confirm the *in vitro* observations and design more and probably better chemotherapeutic compounds.

Dietary constituents in the relevant foods must be sufficient to attain the cellular concentrations that display sufficient bioactivity and chemopreventive capacity (Juan et al., 2008). Presence of high amounts of chemopreventive compounds in plant foods such as the potato of commerce would increase bioavailability of the bioactive phytochemicals. Therefore, crop improvement or breeding to increase health-promoting phytochemicals in plant foods is important.

CHAPTER VI

CONCLUSIONS

Results from this investigation show that antioxidant activity measured by the ABTS assay, total phenolic content, and specific gravity are governed more by genetic factors than environmental conditions. More than 50% of the variability in antioxidant activity (ABTS assay), total phenolic content, and specific gravity was attributed to the cultivar main effect. As for antioxidant activity (DPPH assay), location, season, and cultivar main effects are equally influential in variability of antioxidant activity. Interactions of cultivar, location, and season effects were also significant, and these may obscure progress in breeding for high antioxidants.

There was no significant relationship between antioxidant activity and specific gravity, or between total phenolic content and specific gravity. Also, there was no significant correlation between any of the individual phenolic compounds and specific gravity. Therefore, breeding for high antioxidants and phenolic compounds in potato tubers would not compromise tuber quality in terms of specific gravity.

Accessions of *S. jamesii* and *S. microdontum* species exhibited higher levels of antioxidants, phenolics, and glycoalkaloids than the commonly grown cultivars. Antioxidant activity in *S. jamesii* accessions ranged from 173 (PI 592408) to 961 μg TE/gfw (PI 620875), and 1,383 (PI 592408) to 3,513 (PI 275172) μg TE/gfw, for the DPPH and ABTS assays, respectively. Values in *S. microdontum* ranged from 202 (PI 558097) to 1,535 (PI 498127) μg TE/gfw, and from 1,084 (PI 558097) to 6,288 (PI 498127) μg TE/gfw, for the DPPH and ABTS assays, respectively. Total phenolic

content in *S. jamesii* accessions ranged from 49.7 (PI 592408) to 161 (PI 595775) mg CGA/100gfw, and in *S. microdontum* from 51 (PI 558097) to 269 (PI 498127) mg CGA/100gfw.

High amounts of α-solanine and α-chaconine were found in *S. jamesii* and *S. microdontum* accessions. Tomatine and dehydrotomatine were identified and quantified only in some *S. microdontum* accessions. Generally, amounts of glycoalkaloids in *S. microdontum* were higher than in *S. jamesii* accessions. Most (95%) of the *S. jamesii* accessions exhibited glycoalkaloid levels less than 20 mg/100g, while only two accessions of *S. microdontum* were below this value. The following *S. jamesii* accessions; PI 585116, PI 592413, PI 592397, PI 595778, PI 605371, PI 620870, PI 592410, and PI 603054 exhibited total glycoalkaloid levels close to or greater than the safety limit (20 mg/100g). Only two accessions- PI 473171 and PI 500041 of *S. microdontum* exhibited total glycoalkaloid levels less than 20 mg/100g. Therefore, most *S. jamesii* accessions and the two accessions of *S. microdontum* can potentially be used in breeding without increasing amounts of glycoalkaloids in the progenies, since they contain low levels of glycoalkaloids.

Principal component analysis results showed that there is no significant linear relationship between glycoalkaloids and antioxidant activity, or between glycoalkaloids and phenolic content. Therefore, using wild accessions in breeding for high antioxidant activity and total phenolics would not necessarily increase glycoalkaloids in the developed potato progenies if selected parental materials (accessions) are low in glycoalkaloids.

Tuber extracts from *S. jamesii* accessions inhibited proliferation of colon (HT-29) and prostate (LNCaP) cancer cell lines. The anti-proliferation activity exhibited by the tuber extracts is not due to necrosis, because the amount of LDH released from cells incubated with tuber extracts was not significantly different from that released by cells incubated without tuber extracts (DMSO as a control). Also, the results indicate that accessions of *S. jamesii* are not necessarily cytotoxic to HT-29 colon and LNCaP prostate cancer cell lines.

Colon (HT-29) cancer cell lines were more responsive to tuber extract treatment than prostate (LNCaP) cancer cell lines. Proliferation of colon (HT-29) cancer cells was significantly inhibited by all extracts at 5 μg/ml but a concentration of 10 μg/ml was required for all accessions to inhibit proliferation of LNCaP prostate cancer cells.

Correlations between anti-proliferation and levels of antioxidants, phenolics, and glycoalkaloids in the extracts were not significant. This suggests that compounds, other than the ones measured, may also be contributing to anti-proliferative effects of potato tuber extracts. Antioxidants, phenolics, and glycoalkaloids, together with other compounds present in tuber extracts, may be acting competitively, additively, and/or antagonistically to inhibit proliferation of colon and prostate cancer cells.

In summary, crop improvement or breeding to increase health-promoting phytochemicals in plant foods is necessary in order to boost the bioavailability of the active phytochemicals. However, some of the health-promoting compounds such as glycoalkaloids are required in very low amounts as they are toxic when consumed in

larger quantities. Therefore breeding strategies should ensure that such phytochemicals are not increased but maintained at the necessary low levels.

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