THESIS

EFFECT OF GENOTYPE, STORAGE AND PROCESSING ON THE POLYPHENOLIC CONTENT, COMPOSITION, IN VITRO ANTI-CANCER ACTIVITY AND SENSORY ATTRIBUTES OF COLORED-FLESH POTATOES

Submitted by

Gaurav Madiwale

Department of Food Science and Human Nutrition

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2012

Master's Committee:

Advisor: Jairam Vanamala
Co-Advisor: Martha Stone

Lavanya Reddivari
Marisa Bunning
David Holm

ABSTRACT

EFFECT OF GENOTYPE, STORAGE AND PROCESSING ON THE POLYPHENOLIC CONTENT, COMPOSITION, IN VITRO ANTI-CANCER ACTIVITY AND SENSORY ATTRIBUTES OF COLORED-FLESH POTATOES

The potato (*Solanum tuberosum* L.) is the world's third largest food crop with *per capita* consumption of ~126 lbs. annually in the US. The 2010 US Potato Board Report revealed that over the past ten years, while consumption of traditional potatoes (mashed, baked, fried, steamed, boiled and french fries) declined, specialty/colored potato consumption increased by 17%, possibly due to their putative health benefits. Specialty/colored potatoes, which are rich in anti-proliferative and pro-apoptotic anthocyanins and/or carotenoids, can be an attractive "delivery system" for these bioactive compounds in humans. However, colored potatoes can undergo 3-6 months of storage before processing/consumption and the effect of storage and processing on their anti-cancer properties remains unknown. We hypothesized that potatoes retain polyphenolic content (TP), antioxidant activity (AA) and chemopreventive properties against early stage (HCT-116) and advanced stage (HT-29) human colon cancer cells even after 3 months of storage and processing (baking and chipping). To test this hypothesis, we utilized white-, yellow- and purple-fleshed potato clones and tested their phenolic (Folin-Ciocalteu) and anthocyanin (pH-differential) content, antioxidant activity (ABTS and DPPH), metabolite profile (UPLC-MS), anti-cancer properties (cell proliferation via cell counting and BrdU assays, and apoptosis via Caspase-Glo 3/7

assay), and sensory attributes (9-point hedonic scale). Purple-fleshed potatoes had higher phenolic content and antioxidant activity as compared to their white- and yellow-fleshed counterparts. The antioxidant activity of all clones increased with storage; however, an increase in total phenolic content was observed only in purple-fleshed clones. Baking caused minimal losses while chipping reduced the phenolic and anthocyanin content, and antioxidant activity of the potatoes. With storage, total phenolic and anthocyanin content, and antioxidant activity increased in baked samples while in the chipped samples, they remained constant. Principal component analysis of approximately 1600 peaks obtained by UPLC-MS analysis revealed that storage caused a shift in the metabolite profiles of potato clones. In general, ethanol extracts of uncooked, baked and chipped samples suppressed proliferation and elevated apoptosis ($p < 0.05$) in human colon cancer cell lines (HCT-116, HT-29). However, chipped samples did not have any effect on HT-29 cell lines. Anti-proliferative and pro-apoptotic properties of baked potatoes were similar to that of raw potatoes while chipping caused a significant reduction in the biological activity. Storage generally negatively affected the anti-cancer properties of the potato extracts. Sensory analysis revealed comparable acceptance of purple-fleshed baked and chipped potatoes when compared with traditional cultivars. Consumers were willing to pay a premium for colored-flesh potatoes if they were educated on their potential health benefits. Phenolic content and antioxidant activity of purple-fleshed potatoes, after baking, were comparable with those of anthocyanin-rich berries. Hence, locally grown purple-fleshed potatoes can be a healthier choice as they possess greater levels of bioactive compounds and in vitro anti-cancer properties even after processing as compared to their white- and yellow-fleshed counterparts.

DEDICATION

This thesis is dedicated to my parents GAURI and PRADEEP, and my brother, SHREYANSH whose unconditional love and support made this possible.

ACKNOWLEDGEMENT

I would like to thank my advisor Prof. Jairam Vanamala for seeing great potential in me and giving me the chance to work in his laboratory to pursue my graduate studies. His encouragement, guidance and timely advice helped me accomplish this great task. His passion for science made me push harder each day and complete my work with pure dedication. I cannot really express how grateful I am to Dr. Lavanya Reddivari. She has been endlessly patient with me and made me adept in all my technical skills. She is a crucial element in this thesis and was actively involved right from experimental designing till writing up the results.

My co-advisor, Prof. Martha Stone has been a great pillar of strength for me. I cannot thank her enough for her advice and support towards any decisions I made. She has been very comforting and accommodating thus making me sail through my graduate life with ease. Heartfelt thanks to Prof. Marisa Bunning for serving on my committee and helping me with my sensory evaluation studies. She was very supportive throughout my time at CSU.

I would like to express my heartfelt thanks to Dr. David Holm for serving as my external committee member and supplying the potatoes required for this study, and Fahrettin Goktepe and Steven Keller for helping with their processing. I would like to thank all my lab members, especially Sridhar Radhakrishnan for always helping me with my research work. All the potato processing work would not have been possible without his support. I am thankful to Corey Broeckling, and Jim zumBrunnen for their help with UPLC-MS and statistical analysis, respectively.

Big thanks to Prof. Chris Melby for his endless support, motivation and comfort throughout my CSU life. In spite of being the Department Chair, he made sure all the students were enjoying what they were doing and personally addressed their concerns. I hope he has a lot more mountaineering ahead of him. I wish to thank every member of the Department of Food Science and Human Nutrition, CSU for their continuous help and support, and for making the department a great place to be I would also like to express my gratitude towards all my friends especially Priya Naik and Bhavesh Khemka for putting up with my craziness and making my time in Fort Collins a memorable one. I would also like to thank all my dear friends and teachers back home in India. They helped me live my past, shape my present and direct my future. Heartfelt thanks for those whom I have missed to acknowledge.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth-most extensively grown and consumed food crop across the world after wheat, rice and maize (FAO 2009). Potato is a carbohydrate-rich, versatile vegetable prepared and served in a variety of ways world-wide. In the developing world, the poorest and undernourished families depend on potatoes as a major source of food and nutrition because of its highly available dietary energy. The potato is inexpensive to buy and easy to grow, and can give stable yield under conditions where other crops might fail. It is suitable to places where land is limited and labor is plentiful; conditions which characterize most of the developing parts of the world (Lutaladio and Castaldi 2009). Freshly harvested potatoes contain approximately 80% water and 20% dry matter. Starch accounts for 60-80% of the total dry matter, and the protein content is similar to that of cereals on a dry-weight basis (Lutaladio and Castaldi 2009). Potatoes are a rich source of iron and this, coupled with the presence of high vitamin C content, helps in its absorption. It is also a good source of B-complex vitamins, potassium, phosphorus and magnesium (Burlingame et al. 2009). In addition, the potato has no fat and is source of many phytochemicals. Potato by itself is not very high in these phytochemicals, however, due to its high consumption it is considered the third largest source of phenolic compounds in the human diet after oranges and apples (Chun et al. 2005). Thus potatoes can act as 'delivery mechanisms' for bioactive compounds. Colored-flesh potatoes are gaining popularity due to the potential health benefits of anthocyanins.

Previous researchers used either uncooked or baked potatoes with little emphasis on the effect of storage and processing on biological activity. It is known that storage and processing changes the physical and chemical composition of foods (Spanos et al. 1990; Price et al. 1997), thus, affecting their antioxidant activity (Nicoli et al. 1999; Dewanto et al. 2002). Raw potato phenolic content has been extensively studied (Al-Saikhan et al. 1995; Reyes et al. 2005; Stushnoff et al. 2008; Rumbaoa et al. 2009), but potatoes are almost always consumed after processing (baked, chipped, fried, boiled or microwaved) making it critical to understand the effect of such processing techniques on the activity and composition of bioactive compounds in potatoes. Potatoes are stored for months, sometimes up to one year before they are processed (Herrman et al. 1996). Indeed, post-harvest processes might suppress the *in vivo* anti-colon cancer activity of fruits and vegetables (Vanamala et al. 2006). Hence, it is necessary to determine the combined effects of storage and processing on the anti-cancer activity of potatoes. For consumers, sensory perception is of utmost importance. Hence, it is necessary to understand how inter-clonal differences, post-harvest storage and total phenolic content influence the sensory parameters of baked and chipped potato samples. Berries are the most popular source of anthocyanins, which have many health-benefits associated with them (Meyers et al. 2003; Bagchi et al. 2004; Olsson et al. 2004). However, many populations are unable to consume berries due to their high cost with respect to other fruits and vegetables, including potatoes. Hence, it is important to find how berries compare with colored-flesh potatoes.

Objectives:

1. Determine the effect of genotype, storage time and processing (baking and chipping) on the total phenolic content, antioxidant activity and anthocyanin content of potato cultivars using spectrometric methods.
2. Evaluate the effect of genotype on phenolic composition. Screen the potato cultivars based on the techniques mentioned in Objective 1 and perform UPLC-MS analysis on the promising samples.
3. Study the effect of uncooked and processed potato phenolics on cell proliferation and apoptosis of HCT-116 and HT-29 human colon cancer cell lines.
4. Conduct sensory analysis to understand how inter-clonal differences, post-harvest storage and total phenolic content influence the sensory parameters of baked and chipped potato samples.
5. Quantify the phenolic and anthocyanin content of popular anthocyanin-rich berries such as blueberries, strawberries, raspberries and grapes, and compared them with baked and chipped colored-flesh potatoes.

CHAPTER II

REVIEW OF LITERATURE

1. Introduction to the Potato

The United Nations declared 2008 as the 'International Year of the Potato' to increase the world's focus on the potato for its role in providing food security and alleviating poverty. In the developing world, the poorest and undernourished families depend on potatoes as a major source of food and nutrition because of its highly available dietary energy. The potato is cheap and easy to grow and can give stable yield under conditions where other crops might fail. It is suitable to places where land is limited and labor is plentiful; conditions which characterize most of the developing parts of the world. Potatoes are a rich source of vitamins, minerals and phenolic compounds that play an important role in human health. Table 2.1. shows the chemical composition of potatoes (Leszczyński 1989). The phenolics, which are secondary metabolites, have been the subject of interest for researchers because of their promising role as bioactive compounds.

1.1. History and Significance of the Potato

The first potatoes were grown almost 7000 years ago near Lake Titacaca in the Andes region of Peru (Spooner et al. 2005). The potato soon became a part of Andean culture and religion with many myths and legends surrounding it. The Spanish introduced the potato to Europe in the 16th century when they arrived in Peru in search of gold. Before potatoes became a staple food across Europe, they were considered fit only for consumption by the famished humans and livestock. Steadily their consumption increased all over Europe and this eventually spread all over the world. Potatoes played a

Table 2.1. Typical composition of potato tubers (Leszczyński 1989)

Substance	Content (%)	
	Range	Mean
Dry matter	13.1 - 36.8	23.7
Starch	8.0 - 29.4	17.5
Reducing sugars	0.0 - 5.0	0.3
Total sugar	0.05 - 8.0	0.5
Crude fiber	0.17 - 3.48	0.71
Pectic substances	0.2 - 1.5	-
Total nitrogen	0.11 - 0.74	0.32
Crude protein (total nitrogen x 6.25)	0.69 - 4.63	2.00
Protein nitrogen in total nitrogen	27.3 - 73.4	54.7
Amide nitrogen	0.029 - 0.052	-
Amino acid nitrogen	0.065 - 0.098	-
Nitrates	0.0 - 0.05	-
Lipids	0.02 - 0.2	0.12
Ash	0.44 - 1.87	1.10
Organic acids	0.4 - 1.0	0.6
Ascorbic acid and dehydroascorbic acid*	1.0 - 54.0	10.0 - 25.0
Glycoalkaloids*	0.2 - 41.0	3.0 - 10.0
Phenolic compounds*	5.0 - 30.0	-

*In mg/100 gfw

key role in preventing scurvy in early European sailors and prevented deaths due to starvation. These same sailors, who carried potatoes as a major food source, introduced potatoes to China, India and Japan in the 17th century. In Europe and North America, the initially cultivated potatoes were grown from a few, genetically similar cultivars. As a result, the potatoes became vulnerable to pest and disease attack. In the 1840s, potato blight hit Europe and spread virally across the continent. The Irish working class was solely dependent on potatoes as the primary source of food. When the crop failed, it led to famine conditions leading to the death of more than a million people and massive migrations to Britain and North America. After this Great Irish Famine, potato was considered a crop suitable only for livestock across America till an effective fungicide

against blight was discovered by French botanist Alexander Millardet in 1883 (USPB 2011).

Potatoes arrived in the United States in 1621 as a gift sent by the Governor of Bermuda, Nathaniel Butler, to Francis Wyatt the Governor of Virginia. The initial cultivation was done in New Hampshire by early Scottish-Irish immigrants. There on, the crop spread all across America. Idaho, currently the largest producer of potatoes in the United States, started producing potatoes in 1836. But, it was only with the development of the Russet Burbank cultivar in 1872 that the Idaho potato industry actually began to flourish (USPB 2011).

The potato emerged as a global food in the 20th century. The former Soviet Union and other east European countries started cultivating potatoes extensively. Even today, the potato is the primary production crop in many eastern European nations. It became widely utilized as a snack food as well. Many food companies started making french fries and chips, and potato usage spread all across the world.

In 1995, potatoes became the first vegetable to be grown in space. Scientists from NASA and University of Wisconsin, Madison developed these potatoes to serve as a food source for astronauts on long space missions. From being called a poisonous devil's apple to being enjoyed in the form of french fries and chip, the potato has surely come a long way since its first cultivation in the Andes.

1.2. Potato Production

Potato (*Solanum tuberosum* L.) is one of the most extensively grown food crops across the world. Its world-wide production is ranked fourth after rice, wheat and maize. In 2009, the world production of potatoes exceeded 329 million metric tons spread over

18 million hectares of land (FAO 2009). China and India are the top two potato producers, producing more than a third of the world's potatoes, followed by Russia, Ukraine and the United States of America.

In the United States, the total potato production in 2009 was 19.5 million metric tons. Of these, approximately 60% of the potatoes were processed into various forms including chips, frozen french fries, dehydrated, canned and other products, 27% were sold as table stock and the remaining were feed stock, other products and non-sales. The total value of the potatoes sold in the United States in 2009 was estimated to be $ 3.26 billion; 7% higher than the previous year (USDA-NASS 2010). Almost 90% of the potatoes in the United States are planted in spring and harvested in fall. Potatoes harvested in the other seasons account for the remaining 10%. Idaho, where the Russet Burbank cultivar is most commonly grown, is the largest producer of potatoes, followed by Washington, Wisconsin and Colorado.

In 2009, Colorado produced 1 million metric tons of potatoes covering approximately 24,000 hectares of land. Ninety-two per cent of Colorado's potatoes are produced in San Luis Valley in southern Colorado (Anonymous 2011).

1.3. Potato Cultivars

Early Peruvian farmers developed four recognized potato species. Today, although the potato grown worldwide belongs to just one biological species, *Solanum tuberosum* L., there are more than 5000 cultivars known and cultivated around the world (Lutaladio and Castaldi 2009). These cultivars come in different colors, shapes, sizes and vary in taste, texture, and cooking characteristics (FAO 2008). Most of them are classified into four major groups - russets, reds, yellows and specialty. Russets are

characterized by their even, oval shape, brown net-textured skin, shallow eyes with flesh color varying from creamy white to light golden. Red potatoes are generally characterized by a rosy skin and a white flesh with a texture that is firm, smooth and moist. Yellow cultivars include Yukon Gold, which is a common baking cultivar. Specialty cultivars include colored cultivars and fingerlings. Potatoes may also be classified based on their waxiness. Waxy potatoes, which are good for boiling, have around 16-18% starch. Baking potatoes generally have more starch (20-22%), which gives them a mealy and floury texture.

Colorado grows around 100 potato cultivars and continues to develop new cultivars. Russets are the most popular group of potatoes grown in Colorado. Some popular red cultivars include Colorado Rose, Rio Colorado, and Sangres. Among the yellows, Yukon Gold is a popular cultivar. Fingerlings, All Blue, Mountain Rose, and Purple Majesty are some popular specialty cultivars (Anonymous 2011).

2. The Potato Plant

2.1. Plant Physiology

The potato plant grows about 100 cm tall and produces a tuber, which is actually classified as the stem (Figure 2.1). It belongs to the 'Solanaceae' (nightshade) family of flowering plants which also includes pepper, eggplant and tomato. *S. tuberosum* is further classified into two slightly different subspecies – *andigena*, which is adapted to short day conditions and is predominately cultivated in the Andes and *tuberosum*, which is the most widely cultivated species worldwide and believed to have been descended from European *andigena* and adapted to longer day conditions (Sukhotu and Hosaka 2006).

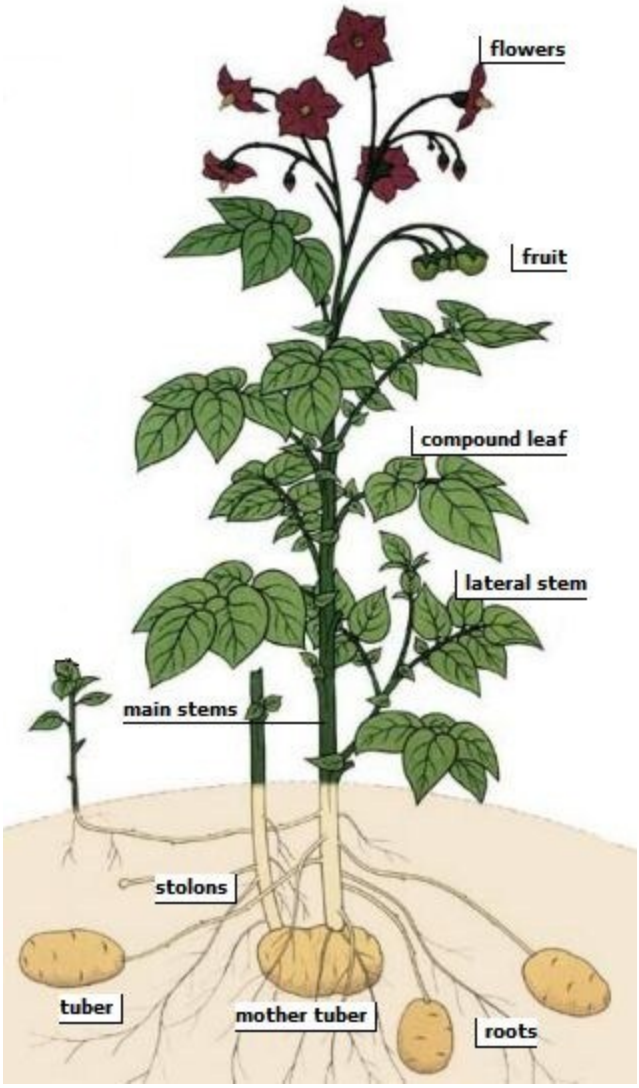


Figure 2.1. The potato plant (FAO 2008)

As the plant grows, it bears flowers which can be white, blue, pink, red or purple in color with yellow stamens. In general, plants bearing white flowers have white skinned tubers while those having colored flowers may have pinkish skins (Winch 2007). The leaves of the plant produce starch, which gets transferred to its underground stems to form tubers. As many as 20 tubers can be formed per plant near the soil surface. The tubers vary in size and shape, the average weight being around 300 g. The nutrients and moisture content of the soil influence the number of tubers reaching maturity.

2.2. Cultivation and Growth

Potato plants can be propagated via true seeds or by vegetative propagation. Commercially, vegetative propagation is the preferred method as it maintains genetic stability. Vegetative propagation will result in plants that grow vigorously, mature quickly and give higher yields. Each tuber can have two to ten buds, which can grow shoots and generate into new plants during favorable conditions (FAO 2008). Post-flowering, potato plants produce small green fruits, each of which can contain up to 300 true seeds. These fruits are inedible as they are high in glycoalkaloids.

The general phases of potato growth have been illustrated in Figure 2.2. In the first stage, the potato tuber is planted in well-drained soil and the tuber begins to develop roots and a shoot. In the second stage, the shoot starts developing leaves and branches through photosynthesis.

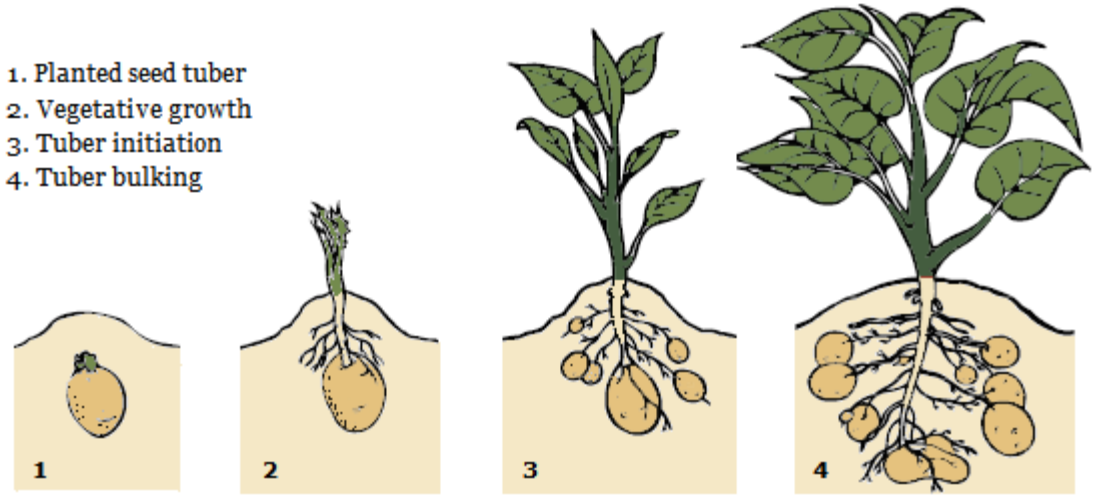


Figure 2.2. Stages of potato plant development (FAO 2008)

The third stage marks the initiation of tuber development followed by the fourth stage, where the tubers start bulking and develop their characteristic shape. The energy generated by the plant through photosynthesis is stored as starch in these tubers. At the

end of the growing season, the plant dies and detaches from the tubers. Tubers are collected using potato harvesters, which collect the plant and surrounding soil, and place it on chains where the tubers are separated from plant and soil debris.

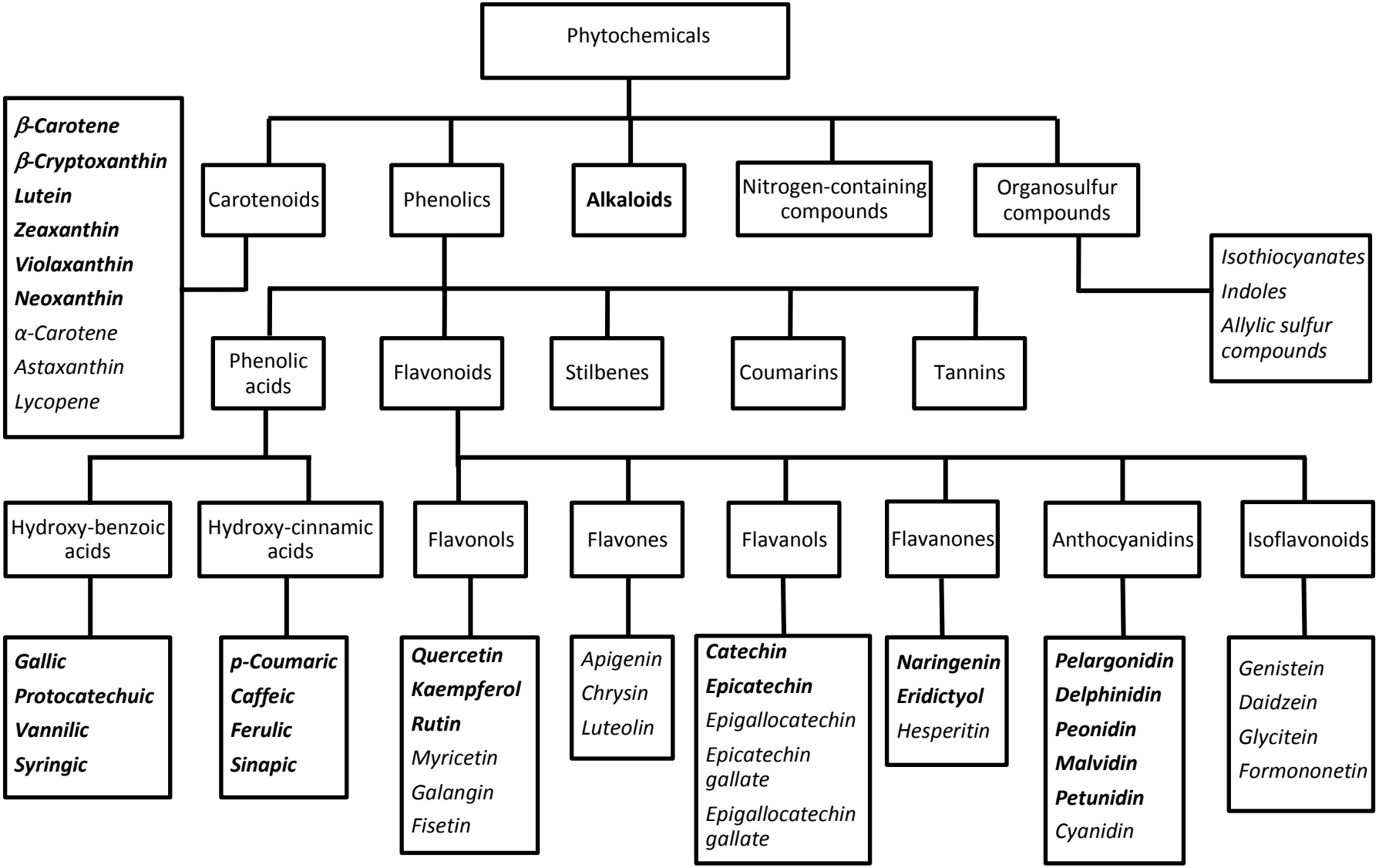
3. Bioactive Compounds found in Potatoes

Bioactive compounds or phytochemicals are secondary plant metabolites found in the potato and other plants that have been the subject of interest for researchers due to their promising role as health-modulators. Phytochemicals can be grouped into five major classes – polyphenols, carotenoids, alkaloids, nitrogen-containing and organo-sulfur compounds (Liu 2004) (Table 2.2.). Among these, polyphenols and carotenoids have been studied the most (Russo 2007). More than 8000 polyphenols have been identified and they can be further categorized into sub-groups comprised of phenolic acids, tannins, stilbenes, coumarins and flavonoids (anthocyanins) (Bravo 1998; Liu 2004). Phenolic acids and flavonoids are the most prominent phytochemical groups present in the potato. These compounds are commonly synthesized from phenylalanine, which is produced via the shikimate pathway (Dixon and Paiva 1995; Häkkinen 2000). The biosynthesis mechanism is shown in Figure 2.3.

3.1. Phenolic acids

Phenolic acids are distributed universally in the plant kingdom. Table 2.2 gives the general classification of phenolic acids. Phenolic acids can be derived from benzoic acid or cinnamic acid. Figure 2.4. gives the structures of some common phenolic acids. In the potato, most of the phenolic acids are present between the cortex and the peel of the potato tuber, and their content reduces towards the center of the tuber (Friedman 1997).

Table 2.2. Classification of dietary phytochemicals (Liu 2004) (Phytochemicals commonly found in potatoes are in '**Bold**')



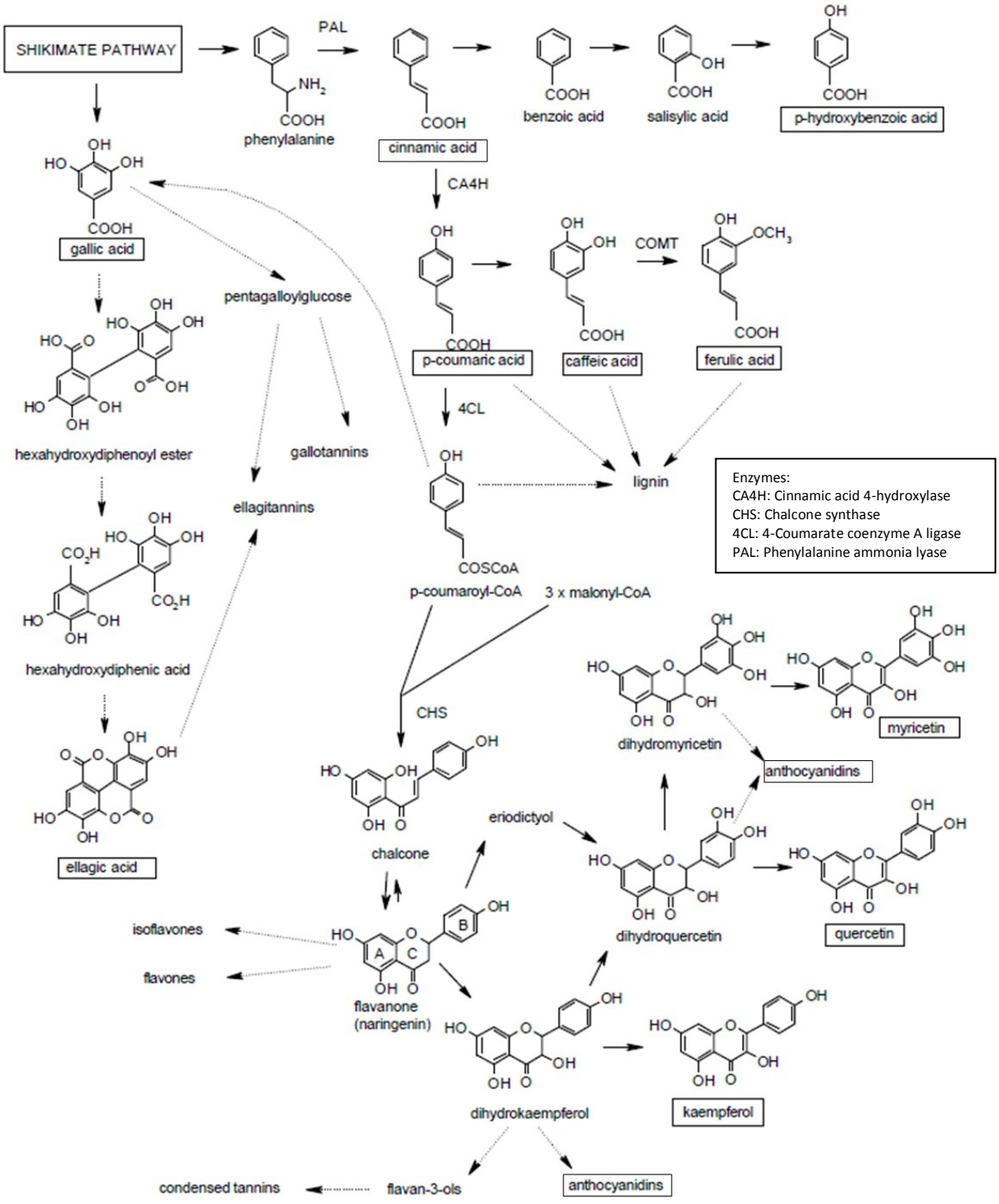
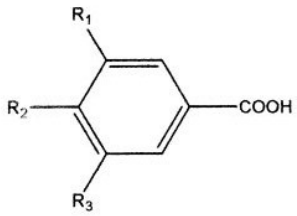


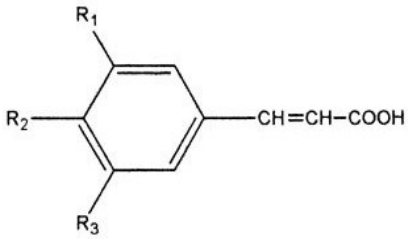
Figure 2.3. Biosynthesis of phenolic acids and flavonoids (Dixon and Paiva 1995; Häkkinen 2000) (Solid arrows indicate well-characterized single-enzyme reactions. Dotted arrows indicate less-characterized multi-enzyme reactions.)

Chlorogenic acid and caffeic acid are two of the most prominent phenolic acids reported in the potato followed by protocatechuic acid, *trans*-cinnamic acid, *para*-coumaric acid, ferulic acid, vanillic acid, gallic acid, syringic acid, and salicylic acid (Lewis et al. 1998; Shakya and Navarre 2006; Reddivari et al. 2007a; Reddivari et al. 2007b).

A



B



Benzoic acid Derivatives	Substitutions		
	R_1	R_2	R_3
p-Hydroxybenzoic	H	OH	H
Protocatechuic	H	OH	OH
Vannilic	CH_3O	OH	H
Syringic	CH_3O	OH	CH_3O
Gallic	OH	OH	OH

Cinnamic acid Derivatives	Substitutions		
	R_1	R_2	R_3
p-Coumaric	H	OH	H
Caffeic	OH	OH	H
Ferulic	CH_3O	OH	H
Sinapic	CH_3O	OH	CH_3O

Figure 2.4. Structure of common phenolic acids derived from benzoic acid (A) and cinnamic acid (B) (Liu 2004)

Chlorogenic acid (Figure 2.5.), which is an ester of caffeic and quinic acids, along with its isomers is the most prominent phenolic acid accounting for up to 90% of the total phenolic compounds in potatoes (Dao and Friedman 1992). 5-caffeoylquinic acid is the major isomer, which is complemented by 3- and 4-caffeoylquinic acids (Friedman 1997).

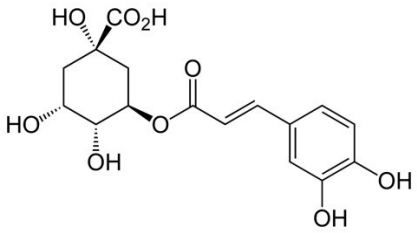


Figure 2.5. Structure of chlorogenic acid

Chlorogenic acid is reported to range from 10 to 19 mg *per* 100 g fresh weight (gfw) potato when measured by ultraviolet spectrophotometry (Dao and Friedman 1992). A wider range, 13.2 to 68.3 mg *per* 100 gfw potato, has also been reported when measured using HPLC (Reddivari et al. 2007a; Reddivari et al. 2007b). Chlorogenic acid content in the skin (100 to 400 mg *per* 100 gfw) is greater than that in the tuber flesh (3 to 90 mg *per* 100 gfw) (Lewis et al. 1998). This wide range can be explained due to intervarietal differences. In a study, a 20-fold difference in the chlorogenic acid content has been observed between the lowest reported (*S. bulbocastanum*) and highest reported (C097226-2R/R) cultivars (Navarre et al. 2011). Pigmented cultivars such as Mountain Rose and Purple Majesty, and non-pigmented cultivars such as Yukon Gold had an approximately 10-fold difference in the chlorogenic acid content (Stushnoff et al. 2008).

Caffeic acid is the second most abundant phenolic acid in the potato. The caffeic acid content can range from 310 to 420 μg *per* 100 gfw potato (Reddivari et al. 2007a; Reddivari et al. 2007b). The caffeic acid content in pigmented cultivars is greater than that in non-pigmented cultivars. A 100-fold difference was observed in the caffeic acid contents of Divina, which is a yellow cultivar and Pollunta chata, which is a purple cultivar (Navarre et al. 2011). The other phenolic acids are present in lower concentrations. In potato peels, protocatechuic acid, vanillic acid and sinapic acid ranged from 10 to 40 mg *per* 100 gfw, 2 to 20 mg *per* 100 gfw, and 2 to 25 mg *per* 100 gfw, respectively. Gallic acid, syringic acid, *para*-coumaric acid, ferulic acid, salicylic acid and cinnamic acid together ranged from 0 to 3 mg *per* 100 gfw. In the tuber flesh, protocatechuic acid ranged from 5 to 20 mg *per* 100 gfw while vanillic acid and *para*-coumaric acid together ranged from 0.5 to 4 mg *per* 100 gfw. The other phenolic acids

ranged from 0 to 1 mg *per* 100 gfw together (Lewis et al. 1998). The values reported in literature are listed in Table 2.3.

Table 2.3. Major phenolic acids found in the potato

Phenolic acid	**Biological sample**	**Reported range *(per 100 gfw)***	**Reference**
Chlorogenic acid	Potato tuber	10 - 19 mg	(Dao and Friedman 1992)
		13 - 68 mg	(Reddivari et al. 2007a; Reddivari et al. 2007b)
		21 - 28.3 mg	(Verde Méndez et al. 2004)
	Potato skin	100 - 400 mg	(Lewis et al. 1998)
	Potato peel	4.4 - 34 mg	(Im et al. 2008)
	Potato flesh	3 - 90 mg	(Lewis et al. 1998)
		0.35 - 12 mg	(Im et al. 2008)
Caffeic acid	Potato tuber	0.31 - 0.42 mg	(Reddivari et al. 2007a; Reddivari et al. 2007b)
		0.73 - 1.12 mg	(Verde Méndez et al. 2004)
	Potato peel	0.39 - 1.20 mg	(Im et al. 2008)
	Potato flesh	0.01 - 0.11 mg	(Im et al. 2008)
Protocatechuic acid	Potato peel	10 - 40 mg	(Lewis et al. 1998)
	Potato flesh	5 - 20 mg	(Lewis et al. 1998)
Vanillic acid	Potato peel	2 - 20 mg	(Lewis et al. 1998)
Sinapic acid	Potato peel	2 - 25 mg	(Lewis et al. 1998)

Health-benefiting properties

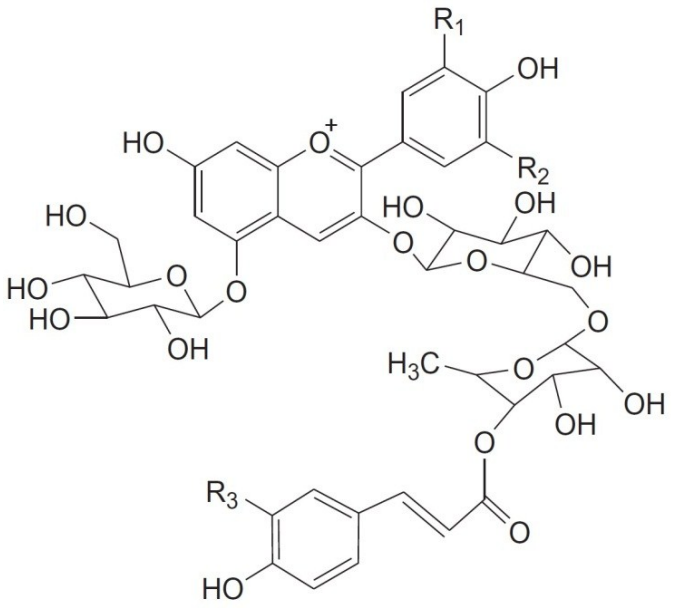
A number of studies have examined the antioxidant, anti-mutagenic, and anti-cancer effects of phenolic acids found in potatoes. Pure soybean oil treated with freeze-dried extracts from peels of six potato cultivars showed a reduction in the peroxide value during storage (Rehman et al. 2004). Potato peel extracts, which are rich in polyphenolic compounds have been shown to have a protective effect on carbon tetrachloride-injured livers of rats. Rats treated with potato peel extracts had their livers protected from

glutathione depletion and reduced a variety of hepatic oxidative stress markers such as aspartate transaminase, alanine transaminase and malondialdehyde (Singh et al. 2008). *Solanum jamesii* tuber extracts showed anti-proliferative and cytotoxic effects against HT-29 human colon cancer, and LNCaP human prostate cancer cell lines (Nzaramba et al. 2009). *In vivo* oxidation of lipoproteins, especially low-density lipoproteins (LDL), has been implicated in causing heart disease. Chlorogenic acid and other polyphenols exhibit strong antioxidant activity towards heart disease-related LDLs. Thus, polyphenols may indirectly reduce the risk of heart disease (Vinson et al. 1995). Chlorogenic acid has been shown to suppress the release of glucose in the blood (Bassoli et al. 2008). Hence, potatoes with low glycemic index may prove beneficial for diabetic patients and result in a lower risk of type II diabetes (Legrand and Scheen 2007). In a cellulose model system, chlorogenic acid competes with nitrite to bind with benzo(*a*)pyrene thus blocking nitrosamine formation (Friedman 1997). It has been reported that chlorogenic acid can inhibit matrix metalloproteinase (MMP-9), an enzyme linked to tumor cell invasion and metastasis (Jin et al. 2005).

3.2. Anthocyanins

Anthocyanins are water soluble flavanoids conferring plants colors such as red, blue and purple (Wang et al. 2012). Chemically, anthocyanins are glycosylated polyhydroxy and/or polymethoxy derivatives of the 2-phenylbenzopyrylium of flavylium salt (Mazza and Miniati 1993; von Elbe and Schwartz 1996). Glycosylated anthocyanins can appear acylated with aromatic acids such as *para*-coumaric, caffeic, ferulic, sinapic, gallic or *para*-hydroxybenzoic acids, and/or aliphatic acids such as malonic, acetic,

malic, succinic or oxalic acids (Figure 2.6.) (Giusti and Wrolstad 2003; Lachman et al. 2009).



	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Peonidin	OCH_3	H
Delfinidin	OH	OH
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

	R_3
Ferulic acid	OCH_3
Caffeic acid	OH
P-cumaric acid	H

Figure 2.6. Structure of major anthocyanins found in red and purple-fleshed potatoes (Ieri et al. 2011)

Many glycosylated anthocyanins acylated with *para*-coumaric and ferulic acid have been reported in colored potatoes. 3-rutinoside-5-glucoside and 3-rutinoside derivatives of pelargonidin, petunidin, malvidin, cyanidin, peonidin and delphinidin have been reported in colored potatoes (Lewis et al. 1998; Eichhorn and Winterhalter 2005). Purple-fleshed potatoes contain anthocyanins such as petunidin- and malvidin-3-rutinoside-5-glycosides acylated with *para*-coumaric and ferulic acid while red-fleshed potatoes have pelargonidin- and peonidin-3-rutinoside-5-glycosides acylated with *para*-coumaric and ferulic acid (Lewis et al. 1998; Naito et al. 1998). This has been confirmed in another study which found pelargonidin-based anthocyanins in the highest

concentration in Mountain Rose, a red-fleshed cultivar and petunidin-3-coum-rutinoside-5-glucoside in Purple Majesty, a purple-fleshed cultivar (Stushnoff et al. 2008). In colored potatoes, anthocyanins can range from 17-20 mg *per* 100 g in red-fleshed potatoes and 20-38 mg *per* 100 g in purple-fleshed potatoes (Brown et al. 2005). Anthocyanins found in red and purple-fleshed potatoes have been listed in Table 2.4.

Health-benefiting properties

Numerous health benefits such as antioxidant activity, anti-cancer and anti-inflammatory properties, have been attributed to consumption of anthocyanin-rich foods (Hidalgo et al. 2012; Tsuda 2012; Wang et al. 2012). The chemopreventive mechanisms of anthocyanins include scavenging-free radicals, reducing cell proliferation, up-regulating/inducing apoptosis and modulating mitogen-activated protein kinase (MAPK) activities (Afaq et al. 2005; Jing et al. 2008; Shin et al. 2009).

Anthocyanin fraction from colored-flesh potatoes induces apoptosis in LNCaP (androgen dependent) and PC-3 (androgen independent) prostate cancer cells via caspase-dependent and independent pathways (Reddivari et al. 2007c). Anthocyanin-rich Mountain Rose cultivar showed greater inhibition of carcinogenesis in rats with chemically-induced breast cancer as compared with white Russet Burbank cultivar (Thompson et al. 2009). A study showed that anthocyanins were anti-inflammatory and lowered plasma concentrations of C-reactive protein, 8-hydrodeoxyguanosine, and interleukin-6 in healthy men on an anthocyanin-rich purple-fleshed potato diet as compared with men fed with a white potato diet (Kaspar et al. 2011).

Table 2.4. Anthocyanins reported in red and purple-fleshed potatoes

Anthocyanin	Potato genotype	Reference
Pet-3-rut-5-glc	Hermanns Blaue	(Eichhorn and Winterhalter 2005)
	Purple Majesty	(Stushnoff et al. 2008)
Pet-3-coum-rut-5-glc	Hermanna Blaue, Shetland Black, Vitelotte	(Eichhorn and Winterhalter 2005)
	I53, Stage II Blue, Urenika, Arran Victory, Blue Derwent, Kowiniwini, Moe Moe, Ngaoutiouti, Old Red, Poiwa, Raupi, Skerry Blue	(Lewis et al. 1998)
	Purple Majesty	(Stushnoff et al. 2008)
Pet-3-caf-rut-5-glc	Vitelotte Noire	(Ieri et al. 2011)
Pet-3-ferul-rut-5-glc	Purple Majesty	(Stushnoff et al. 2008)
Pel-3-rut	Red Flesh, Desirée, Red Rocks	(Lewis et al. 1998)
	Mountain Rose	(Stushnoff et al. 2008)
Pel-3-rut-5-glc	Highland Burgundy Red	(Eichhorn and Winterhalter 2005)
	Mountain Rose	(Stushnoff et al. 2008)
Pel-3-coum-rut-5-glc	Highland Burgundy Red	(Eichhorn and Winterhalter 2005)
	Desirée, I29, O60/1, Red Rascal, Red Rocks	(Lewis et al. 1998)
	Mountain Rose	(Stushnoff et al. 2008)
Pel-3-ferul-rut-5-glc	Mountain Rose	(Stushnoff et al. 2008)
Peo-3-rut-5-glc	Shetland Black	(Eichhorn and Winterhalter 2005)
Peo-3-coum-rut-5-glc	Shetland Black	(Eichhorn and Winterhalter 2005)
	Red Flesh	(Lewis et al. 1998)
	Mountain Rose, Purple Majesty	(Stushnoff et al. 2008)
Mal-3-rut-5-glc	Vitelotte	(Eichhorn and Winterhalter 2005)
	Vitelotte Noire	(Ieri et al. 2011)
Mal-3-coum-rut-5-glc	Vitelotte	(Eichhorn and Winterhalter 2005)
	Stage II Blue, Urenika, Kowiniwini, Skerry Blue	(Lewis et al. 1998)
	Mountain Rose	(Stushnoff et al. 2008)
Mal-3-caf-rut-5-glc	Vitelotte Noire	(Ieri et al. 2011)
Mal-3-ferul-rut-5-glc	Vitelotte Noire	(Ieri et al. 2011)
Del-3-coum-rut-5-glc	Purple Majesty	(Stushnoff et al. 2008)
	Vitelotte Noire	(Ieri et al. 2011)

Bioactive compounds in the potato are affected by genotype, environment, storage conditions and processing. The effect of these parameters will be discussed in further details in the following chapters. Figure 2.7. gives an overview of the parameters discussed.

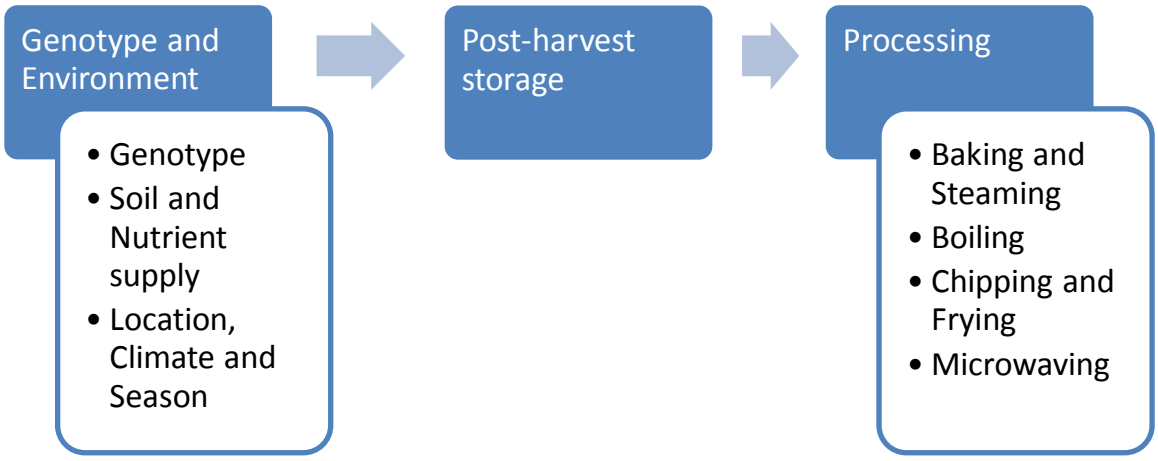


Figure 2.7. Overview of parameters affecting bioactive compounds in the potato

4. Effect of Genotype and Environment on Potato Bioactives

4.1. Genotype

Around 8000 years ago, early Peruvian farmers developed four recognized potato species. Today, although the potato grown worldwide belongs to just one biological species, *Solanum tuberosum* L., there are more than 5000 cultivars known and cultivated around the world (Lutaladio and Castaldi 2009). These cultivars come is different colors, shapes, sizes and vary in taste, texture, and, cooking characteristics (FAO 2008). Most of them are classified into four major groups - russets, reds, yellows and specialty. The potato genotype has a significant influence on the profile of phenolic compounds and their quantity. A study screening hundreds of cultivated and wild potato species reported a 15-fold difference in their phenolic contents (Navarre et al. 2009). Another study

looking at 74 Andean potato genotypes reported an 11-fold variation in their phenolic content (André et al. 2006). The total phenolic content of potatoes can range from 28 to 400 mg *per* 100 g potato depending on the cultivar (Reddivari et al. 2007b; Reddivari et al. 2007a; Stushnoff et al. 2008). It has been reported that Granola and Russet Norkotah cultivars had almost twice the phenolic concentration as compared with the Yukon Gold cultivar (Al-Saikhan et al. 1995).

The total phenolic content of colored-flesh potatoes is more than their white-fleshed counterparts (Jansen and Flamme 2006; Stushnoff et al. 2008; André et al. 2009). Even among the colored-flesh cultivars, violet- and purple-fleshed potatoes had higher total phenolic content as compared to red-fleshed cultivars (Jansen and Flamme 2006; Reddivari et al. 2007a; Reddivari et al. 2007b).

4.2. Soil and Nutrient supply

Nitrogen fertilizers applied at 100 and 200 kg/ha did not influence the anthocyanin content of 23 potato genotypes (Jansen and Flamme 2006). A similar observation was reported in another study which tested effect of fertilizers with varying levels of nitrogen, phosphorus, sodium and magnesium on the carotenoid content of potatoes (Kotíková et al. 2007). The sale of organic foods has increased between 17% and 21% each year since 1997 (Bellows et al. 2008). However, a study comparing organically and conventionally grown potatoes did not find a difference in their antioxidant activity (Rosenthal and Jansky 2008). This was confirmed by another study which reported no difference in the polyphenolic content or antioxidant activity of conventionally and organically grown potatoes with polyphenols from conventionally

grown potatoes being more resistant to losses due to cooking as compared with their organic counterparts (Faller and Fialho 2009).

4.3. Location, Climate and Season

Researchers have reported the effect of location and climate on the bioactive compounds in potatoes. However, the results have been varied due to the interplay of numerous factors such as temperature, rainfall, soil, altitude and light conditions at a given location influencing the bioactive compounds in the potato. Reddivari et al. (2007b) studied the effect of two locations – Dalhart and McCook, both in Texas, USA, which differ in their altitude, latitude, mean annual temperature, rainfall and production season, on the bioactive compounds of 25 potato genotypes. They reported that the genotypes grown in McCook had greater antioxidant activity and total phenolic content but lower total carotenoid content than those grown in Dalhart. A similar observation was reported for potatoes grown in Colorado and Texas. Colorado-grown potatoes had a higher anthocyanin (2.5 times) and phenolic (1.4 times) content as compared with Texas-grown potatoes possibly due to the cooler climate and longer days (Reyes et al. 2004). Brown et al. (2008) also observed higher anthocyanin content in genotypes grown at higher altitudes but the carotenoid content and antioxidant capacity did not vary.

However, many researchers have reported insignificant influence of location on potato bioactives. Colored-flesh potatoes grown in two locations in Germany did not show a difference in their anthocyanin content (Jansen and Flamme 2006). Similarly, the carotenoid content of potatoes grown in two locations with varying altitude in Czech Republic did not differ (Kotíková et al. 2007). Ezekiel et al. (2008) studied two

genotypes grown at three locations in India having similar altitude but different mean temperatures and found no differences in the total phenolic content.

Similar to the effects of location, seasonal influence has resulted in varied outcomes with respect to potato bioactives. Reddivari et al. (2007b) reported significant influence of year on the total phenolic content and total carotenoid content of 25 specialty potato genotypes. However, a consistent trend was not observed for all the genotypes. Total phenolic and carotenoid content increased in some genotypes while a decrease was seen for others. Another study observed variations in the total phenolic content and antioxidant activity of white-, yellow-, red- and purple-fleshed potato genotypes over a five year period (Stushnoff et al. 2008). Three other studies also reported significant influence of production year on the potato bioactives (Kotíková et al. 2007; Rosenthal and Jansky 2008; André et al. 2009). Contrary to this, Jansen and Flamme (2006) reported no influence of year in the anthocyanin content of 23 colored-flesh potato genotypes. This was observed in spite of the different weather conditions in the two years.

Thus, it is essential to understand the effects of environment and genotype on the bioactive compounds in potatoes. It has been observed that the genotypic factors are more prominent as compared to environmental factors (Reddivari et al. 2007b). Efforts should be focused towards breeding cultivars that are more resistant to the influence of environment thus making the quantification of potato bioactive compounds predictable for a given genotype.

5. Effect of Post-harvest Storage on Potato Bioactives

Harvested potatoes can be stored for up to one year before being processed or consumed (Herrman et al. 1996). This makes it critical to understand the effect of storage on the bioactive compounds in potatoes. It has been observed that storage generally increases the phenolic content of potatoes. However, prolonged storage can also cause a decrease or maintain the level of phenolic content. Stushnoff et al. (2008) studied the effect of storage (263 days) at 5°C on the total phenolic content of eight pigmented and non-pigmented genotypes. They observed that the phenolic content of some genotypes elevated by up to 100% while there was little or no change for the other genotypes. However, one study reported that storage at 4°C for 135 days in 86% humidity did not alter the anthocyanin content of six genotypes (Jansen and Flamme 2006). Rosenthal and Jansky (2008) reported an increase in the antioxidant activity of potatoes stored at 5.6°C for 5.5 months. Blessington et al (2010) studied the effect of storage at 4°C, 20°C and reconditioning at 20°C after storage at 4°C in eight potato genotypes. They observed that only the reconditioned potatoes had a significant increase in their total phenolic content.

However, the total carotenoid content increased after all three storage regimes. Lewis et al (1999) observed that storage at 4°C for 180 days caused an increase in the anthocyanin concentration of four pigmented genotypes. However, the total phenolic content increased slightly up to 120 days after which it remained constant. However, tubers stored at 10°C, 18°C and 26°C did not show an increase in their anthocyanin concentration.

Low temperature storage can induce phenylalanine ammonia-lyase (PAL), a key regulatory enzyme in the biosynthesis of polyphenols including anthocyanins, which can

cause an increase in the phenolic content (Jiang and Joyce 2003). In colored-flesh potatoes, cold storage conditions are known to cause the conversion of starch to sugar (Isherwood 1976), which can up-regulate genes coding for *dihydroflavonol reductase (DFR)* and *anthocyanidin synthase (ANS)*, which are involved in anthocyanin biosynthesis, and hence potentially cause an increase in the anthocyanin concentration (Vitrac et al. 2000; Gollop et al. 2001; Gollop et al. 2002; Solfanelli et al. 2006).

6. Effect of Processing on Potato Bioactives

It is well known that processing changes the physical and chemical composition of foods (Spanos et al. 1990; Price et al. 1997), thus, affecting their antioxidant activity (Nicoli et al. 1999; Dewanto et al. 2002). Potatoes are almost always consumed after processing (baked, chipped, fried, boiled or microwaved) making it critical to understand the effect of such processing techniques on the activity and composition of bioactive compounds in potatoes. Literature has reported conflicting findings regarding the effect of processing on potato bioactive compounds.

6.1. Baking and Steaming

Dao and Friedman (1992) reported a 100% loss in the chlorogenic acid content of potatoes baked at 212°C for 45 minutes, which suggested that chlorogenic acid is susceptible to heat. However, some studies have observed a reduction but not a complete loss of chlorogenic acid. Im et al. (2008) studied the effect of baking (oven heating) on the chlorogenic acid content of Superior potatoes. They reported 90% to 100% retention of the chlorogenic acid and its isomer. This discrepancy in observations could be attributed to different processing conditions. Im et al. subjected their samples to a milder treatment (200°C for 10 minutes) as compared to Dao and Friedman. Also, unlike Dao and Friedman, Im et al. wrapped their samples in aluminum foil, which potentially

prevented complete loss in the chlorogenic acid content. Furthermore, Dao and Friedman conducted their research using ultra violet spectrometry while Im et al. employed advanced LC-MS/MS method.

Baking led to an increase in the total phenolic content and antioxidant activity of eight potato genotypes. Samples had greater levels of chlorogenic acid, caffeic acid, *para*-coumaric acid and vanillic acid (Blessington et al. 2010). Baking for 30 minutes increased the total phenolic content and chlorogenic acid content in three potato genotypes. If the baking was continued for 45 minutes, the total phenolic content decreased. However, the phenolic content was still more than that of raw potatoes (Navarre et al. 2010). This increase could be due to improvement in the extractability of phenolic compounds as cooking weakens the matrix, and inactivates enzymes that use phenolic compounds as substrate (Ezekiel et al. 2011). The effects of baking cannot be generalized for all potato clones as they differ depending on potato genotype. It was seen that baking significantly altered the total phenolic content of Dakota Pearl cultivar but not of the Nordonna cultivar. The total phenolic content of Red Norland cultivar is altered approximately equally by baking (Xu et al. 2009). Hence, it is critical to determine the effects of baking for every cultivar for a range of temperature-time combinations.

Steam heating on 'high' for 10 minutes lead to a retention of 45-65% of chlorogenic acid and its isomer (Im et al. 2008). Another study reported that steam-cooking of potato strips caused a 58% loss in the chlorogenic acid content (Tudela et al. 2002). However, an increase in the phenolic content has also been reported. Steaming for 15 minutes increased the total phenolic content and chlorogenic acid content in three

potato genotypes (Navarre et al. 2010). Im et al. used "plugs" of potatoes, while Navarre et al steamed whole potatoes with the skin on, which could explain their observations.

6.2. Boiling

Boiling reduced or, in some cases, retained, or enhanced the total phenolic content and antioxidant activity of potato genotypes with respect to uncooked samples. Potatoes boiled in water for 30 minutes had a 60% loss in their chlorogenic acid content (Dao and Friedman 1992). It was also reported that the loss of chlorogenic acid and its isomer were dependent on the salt concentration in the water. One per cent salt led to 20-40% loss while 3% salt caused 70% and 40% loss in chlorogenic acid and its isomer respectively (Im et al. 2008). Researchers found that boiling for 20 minutes did not alter the phenolic acid content but significantly decreased the anthocyanin content of colored-flesh cultivars (Mulinacci et al. 2008). Other researchers reported that boiling for 18 minutes caused an increase in the total phenolic content and chlorogenic acid content in two white-fleshed and one purple-fleshed potato genotypes (Navarre et al. 2010). Boiled samples had greater levels of chlorogenic acid, caffeic acid and vanillic acid (Blessington et al. 2010). A possible reason suggested was the increase in the extractability of phenolic compounds from cooked samples, similar to the observations for baked potato samples.

6.3. Chipping and Frying

Literature reports conflicting observations on the effect of frying on potato bioactive compounds, similar to baking or boiling the potatoes. Frying and sautéing led to 20% to 30% loss in the content of chlorogenic acid and its isomer (Im et al. 2008). Potato strips fried in sunflower oil at 190°C for four minutes led to a loss of 76% in the chlorogenic acid content and 66% in case of caffeic acid derivatives (Tudela et al. 2002).

As seen for baked or boiled potatoes, an increase in the total phenolic content post-frying has also been reported. Frying led to an increase in the total phenolic content and antioxidant activity of eight potato genotypes. Samples had greater levels of chlorogenic acid, caffeic acid, *para*-coumaric acid, vanillic acid (Blessington et al. 2010). In the Piccolo cultivar, stir-frying for 18 minutes led to an increase in the total phenolic content (Navarre et al. 2010). Both these reports also claim that the extractability of phenolic compounds improved after frying which led to an increase in the total phenolic content. An increase in antioxidant activity was also observed which could be due to the presence of antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and tocopherols added to commercial cooking oils to prevent rancidity.

6.4. Microwaving

Microwaving led to an increase in the total phenolic content and antioxidant activity of eight potato genotypes. Samples had greater levels of chlorogenic acid, caffeic acid, *para*-coumaric acid, vanillic acid and (-) epicatechin (Blessington et al. 2010). Microwaving resulted in a 45% loss in the chlorogenic acid content of potatoes (Dao and Friedman 1992). A recent study reported a loss of 40% in the chlorogenic acid content and 20% in its isomer (Im et al. 2008). It was also observed that these losses decreased with the lower microwave power level (Barba et al. 2008). In purple-fleshed cultivars, microwaving did not alter the phenolic acid content but there was a significant decrease (16-29%) in the anthocyanin content (Mulinacci et al. 2008). Microwaving increased the total phenolic content and chlorogenic acid content in two white-fleshed and one purple-fleshed potato genotypes (Navarre et al. 2010).

The above review of literature indicates that the content, composition and bioactivity of potato bioactive compounds depend on genotype, environment, storage, and processing method. Better understanding of how these variables interact with each other might help with development of mathematical models to predict the bioactive compounds levels and bioactivity of the potato products.

CHAPTER III

STORAGE ELEVATES PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY BUT SUPPRESSES ANTI-PROLIFERATIVE AND PRO-APOPTOTIC PROPERTIES OF COLORED-FLESH POTATOES AGAINST HUMAN COLON CANCER CELL LINES

Abstract

Colored-flesh potatoes are an excellent source of health-benefiting dietary polyphenols, but are stored for up to 3-6 months before consumption. This study investigated the effect of simulated commercial storage conditions on antioxidant activity (DPPH, ABTS), phenolic content (FCR) and composition (UPLC-MS), and anti-cancer properties (an early (HCT-116) and advanced stage (HT-29) human colon cancer cell lines) of potato bioactive compounds. Extracts from seven potato clones of differing flesh colors (white, yellow and purple) before and after 90 days of storage were used in this study. Antioxidant activity of all clones increased with storage however an increase in total phenolic content was observed only in purple-fleshed clones. Advanced purple-fleshed selection CO97227-2P/PW had greater levels of total phenolics and monomeric anthocyanins, and antioxidant activity and a diverse anthocyanin composition as compared with Purple Majesty. Purple-fleshed potatoes were more potent in suppressing proliferation and elevating apoptosis of colon cancer cells compared with the white- and yellow-fleshed potatoes. The extracts from both fresh and stored potatoes (10 - 30 µg/ml) suppressed cancer cell proliferation and elevated apoptosis compared with the solvent control, but these anti-cancer effects were more pronounced with the fresh potatoes. Storage duration had a strong positive correlation with antioxidant activity and

percentage of viable cancer cells and a negative correlation with apoptosis induction. These results suggest that though the antioxidant activity and phenolic content of potatoes were increased with the storage, the anti-proliferative and pro-apoptotic activities were suppressed. Thus, while assessing the effects of farm to fork operations on health-benefiting properties of plant foods, it is critical to use quantitative analytical techniques in conjunction with *in vitro* and/or *in vivo* biological assays.

1. Introduction

The potato (*Solanum tuberosum* L.) is the fourth most important food crop worldwide and is an important vegetable crop in the United States with *per capita* consumption of approximately 54 kg (Burlingame et al. 2009). Potatoes are a good source of carbohydrates, minerals and vitamins, and also rich in antioxidant polyphenols and carotenoids. Colored-flesh potatoes are gaining popularity among consumers due to greater levels of phenolic acids, anthocyanins and carotenoids. The total phenolic content of colored-flesh potatoes (90 to 400 mg GAE/100 gfw) (Stushnoff et al. 2008) is generally comparable to that of common berries such as strawberries, blueberries and cranberries (100 to 412 mg GAE/100 gfw) (Zheng and Wang 2002; Zheng et al. 2007; You et al. 2010). However, potatoes are relatively inexpensive and can be consumed in larger quantities in one meal, and hence can contribute to maintaining a healthy population.

Chlorogenic acid, caffeic acid, and ferulic acid are among the prominent phenolic acids present in the potato, while para-coumaric acid, sinapic acid, and vanillic acid are present in minor quantities (Mattila and Hellström 2007). Purple-fleshed potatoes contain anthocyanins such as petunidin- and malvidin-3-rutinoside-5-glycosides acylated with

para-coumaric and ferulic acid while red-fleshed potatoes have pelargonidin- and peonidin-3-rutinoside-5-glycosides acylated with para-coumaric and ferulic acid (Lewis et al. 1998; Naito et al. 1998). Violaxanthin, antheraxanthin, lutein and zeaxanthin are the major carotenoids found in potatoes (Breithaupt and Bamedi 2002; Morris et al. 2004).

A number of researchers have examined the antioxidant, anti-mutagenic, and anti-cancer effects of potato polyphenols. Pure soybean oil treated with freeze-dried extracts from peels of six potato cultivars showed a reduction in the peroxide value (Rehman et al. 2004). In our previous study, *Solanum jamesii* tuber extracts showed anti-proliferative and cytotoxic effects against HT-29 human colon cancer, and LNCaP human prostate cancer cell lines (Nzaramba et al. 2009). Chlorogenic acid and other polyphenols also exhibit strong antioxidant activity towards heart disease-related LDLs and thus may indirectly reduce the risk of heart disease (Vinson et al. 1995). Red- and purple-fleshed potato cultivars showed greater antioxidant potency suggesting a role of anthocyanins as antioxidants (Onyeneho and Hettiarachchy 1993). We have previously reported that anthocyanin fractions from potato extracts were pro-apoptotic and induce apoptosis via both caspase-dependent and -independent pathways in LNCaP (androgen-dependent) and PC-3 (androgen-independent) prostate cancer cell lines (Reddivari et al. 2007c). The chemopreventive mechanisms of anthocyanins include scavenging-free radicals, reducing cell proliferation, up-regulating/inducing apoptosis and modulating mitogen-activated protein kinase (MAPK) activities (Afaq et al. 2005; Jing et al. 2008; Shin et al. 2009). A recent study using healthy men, showed that potato anthocyanins were anti-inflammatory and lowered plasma concentrations of C-reactive protein, 8-hydrodeoxyguanosine, and

interleukin-6 in men consuming anthocyanin-rich purple potatoes compared with white potatoes (Kaspar et al. 2011).

Literature suggests that cold storage (~5 °C) of potatoes either leads to an increase in the phenolic content or keeps it constant (Mqndy et al. 1966; Lewis et al. 1999; Stushnoff et al. 2008; Blessington et al. 2010). A similar observation has been reported for antioxidant activity (Spychalla and Desborough 1990; Mizuno et al. 1998; Rosenthal and Jansky 2008). Little information is available on effect of storage on bioactivity and how analytical data (total phenolic and anthocyanin content, antioxidant activity) correlates with bioactivity (cell proliferation and apoptosis) before and after prolonged storage. In this study, we have investigated the effects of storage on the content, composition and antioxidant activity of potatoes, and on potato anti-cancer properties using HCT-116 and HT-29 colon cancer cell lines.

Antioxidant activity of seven potato clones, White – Atlantic, Yellow – Yukon Gold, CO97232-2R/Y, AC97521-1R/Y, and Purple – Purple Majesty, CO97215-2P/P and CO97227-2P/PW increased with storage. Phenolic content increased initially with storage followed by a decrease. But, after 90 days of storage, purple-fleshed potatoes showed significantly higher levels of phenolics compared with initial levels. Fresh purple-fleshed potato extracts showed more potent anti-proliferative and pro-apoptotic properties in HCT-116 and HT-29 cell lines compared with extracts of white- and yellow-fleshed cultivars. Storage reduced the anti-proliferative and pro-apoptotic properties of all clones tested. In summary, even though the content of phenolics increased, the anti-cancer activity decreased with storage. These findings suggest that it is critical to use analytical

techniques in conjunction with *in vitro* and/or *in vivo* functional assays in assessing the effects of treatments on health-benefiting properties of plant foods.

2. Materials and Methods

2.1. Chemicals

Solvents for the extractions were purchased from the Department of Central Receiving, Colorado State University (Fort Collins, CO). Reagents and chemicals for total phenolics, monomeric anthocyanins and antioxidant activity assays, and phenolic acid standards were procured from Sigma (St. Louis, MO). Gallic acid was purchased from Fisher Scientific (Pittsburgh, PA). Malvidin chloride, peonidin chloride and pelargonidin chloride standards were procured from Indofine Chemicals (Hillsborough, NJ).

For the cell culture assays, McCoy's media, Dulbecco's modified Eagle's medium F-12, bovine serum albumin, and sodium bicarbonate were procured from Sigma (St. Louis, MO). Fetal bovine serum, streptomycin/penicillin mix and charcoal powder were obtained from Fisher Scientific (Pittsburgh, PA).

2.2. Potatoes

Seven potato clones – commercial cultivars (Atlantic, Purple Majesty, and Yukon Gold), and advanced selections (CO97232-2R/Y, AC97521-1R/Y, CO97215-2P/P, and CO97227-2P/PW) were grown at San Luis Valley Research Center – Colorado State University, Center, CO. The potatoes were grown in Dunul cobbly sandy loam soil for a growth period of 100-110 days; starting from mid-May till October. Vine killing was done approximately three weeks before harvesting using sulfuric acid. The potatoes were reconditioned for three weeks to allow sugar-starch conversion and then stored at 3 ± 1

°C. This was considered as 'Day 0.' Potatoes were randomly placed in numbered bags and weighed at Day 0 and then subsequently at monthly intervals before sampling for analysis to obtain moisture loss data. Tubers showed no signs of visual deterioration or loss of firmness even at Day 90.

2.3. Preparation of Potato Extracts

Potato samples (10 g) were homogenized with 25 ml of 80% ethanol acidified with formic acid (0.1% v/v). The homogenized samples were poured into chloroform-resistant tubes and vortexed every 15 minutes for an hour. Then 15 ml chloroform was added to the tubes and they were vortexed every 10 minutes for half an hour. The tubes were then centrifuged at 4000 rpm for 10 minutes and stored overnight to allow layer separation. Supernatants (~15 ml) were collected and stored at -20°C until further analyses. All potato samples were corrected for moisture loss during storage. To minimize intraclone variability for each clone, eight randomized tuber samples were taken at each time point and extracted separately for further analyses.

2.4. Quantification of Total Phenolics

Total phenolic content of the potato extracts was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al. 1999). In a 96-well microplate, 35 µl of extract was combined with 150 µl of 0.2 M Folin-Ciocalteu reagent and allowed to react for 5 minutes. Then 115 µl of sodium carbonate solution (7.5% w/v) was added and the mixture was allowed to react for 30 minutes at 45 °C and cooled for one hour. The absorbance was read at 765 nm using a microplate reader (Synergy-2, BioTek Instruments Inc., VT, U.S.) and expressed as milligrams of gallic acid equivalents *per* 100 g of fresh potato sample (mg GAE/100 gfw).

2.5. Quantification of Total Monomeric Anthocyanin Content

The total monomeric anthocyanin content was determined by pH differential method (Wrolstad 1976). Buffers of pH 1.0 and pH 4.5 (290 µl of each) were separately added to 10 µl of the purple-fleshed potato extracts. After 15 minutes, absorbance was measured at 525 and 700 nm using a microplate reader (Synergy-2, BioTek Instruments Inc., VT). The difference in absorbance (A) at different pH values and wavelengths was obtained using the equation below.

$$A = (A_{525} - A_{700})_{pH1.0} - (A_{525} - A_{700})_{pH4.5}$$

Monomeric anthocyanin concentration (MAC) was calculated using an extinction coefficient (ε) of 26,900 l/cm/mol, molecular weight (MW) of 449.2 g/mol, standard path length of 1 cm and a dilution factor (DF) of 10 using the formula below.

$$\text{MAC (mg/l)} = (A \times MW \times DF \times 1000) / (\varepsilon \times 1)$$

Anthocyanin content was reported as mg cyanidin-3-glucoside *per* 100 g of fresh potato sample (mg C-3-G equivalents/100 gfw).

2.6. Antioxidant Activity Analysis

The antioxidant activity was measured using modified 2,2-diphenyl-1-picryhydrazyl radical (DPPH) assay (Blois 1958) and modified 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay (Awika et al. 2003; Reddivari et al. 2007a). For the DPPH assay, freshly prepared 285 µl of diluted DPPH solution (240 µg/ml) was added to 15 µl of ethanol extracts in a 96-well microplate, and allowed to react for 30 minutes. The absorbance was measured at 517 nm using a microplate reader (Synergy-2, BioTek Instruments Inc., VT), and compared with trolox standards. The

antioxidant activity was calculated as mg trolox equivalent *per* 100 g of fresh potato sample (mg TE/100 gfw).

For the ABTS assay, equal volumes of 3 mM ABTS radical and 8 mM potassium persulfate were allowed to react in the dark for at least 16 hours at room temperature to form the mother solution. Then 5 ml of this mother solution was mixed with 145 ml of phosphate buffer (pH 7.4) to make the working solution. In a 96-well microplate, 290 µl of the ABTS working solution was mixed with 10 µl of ethanol extracts and allowed to react for 30 minutes. The absorbance was measured at 734 nm using a microplate reader (Synergy-2, BioTek Instruments Inc., VT). The antioxidant activity of the samples was expressed as mg trolox equivalent *per* 100 g of fresh potato sample (mg TE/100 gfw).

2.7. Ultra Performance Liquid Chromatography (UPLC) and Mass Spectrometry

Potato extracts (2 µl) were injected in a Waters Acquity UPLC system (Waters Corporation, Milford, MA) using a HSS T3 column (1.8 µm, 1.0 x 100 mm), and a gradient from solvent A (100% water + 0.1% formic acid) to solvent B (95% methanol, 5% water, 0.1% formic acid). Injections were made in 100% A, which was held for 2 minutes, followed by a 13 minute linear gradient to 100% B, followed by a 2 minute hold at 100% B. The column was returned to starting conditions over 0.1 minutes, and allowed to reequilibrate for 2.9 minutes. Flow rate was kept constant at 140 µl/min for the duration of the run. The column and the auto sampler were held at 50 °C and 5 °C, respectively.

Column eluent was infused into a Micromass Q-Tof Micro mass spectrometer (Waters Corporation, Milford, MA) fitted with an electrospray source. Data was collected in positive ion full scan mode, scanning from m/z 50-1200 at a rate of 2 scans *per* second

with an interscan delay of 0.1 second. Calibration was performed prior to sample analysis via infusion of sodium formate solution, with mass accuracy within 3 µl/l (ppm). The capillary voltage was held at 2200 V, the source temperature at 130 °C, and the desolvation temperature at 300 °C with a nitrogen desolvation gas flow rate of 300 l/hr. The quadrupole was held at collision energy of 7 volts.

Peak detection was performed using MarkerLynx software (Waters MassLynx, v 4.1, Milford, MA). Peak areas were exported to SIMCA-P+ (Umetrics AB, v12.0, San Jose, CA) for principal component analysis. Data were scaled to unit variance and mean centered before principal component analysis.

2.8. Human Colon Cancer Cell Lines

HCT-116, p53+/+ cells were a generous gift from Dr. Bert Vogelstein and HT-29 cells were purchased from ATCC (Manassas, VA). The cells were maintained at 37 °C in a humidified 5% CO_2 incubator in McCoy's media supplemented with sodium bicarbonate (2.2 g/l), fetal bovine serum (50 ml/l), and streptomycin/penicillin mix (10 ml/l).

2.9. Cell Proliferation

Cell proliferation was assessed via BrdU assay (Cell Signaling Technology, MA) and cell counting using an automated cell counter (Nexcelom Bioscience, Lawrence, MA). Briefly, HCT-116 or HT-29 cells were grown in 96 well plates at 4000 cells *per* well in Dulbecco's modified Eagle's medium F-12 (DMEM). After 24 hours, the cells were treated with potato extracts diluted in DMEM having final phenolic concentrations of 10, 20 and 30 µg GAE/ml. The treatments were added in triplicates at the volume of 1ml *per* well and then allowed to incubate for 24 hours. At the end of the incubation

period, cell viability was assessed by quantifying the amount of 5-bromo-2'-deoxyuridine (BrdU) incorporated into cellular DNA of proliferating cells using an anti-BrdU antibody. For cell counting, cells were plated at 50,000 cells *per* well in a 12-well plate and treated as above and reported as *per cent* reduction with respect to control.

2.10. Apoptosis

Apoptosis was measured using the Caspase-Glo 3/7 assay (Promega Corporation, Madison, WI). After 24 hour incubation with the extracts, HCT-116 and HT-29 cells were counted and 15,000 cells were added *per* well to a 96-well microplate and the volume was made up to 200 µl using DMEM. Caspase-Glo 3/7 reagent (100 µl) was added to each well and the plate was placed on a shaker at 300 rpm for 5 minutes. The plate was incubated in the dark at room temperature and luminescence was measured after 30 minutes. Cells undergoing apoptosis have a higher caspase-3 and caspase-7 activity, which results in a stronger luminescence signal.

2.11. Statistical Analysis

Fisher's protected t-tests using the Least Squares Means test, which was used for comparing group differences with $p < 0.05$ being considered as a statistically significant difference, and Pearson correlation coefficients were calculated using SAS Statistical Analysis System, v.9.2 (SAS Institute Inc., Cary, NC). All results have been expressed as mean ± standard error.

3. Results and Discussion

3.1. Total Phenolic Content

Total phenolic content of seven clones (white-, yellow- or purple-fleshed) measured at 0, 30, 60 and 90 days of storage using Folin-Ciocalteu reagent assay ranged

from 25.6 ± 0.4 to 268.6 ± 3.3 mg GAE/100 gfw. This is in accordance with previous studies reporting that the total phenolic content of potato cultivars ranged from 90 to 400 mg GAE/100 gfw (Stushnoff et al. 2008) and 76 to 181 mg chlorogenic acid equivalents/100 gfw (Reyes et al. 2005). The phenolic content primarily depends upon the genotype and slight variations with in the genotype reported by different authors may be due to differences in the growing location, method of extraction and sample preparation as vigorous extraction methods can lead to an increase in the phenolic content (Rumbaoa et al. 2009). The rank order for the phenolic content was purple-fleshed clones

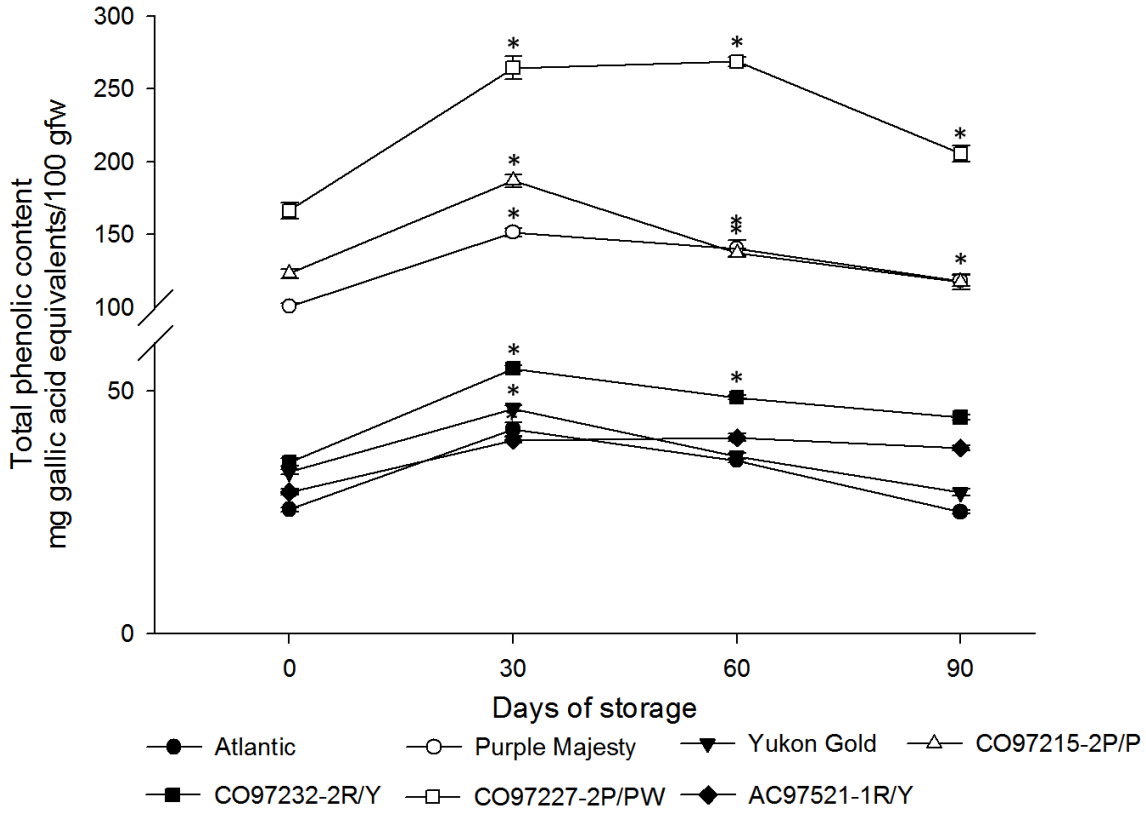


Figure 3.1. Initial storage elevated total phenolic content in potato clones. Total phenolic content of the potato extracts was measured by Folin-Ciocalteu reagent assay as described in Materials and Methods, and expressed as mg gallic acid equivalents/100 gfw. The letters (P/P, P/PW and R/Y) after some of the advanced selections denote skin/flesh color. P: purple; PW: purple with white patches; R: red; Y: yellow. *Indicates significant differences ($p < 0.05$) in the phenolic content compared with the initial time point. Results are presented as mean ± SE of 8 replicates for each time point.

followed by yellow-fleshed clones and finally the white-fleshed cultivar. The phenolic content of purple-fleshed clones was approximately six to eight times greater than that of Atlantic because of the presence of anthocyanins along with phenolic acids.

A significant increase ($p < 0.05$) in the phenolic content was observed after 30 days of storage for most clones (Figure 3.1.), followed by a decline either to initial levels or to significantly above the initial levels after 90 days of storage depending upon the clone. CO97227-2P/PW had the greatest phenolic content among all clones tested. At Day 0, its phenolic content was 166.2 ± 5.6 mg GAE/100 gfw which increased to 268.6 ± 3.2 mg GAE/100 gfw at 60 days of storage and finally reduced to 205.4 ± 5.5 mg GAE/100 gfw at 90 days of storage. The lowest phenolic content was seen in the Atlantic cultivar (25.6 ± 0.4 mg GAE/100 gfw) at Day 0.

In plants, environmental stresses such as low temperature storage, strong light, wounding or pathogen attacks have been shown to induce generation of phenolic compounds via the phenylpropanoid pathway (Dixon and Paiva 1995). In potatoes, low temperature storage (Rhodes and Wooltorton 1978), light (Percival and Baird 2000), wounding (Reyes et al. 2007) and disease (Smith and Rubery 1981) can cause an increase in the phenolic content. Low temperature storage-induced activation of phenylalanine ammonia-lyase (PAL), a key regulatory enzyme in the biosynthesis of polyphenols including anthocyanins (Jiang and Joyce 2003), and *de novo* synthesis of secondary metabolites (Lewis et al. 1999) may be responsible for an initial increase in the phenolic content with storage. Between 30 - 90 days of storage, a decreasing trend in phenolic content may be due to degradation of the polyphenolic compounds, especially chlorogenic acid (Rhodes and Wooltorton 1978).

3.2. Total Monomeric Anthocyanin Content

The anthocyanin content of purple-fleshed clones ranged from 20.9 ± 0.2 to 110.3 ± 1.4 mg C-3-G equivalents/100 gfw (Figure 3.2.). It has been documented that the anthocyanins in purple-fleshed cultivars can range from 11 to 174 mg C-3-G equivalents/100 gfw (Reyes et al. 2005). This is in line with our current observation. CO97227-2P/PW had the highest anthocyanin content among all cultivars irrespective of

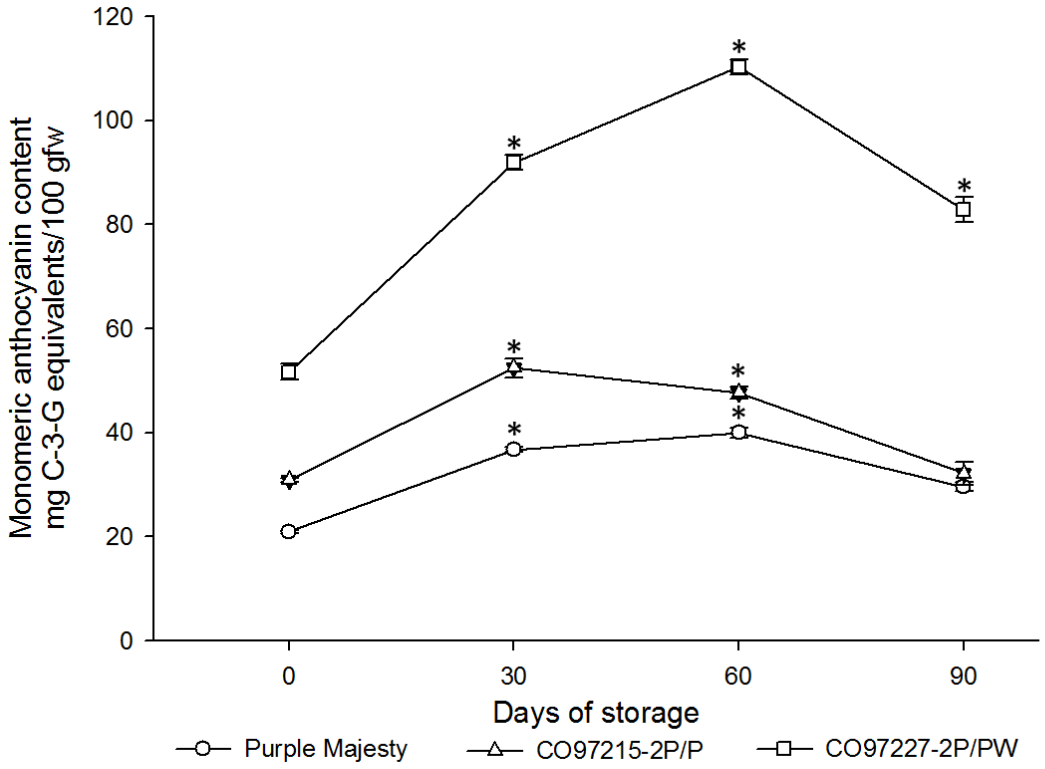


Figure 3.2. Initial storage caused *de novo* synthesis of anthocyanins in purple-fleshed clones. The trend is similar to the total phenolic content suggesting that anthocyanins are major polyphenolic compounds present in purple-fleshed potatoes. Monomeric anthocyanin content was measured using the pH-differential method as described in Materials and Methods, and expressed as mg cyanidin-3-glucoside equivalents/100 gfw. The letters (P/P and P/PW) after some of the advanced selections denote skin/flesh color. P: purple; PW: purple with white patches. *Indicates significant differences ($p < 0.05$) in the anthocyanin content compared with the initial time point. Results are presented as mean ± SE of 8 replicates for each time point.

the storage time. The initial anthocyanin content of CO92772-2P/PW was 51.7 ± 1.5 mg C-3-G equivalents/100 gfw which then increased to 110.3 ± 1.5 mg C-3-G equivalents/100 gfw at Day 60 and gradually decreased to 82.9 ± 2.4 mg C-3-G equivalents/100 gfw at Day 90. Purple Majesty, which showed lowest anthocyanin content among the three clones tested, had 20.9 ± 0.2, 40 ± 1 and 29.6 ± 0.9 mg C-3-G equivalents/100 gfw at 0, 60 and 90 days, respectively.

The anthocyanin content followed a trend similar to that of the phenolic content, suggesting that anthocyanins contribute to a major portion of polyphenols in purple-fleshed potatoes. Cold storage conditions are known to cause the conversion of starch to sugar (Isherwood 1976), which can up-regulate genes coding for *dihydroflavonol reductase (DFR)* and *anthocyanidin synthase (ANS)*, which are involved in anthocyanin biosynthesis, and hence potentially cause an increase in the anthocyanin concentration (Vitrac et al. 2000; Gollop et al. 2001; Gollop et al. 2002; Solfanelli et al. 2006). Also, as suggested for the phenolic content, the initial increase might be due to enhanced PAL activity (Jiang and Joyce 2003) and *de novo* synthesis of anthocyanins (Lewis et al. 1999) during storage.

3.3. Antioxidant Activity

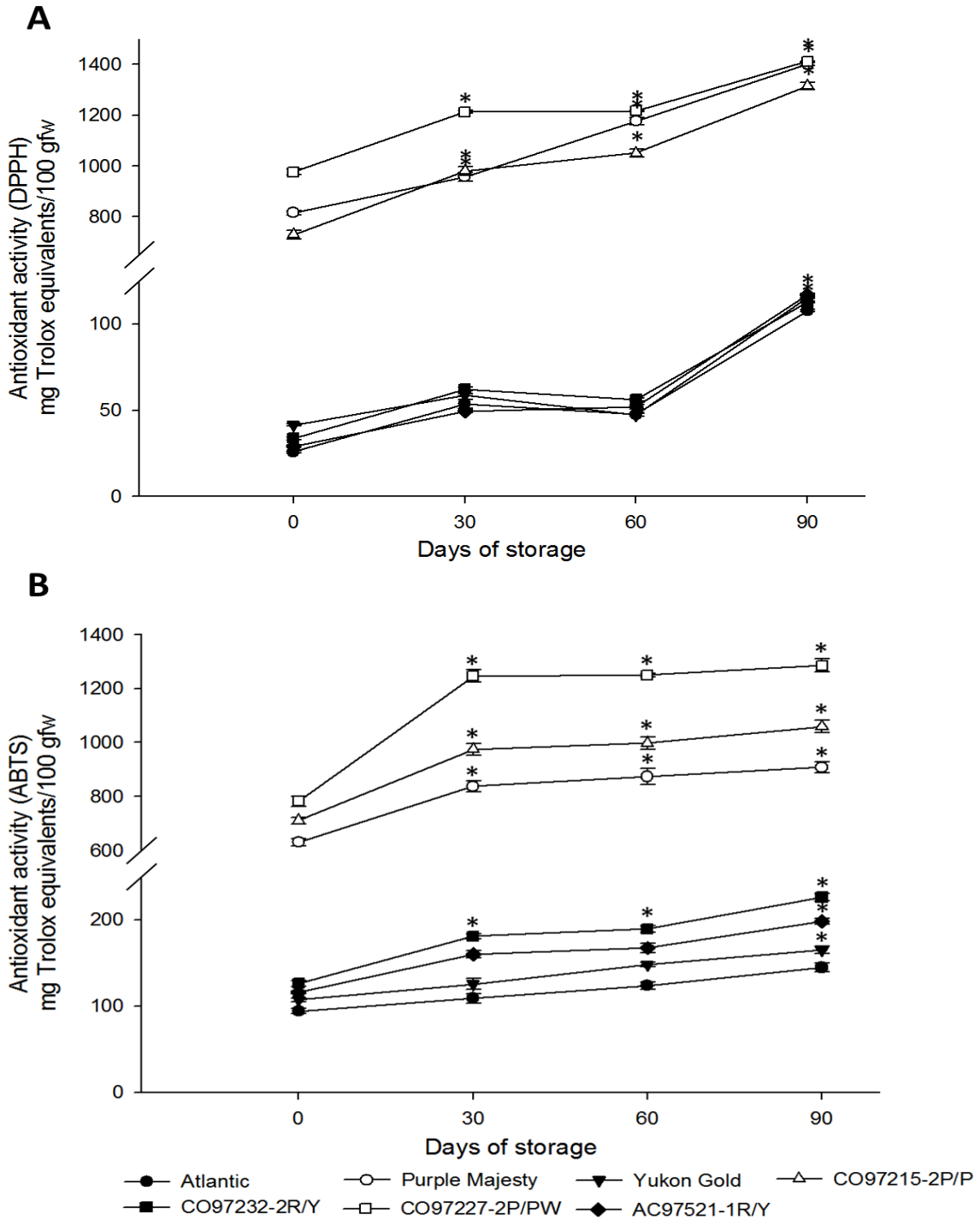
Antioxidant activity measured by the DPPH and ABTS assays, showed an increase with storage (Figure 3.3). The antioxidant capacity measured by DPPH assay for fresh potatoes ranged from 25.8 ± 0.6 mg TE/100 gfw at Day 0 to 107.8 ± 0.4 mg TE/100 gfw at Day 90 for Atlantic and 976.2 ± 11.46 mg TE/100 gfw at Day 0 to 1412.2 ± 1.3 mg TE/100 gfw at Day 90 for the advanced selection CO97227-2P/PW, which showed the highest antioxidant activity among the seven clones tested (Figure 3.3 A). For the

ABTS assay, the range was from 94 ± 3.2 mg TE/100 gfw at Day 0 to 144.4 ± 4.9 mg TE/100 gfw at Day 90 for Atlantic and 782.3 ± 19.2 mg TE/100 gfw at Day 0 to 1285.4 ± 25.1 mg TE/100 gfw at Day 90 for CO97227-2P/PW (Figure 3.3. B). The antioxidant values at Day 90 were significantly higher compared with Day 0 for all clones irrespective of the tuber flesh color.

The DPPH and ABTS methods measure the antioxidant activity as a result of their respective radical quenching ability. It has been reported that the antioxidant potential of pigmented cultivars can be two to eight times higher than the non-pigmented cultivars because of presence of anthocyanins and/or carotenoids along with the phenolic acids (Brown 2004; Stushnoff et al. 2008). In this study, CO97227-2P/PW had approximately a 10-fold greater antioxidant activity than the white cultivar. At 90 days of storage, the antioxidant activity had increased to its maximum for the duration of the study though there was a trend towards reduction in the phenolic content between 30 – 90 days. These results indicate the contribution of some of the non-phenolic compounds such as vitamins and minerals to the antioxidant activity (Gliszczynska-Swiglo 2006; Shenkin 2006).

3.4. UPLC-MS profile of Phenolic Compounds

Based on the data from total phenolic and anthocyanin content, and antioxidant activity assays, four clones were selected for phenolic profile screening. Of the four selected, three were commercially available cultivars representative of their color – Atlantic (white), Purple Majesty (purple) and Yukon Gold (yellow) – and the fourth one was CO97227-2P/PW, a purple-fleshed advanced selection, which had the highest phenolic content, antioxidant activity and anthocyanin content. Chlorogenic acid was the most abundant phenolic acid in most clones (Table 3.1). It has been reported that



Figures 3.3. Antioxidant activity of potatoes as assessed by DPPH (A) and ABTS (B) assays, respectively. Antioxidant activity was expressed as mg trolox equivalents/100 gfw. The letters (P/P, P/PW and R/Y) after some of the advanced selections denote skin/flesh color. P: purple; PW: purple with white patches; R: red; Y: yellow. *Indicates significant differences ($p < 0.05$) in the antioxidant activity compared with the initial time point. Results are presented as mean ± SE of 8 replicates for each time point.

chlorogenic acid may account for 90% of the total phenolic content in potatoes (Dao and Friedman 1992). However, in this study only chlorogenic acid was measured but not its isomers. The chlorogenic acid content of Purple Majesty was approximately 35 times greater than that of Yukon Gold at Day 0 which, at Day 90 reduced to approximately 12-fold. A previous study has reported an approximately ten-fold difference in the chlorogenic acid concentration in pigmented cultivars such as Mountain Rose and Purple Majesty, and non-pigmented cultivars such as Yukon Gold (Stushnoff et al. 2008). Another study observed a 20-fold difference in the chlorogenic acid content (Navarre et al. 2011). In Atlantic and Yukon Gold cultivars, 90 days storage increased the chlorogenic acid content approximately two-fold and four-fold respectively. Among the purple-fleshed clones, a 1.5-fold increase was observed. Caffeic acid was the second most abundant phenolic acid. We have previously reported that caffeic acid content can range from 310 to 420 µg *per* 100 gfw potato (Reddivari et al. 2007a; Reddivari et al. 2007c). In the current study, the caffeic acid ranged from 580 to 1160 µg *per* 100 gfw potato in the white- and yellow-fleshed cultivars, irrespective of storage. The caffeic acid content in pigmented clones was greater than that in non-pigmented clones. The purple-fleshed clones contained caffeic acid ranging from 5.7 mg to 10.7 mg *per* 100 gfw potato irrespective of storage. A previous study observed a 100-fold difference in the caffeic acid contents of the yellow cultivar, Divina, and the purple cultivar Pollunta chata (Navarre et al. 2011). Storage increased the caffeic acid content in Atlantic, Yukon Gold, Purple Majesty and CO97227-2P/PW clones 1.3, 1.9, 1.5, and 1.8, times respectively. Thus, this increase in the phenolic acids could explain the observed increase in the total phenolic content with storage.

Table 3.1. Phenolic profile of potato extracts and the effect of storage

Compound	Molecular ion M^+ (m/z)	Retention time (mins)	Atlantic		Yukon Gold		Purple Majesty		CO97227-2P/PW	
			Day 0	Day 90	Day 0	Day 90	Day 0	Day 90	Day 0	Day 90
Chlorogenic acid	355	6.11	1.15± 0.02	2.13±0.06	0.42±0.02	1.94±0.13	14.77± 0.13	23.77± 0.31	19.20± 0.65	30.09± 0.26
Caffeic acid	181	6.15	0.87± 0.08	1.16±0.09	0.58±0.03	1.12± <0.01	5.73± 0.33	8.79± 0.45	5.98± 0.21	10.73± 1.13
Ferulic acid	177	7.56	-	0.102± 0.01	0.043± <0.01	0.136± <0.01	-	-	-	-
Sinapic acid	207	7.76	-	0.041± <0.01	-	0.023± <0.01	-	-	-	-
Pet-3-rut-5-glc	787	5.79	-	-	-	-	215.4	449.7	297.1	761.8
Mal-3-rut-5-glc	801	6.19	-	-	-	-	31.06	56.51	84.05	184.1
Peo-3-coum-rut-5-glc isomer	917	7.70	-	-	-	-	0	0	128.9	297.2
Pet-3-coum-rut-5-glc	933	7.92	-	-	-	-	6574	10720	11034	12026
Pel-3-coum-rut-5-glc	887	8.11	-	-	-	-	0	0	1224	1514
Peo-3-coum-rut-5-glc	917	8.21	-	-	-	-	306.8	416.2	7527	6225
Mal-3-coum-rut-5-glc	947	8.31	-	-	-	-	668.7	834.2	1949	2516

The phenolic acid values are expressed as mg/100 gfw potato. The anthocyanins have been reported as area under the curve. Values are presented as mean ± SE of six replicates

Purple-fleshed clones also contained anthocyanins in their glycosylated form; some of them were acylated with para-coumaric acid. Pel-3-coum-rut-5-glc was observed only in CO97227-2P/PW. CO97227-2P/PW also had approximately a 3-fold higher amount of mal-3-coum-rut-5-glc as compared with Purple Majesty. Pet-3-coum-rut-5-glc was the most abundant anthocyanin in Purple Majesty, followed by mal-3-coum-rut-5-glc and then peo-3-coum-rut-5-glc. This agrees with another study that has reported Purple Majesty anthocyanins in the same order of abundance (Stushnoff et al. 2008). Storage increased the individual anthocyanins 1.2 to 2.5 times in both purple-fleshed clones with the exception of peo-3-coum-rut-5-glc in CO97227-2P/PW. This could explain the observed increase in the monomeric anthocyanin content from Day 0 to Day 90.

Principal component analysis indicated differences in the phenolic profiles among the clones and between the initial and final storage period (Figure 3.4.).

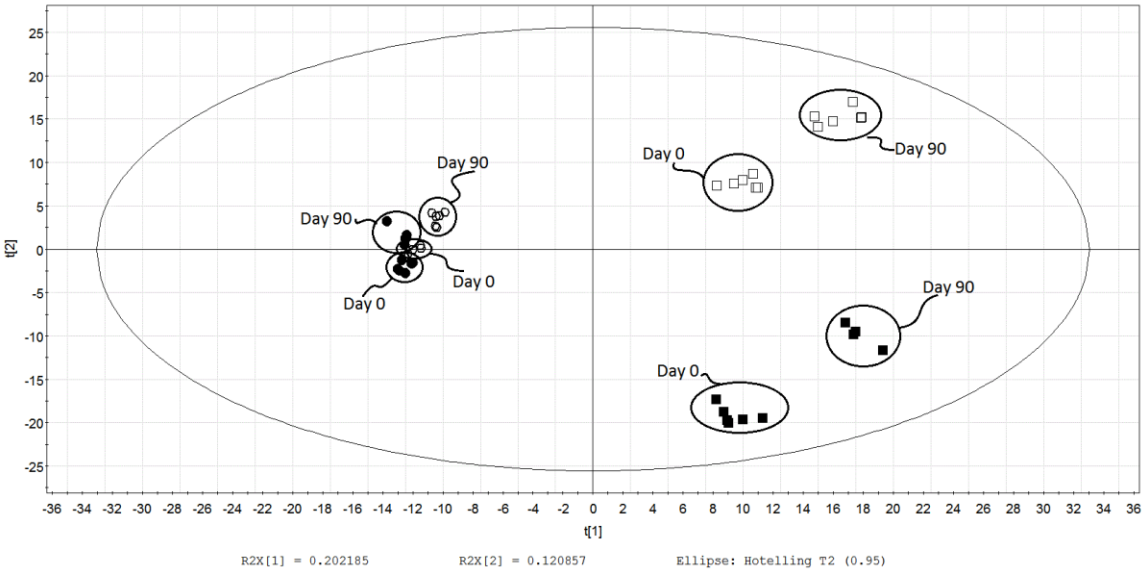


Figure 3.4. Principal component analysis revealed differences in phenolic profiles based on cultivar and storage. The purple-fleshed clones had different profiles than the white- and yellow-fleshed cultivars. The bubbles point out difference in phenolic profiles due to storage. Plot shows all the individual data points. Data are represented for Atlantic (●), Purple Majesty (□), Yukon Gold (○) and CO97227-2P/PW (■) cultivars.

The phenolic profiles of Purple majesty (purple) were not only different from Atlantic (white) and Yukon Gold (yellow) but also from other purple clone CO97227-2P/PW.

3.5. Cell Proliferation and Apoptosis Assays

The potential growth inhibitory effects of the extracts from the four clones (Atlantic, Purple Majesty, Yukon Gold and CO97227-2P/PW) before and after storage were investigated using HCT-116 and HT-29 human colon cancer cell lines. Figures 3.5. and 3.6. illustrate the effects of different concentrations of the extracts (expressed as μg GAE/ml) on proliferation of HCT-116 cells. Number of cells were quantified and reported as percentage reduction with respect to control treatment (only media).

Potato extracts caused a dose-dependent reduction in the number of cells (Figures 3.5. and 3.6.). The potato extracts could also suppress proliferation of HT-29 cells, which is an advanced human colon cancer cell line (Figures 3.7. and 3.8.). However, the efficacy was lower as compared to HCT-116 cell line. The purple-fleshed clones showed more potent anti-proliferative properties compared with the white- and yellow-fleshed cultivars. Significant reduction in the anti-proliferative property was observed for all four clones with storage. CO97227-2P/PW was the most potent clone showing greater than 70% reduction in cell proliferation at 30 μg GAE/ml.

Previous studies have shown reduction in proliferation of cancer cells when treated with potato extracts. Purple Majesty extracts (2%) in the media have been reported to suppress proliferation of MCF7 (estrogen-dependent) and MDAMB468 (estrogen-independent) breast cancer cells by approximately 70% after a five day incubation period (Stushnoff et al. 2008). Phenolic acids identified in potatoes have been implicated in suppression of cancer cell proliferation *in vitro* (Lee and Zhu 2006).

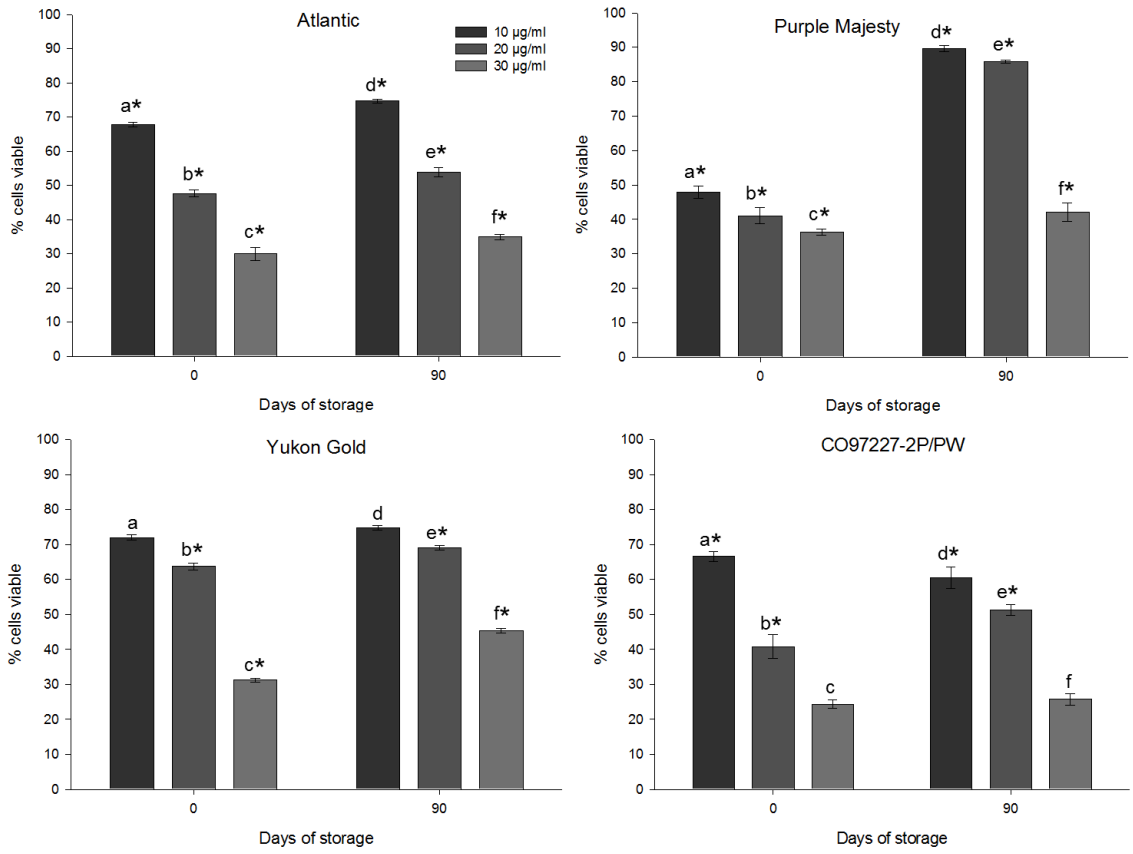


Figure 3.5. Potato extracts suppressed proliferation of HCT-116 cells in a dose-dependent manner. Cell number was measured using a cellometer as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

A similar trend was seen in the elevation of apoptosis (Figures 3.9. and 3.10.). The potato extracts showed a dose-dependent increase in the induction of apoptosis with respect to the control (only media). Duration of storage negatively affected the pro-apoptotic activity of HCT-116 (Figure 3.9.) and HT-29 (Figure 3.10.) cancer cells. Purple-fleshed clones not only had anti-proliferative activity but also caused a greater

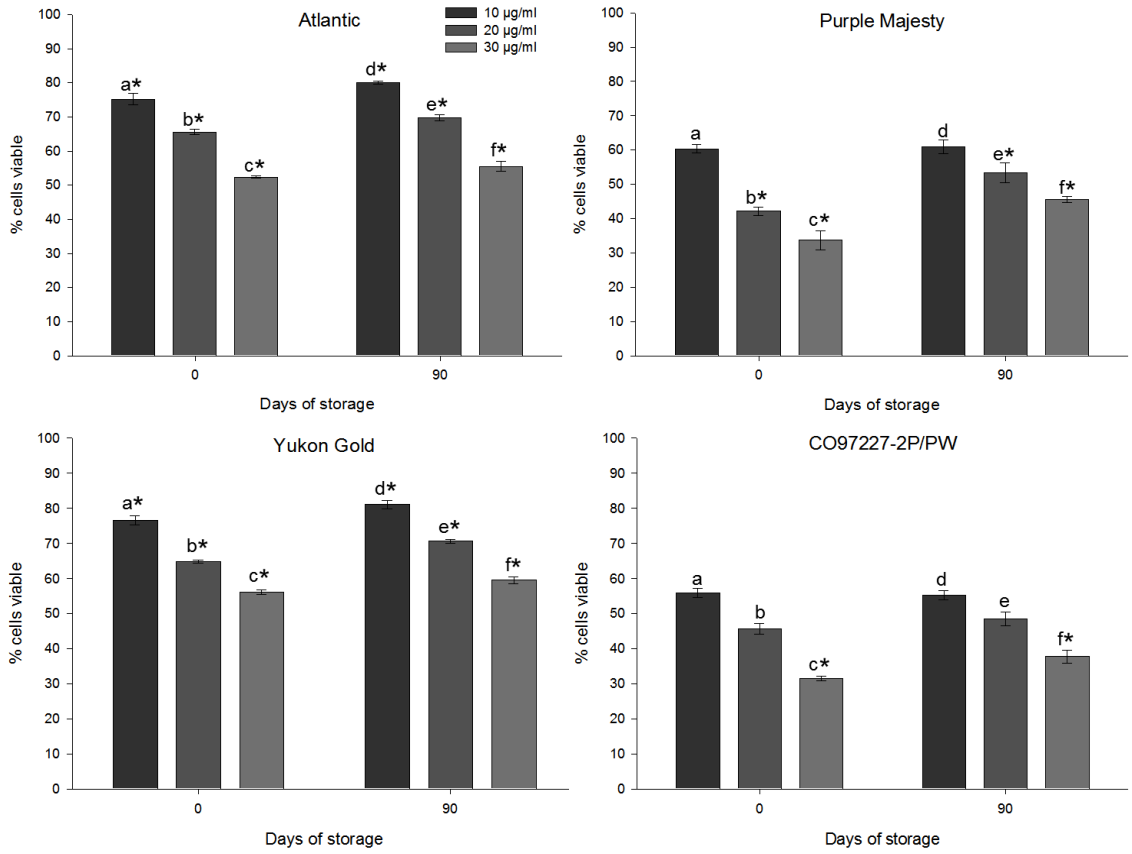


Figure 3.6. Potato extracts suppressed proliferation of HCT-116 cells in a dose-dependent manner. Cell viability was measured using the BrdU assay as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

increase in apoptosis as compared to the white- and yellow-fleshed cultivars. CO97227-2P/PW, with greater total phenolic content and antioxidant capacity, was the most potent clone causing almost a 10-fold increase in the apoptotic cells as compared to the control. The diverse anthocyanin composition of CO97227-2P/PW as seen in the above section may explain the higher anti-cancer activity as compared with Purple Majesty.

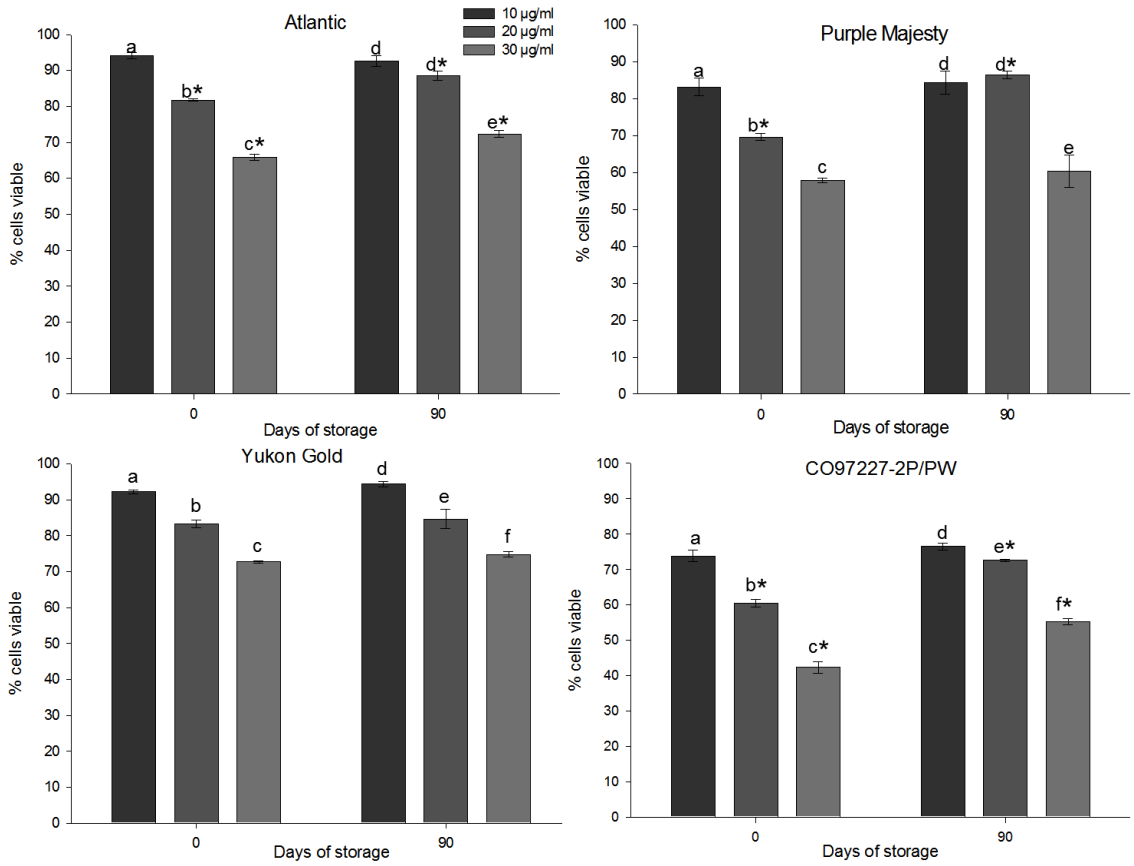


Figure 3.7. Potato extracts suppressed proliferation of HT-29 cells in a dose-dependent manner. Cell number was measured using a cellometer as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

3.6. Correlations

Significant positive correlations were observed for phenolic content and antioxidant activity ($R^2 = 0.90$, $p < 0.0001$) as well as anthocyanin content ($R^2 = 0.89$, $p < 0.0001$). This is in line with a strong positive correlation between the total phenolic and anthocyanin content ($R^2 = 0.91$) as reported by Reyes et al. (2005). Significant positive correlation has been observed between the DPPH and ABTS assays

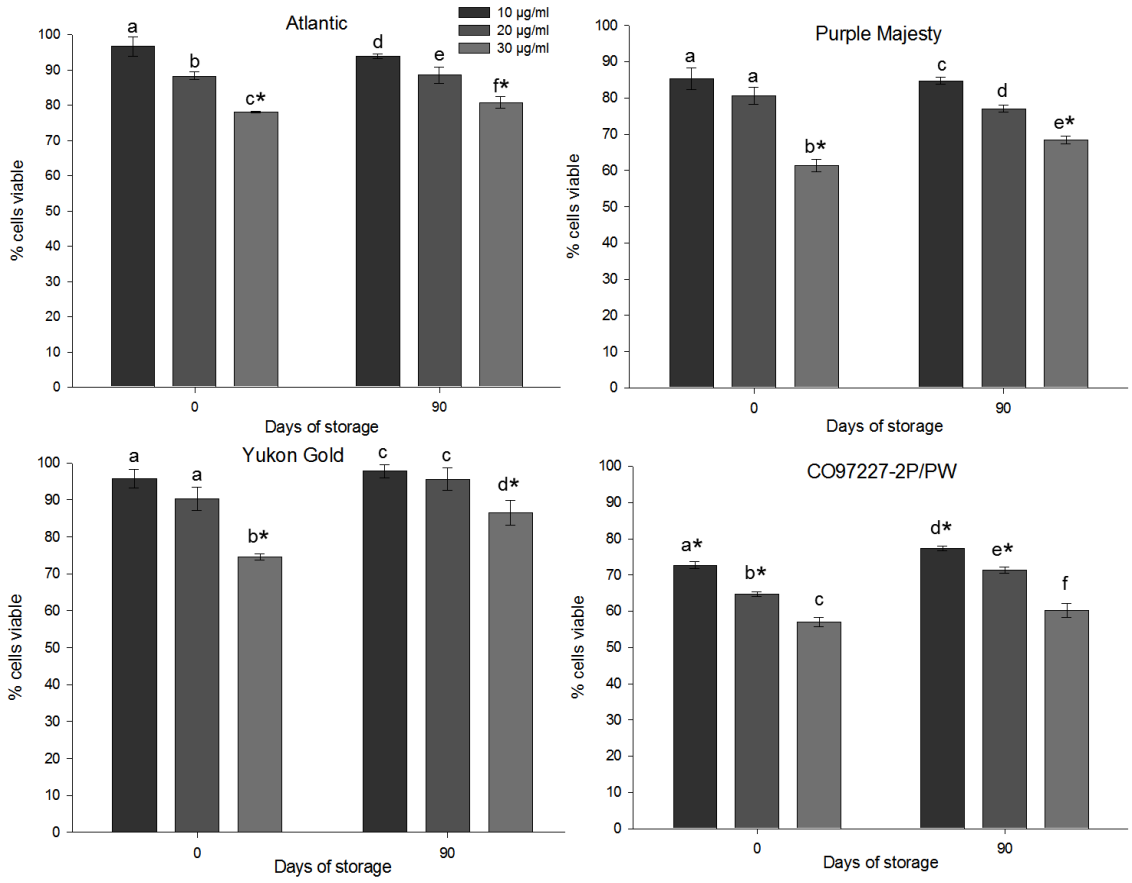


Figure 3.8. Potato extracts suppressed proliferation of HT-29 cells in a dose-dependent manner. Cell viability was measured using the BrdU assay as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

for antioxidant activity measurement (Thaipong et al. 2006; Reddivari et al. 2007a; Dudonné et al. 2009). Similar, correlation ($R^2 = 0.96$, $p < 0.0001$) was observed between DPPH and ABTS assays.

Storage duration was positively correlated with antioxidant activity ($R^2 = 0.79$, $p < 0.02$) of all the genotypes individually. Moderate to strong correlations were observed

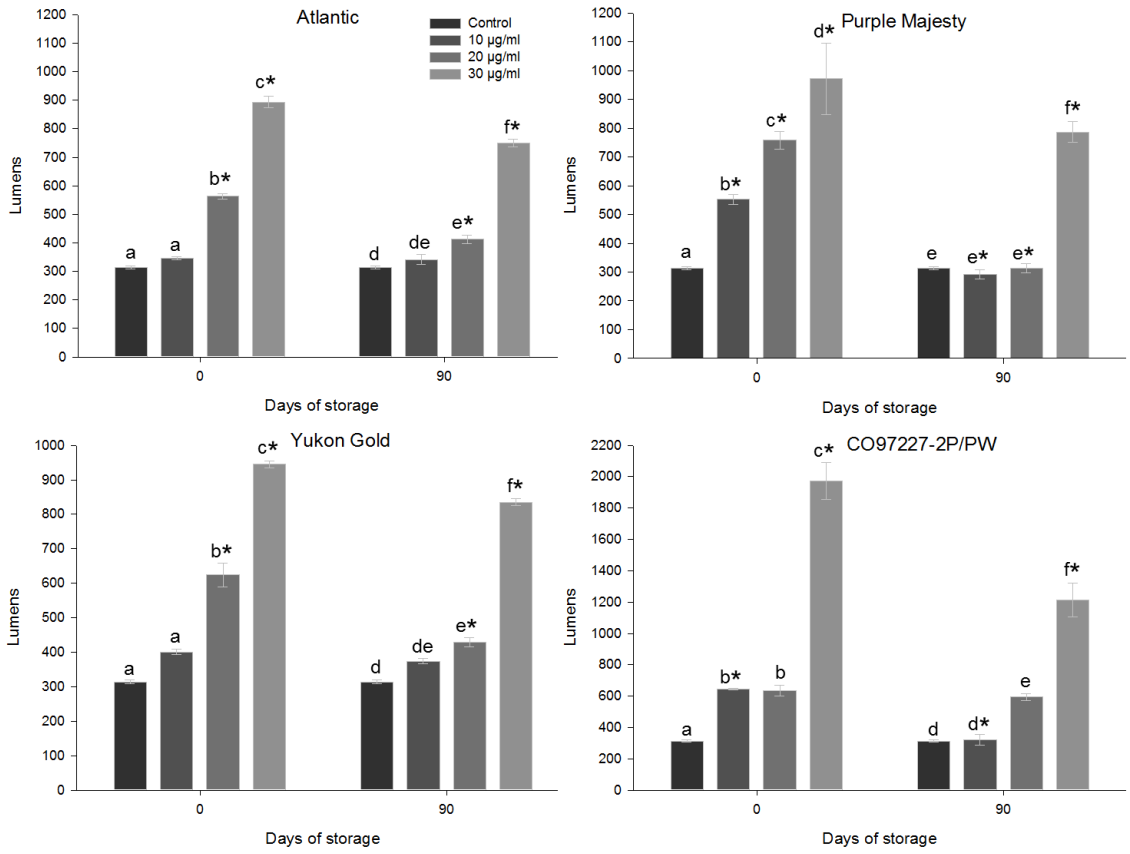


Figure 3.9. Potato extracts induced dose-dependent apoptosis in HCT-116 colon cancer cells. Apoptosis was measured using Caspase-Glo 3/7 assay as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between apoptotic cells at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between apoptotic cells at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

between storage duration and percentage of cancer cells viable for Atlantic ($R^2 = 0.74$, $p < 0.1$ for BrdU and $R^2 = 0.67$, $p < 0.1$ for cell counting) and Yukon Gold samples ($R^2 = 0.46$, $p < 0.36$ for BrdU and $R^2 = 0.99$, $p < 0.001$ for cell counting). However, apoptosis induction exhibited a strong negative correlation with storage duration for Atlantic ($R^2 = -0.93$, $p < 0.01$) and Yukon Gold ($R^2 = -0.95$, $p < 0.01$) samples indicating a loss in the ability to induce apoptosis with storage duration. For the Purple Majesty samples, storage

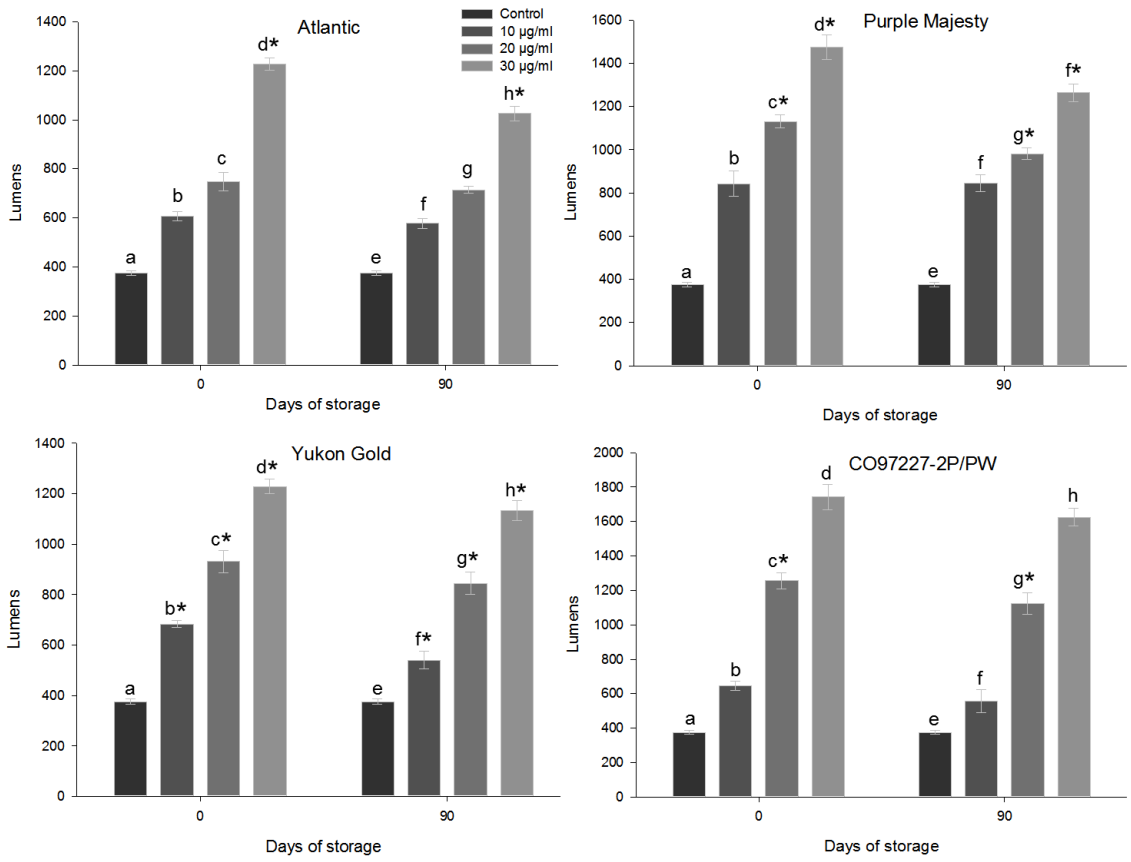


Figure 3.10. Potato extracts induced dose-dependent apoptosis in HT-29 colon cancer cells. Apoptosis was measured using Caspase-Glo 3/7 assay as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between apoptotic cells at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between apoptotic cells at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

duration moderately correlated with percentage of viable cancer cells ($R^2 = 0.90$, $p < 0.01$ for BrdU and $R^2 = 0.66$, $p < 0.1$ for cell counting) and apoptosis ($R^2 = -0.58$, $p < 0.2$). For the CO97227-2P/PW, the correlations were not significant which could indicate that storage duration did not suppress its anti-cancer properties.

CHAPTER IV

COMBINED EFFECTS OF STORAGE AND PROCESSING ON THE COMPOSITION, ANTI-CANCER PROPERTIES AND SENSORY ATTRIBUTES OF COLORED-FLESH POTATOES

Abstract

Potatoes can be stored for up to one year before being processed and consumed. The objective of this study was to determine the extent to which fresh and stored colored-flesh potatoes retain their anti-cancer properties after baking and chipping compared with uncooked potatoes. We utilized white-, yellow- and purple-fleshed potato clones and tested their phenolic and anthocyanin content, antioxidant activity, metabolite profile, anti-cancer properties, and sensory attributes. When compared with uncooked samples, baking or chipping led to significant losses in the phenolic and anthocyanin content, and antioxidant activity of the potatoes. However, with storage, total phenolic and anthocyanin content, and antioxidant activity increased in baked samples while in the chipped samples, they remained constant. Principal component analysis of approximately 1600 peaks obtained by ultra performance liquid chromatography-mass spectroscopy (UPLC-MS) analysis revealed differences among metabolite profiles of baked and chipped white-, yellow- and purple-fleshed clones post-storage. Even though two clones had same flesh color (purple), their metabolite profiles were different. Ethanol extracts of baked and chipped samples, in general, suppressed proliferation and elevated apoptosis ($p < 0.05$) in early stage (HCT-116, p53+/+) and advanced stage (HT-29) human colon cancer cell lines. Anti-proliferative and pro-apoptotic properties of baked potatoes were similar to that of fresh potatoes while chipping caused a significant reduction in the

biological activity. After 90 days of storage, Atlantic and Purple Majesty, in general, showed reduction in the anti-proliferative and pro-apoptotic properties of baked and chipped samples. However, CO97227-2P/PW and Yukon Gold retained their anti-cancer properties. Sensory analysis revealed comparable acceptance of purple-fleshed baked and chipped potatoes when compared with traditional cultivars. Consumers were willing to pay a premium for colored-flesh potatoes if they were educated on their potential health benefits. Phenolic content and antioxidant activity of purple-fleshed potatoes, after baking, were comparable with those of anthocyanin-rich berries. Hence, purple-fleshed potatoes can be a healthier choice as they possess greater levels of bioactive compounds and anti-cancer properties even after processing as compared with white- and yellow-fleshed counterparts.

1. Introduction

The potato (*Solanum tuberosum* L.) is one of the most commonly consumed vegetable crops worldwide. Due to its high consumption it is considered the third largest source of phenolic compounds in the human diet after oranges and apples (Chun et al. 2005). The US Potato Board, through the National Eating Trends Report (2010), revealed that over the past ten years, though the consumption of traditional potatoes (mashed, baked, fried, steamed, boiled and french fries) declined, specialty/colored potato consumption increased by 17%, possibly due to their putative health benefits. Anthocyanin-rich colored-flesh (purple and red) potatoes have up to eight times higher antioxidant capacity compared with white or yellow counterparts (Stushnoff et al. 2008).

The role of potato polyphenols as antioxidants, anti-carcinogenic and anti-mutagenic agents have been reported in numerous studies. Potato polyphenols are

effective against human liver, colon, and prostate cancer cells (Chu et al. 2002; Nzaramba et al. 2009). Chlorogenic acid, in particular, has shown to suppress the proliferation of A549 human lung cancer cell lines and block UVB- or TPA-induced transactivation of AP-1 and NF-κB, which are inflammatory mediators linked to cancer, in JB6 mouse epidermal cell line (Feng et al. 2005). Colored-flesh potatoes are a rich source of anthocyanins with a wide array of health benefits. Colored-flesh potato anthocyanins are toxic to human stomach cancer cells and suppress growth of benzopyrene-induced stomach cancer in mice (Hayashi et al. 2006). We have previously reported that anthocyanin fractions from potatoes induce apoptosis in prostate cancer cell lines through caspase-dependent and -independent pathways (Reddivari et al. 2007c). Researchers recently found lower levels of inflammatory markers such as plasma C-reactive protein, 8-hydrodeoxyguanosine, and interleukin-6, in healthy men who were consuming purple potatoes as compared with those who were consuming white ones (Kaspar et al. 2011).

Previous researchers used either uncooked or baked potatoes with little emphasis on the effect of storage and processing on biological activity. It is known that storage and processing changes the physical and chemical composition of foods (Spanos et al. 1990; Price et al. 1997), thus, affecting their antioxidant activity (Nicoli et al. 1999; Dewanto et al. 2002). Raw potato phenolic content has been extensively studied (Al-Saikhan et al. 1995; Reyes et al. 2005; Stushnoff et al. 2008; Rumbaoa et al. 2009), but potatoes are almost always consumed after processing (baked, chipped, fried, boiled or microwaved) making it critical to understand the effect of such processing techniques on the activity and composition of bioactive compounds in potatoes. Domestic cooking such as microwaving, boiling or frying can result in partial losses in the phenolic content

(Tudela et al. 2002). Specifically, chlorogenic acid has also been shown to undergo degradation after home processing (Im et al. 2008). However, processing has also been reported to cause an increase in the phenolic content and the antioxidant activity of potato (Blessington et al. 2010). Potatoes are stored for months, sometimes up to one year before they are processed (Herrman et al. 1996). Hence, it is necessary to determine the combined effects of storage and processing on the anti-cancer activity of potatoes. The objective of this study was to analyze the extent to which potatoes, especially colored-fleshed ones, retained their anti-cancer activity after post-storage processing as compared with uncooked potatoes.

For the consumer, sensory perception is of utmost importance. Hence, we conducted sensory analysis to understand how inter-clonal differences, post-harvest storage and total phenolic content influence the sensory parameters of baked and chipped potato samples.

Berries are the most popular source of anthocyanins, which have many health-benefits associated with them (Meyers et al. 2003; Bagchi et al. 2004; Olsson et al. 2004). However, many populations are unable to consume berries due to their high cost with respect to other fruits and vegetables, including potatoes. Hence, to find how berries compare with colored-flesh potatoes, we quantified the phenolic and anthocyanin content of popular anthocyanin-rich berries such as blueberries, strawberries, raspberries and grapes, and compared them with baked and chipped colored-flesh potatoes.

2. Materials and methods

2.1. Chemicals

Ethanol for the extractions was purchased from the Central Receiving, Colorado State University (Fort Collins, CO). Phenolic acid standards, reagents and chemicals for spectrophotometric quantitative assays were purchased from Sigma (St. Louis, MO). Gallic acid was acquired from Fisher Scientific (Pittsburgh, PA).

McCoy's 5A modified medium, Dulbecco's modified Eagle's medium F-12, bovine serum albumin, and sodium bicarbonate required for cell culture were obtained from Sigma (St. Louis, MO). Fetal bovine serum, streptomycin/penicillin mix and charcoal powder were procured from Fisher Scientific (Pittsburgh, PA).

2.2. Potatoes and Anthocyanin-rich Fruits

Seven potato clones – commercial cultivars (Atlantic – white-fleshed, Yukon Gold – yellow-fleshed, and Purple Majesty – purple-fleshed), and advanced selections (CO97232-2R/Y, AC97521-1R/Y, CO97215-2P/P, and CO97227-2P/PW) were grown at San Luis Valley Research Center, Colorado State University, Center, CO. For the advanced selections, the two letters separated by a '/' at the end of the name indicate skin color and flesh color respectively (R: Red, Y: Yellow, P: Purple, PW: Purple white). The potatoes were grown in Dunul cobbly sandy loam soil for a growth period of 100-110 days; starting from mid-May till October. Vine killing was done approximately three weeks before harvesting using sulfuric acid. The potatoes were reconditioned at 16 ± 1 °C for three weeks to allow sugar-starch conversion and then stored in a dark room at 3 ± 1 °C. This was considered as 'Day 0'. Each potato clone was placed in four numbered bags weighing 4.5 kg (10 lb) each for every processing method (uncooked, baked,

chipped) and every month (November, December, January, February) of study and their weight was recorded at Day 0 and at monthly intervals subsequently before sampling for analysis.

Organic anthocyanin-rich fruits (blueberries, raspberries, strawberries and grapes) were purchased at Whole Foods Market®, Fort Collins, CO.

2.3. Baking and Chipping of the Potatoes

Potatoes were removed from storage every month from November 2009 (Day 0) till February 2010 (Day 90) and baked in a conventional oven preheated to 204 °C for one hour and fifteen minutes. Before baking each potato was washed with water, dried, wrapped in food-grade aluminum foil and pierced approximately 1.5 cm deep with a knife at approximately 3 cm intervals. The baked potatoes were cooled for 15-20 minutes and diced with skin into pieces weighing 7±1 g and stored at -20 °C until extraction.

Chipping of potatoes was done every 30 days from Day 0 to Day 90. The potatoes were taken out from the storage three weeks before the chipping and reconditioned at 15 °C. Then the potatoes were cleaned under running tap water and then introduced into an industrial chipper (Dito Dean Food Prep, Model TRS 23 with C-2 blade) with 1/16" blade clearance. The raw chips were washed under running warm water for approximately one minute to remove any water-soluble sugars present on the surface, placed in strainer trays to remove excess water and fried in Bakers & Chefs™ Clear Frying Oil at 185 °C till bubbling slowed. The fried chips were placed on paper toweling for absorbing excess oil and then allowed to cool for 10-15 minutes. The chips were then labeled, bagged and stored at -20 °C until extraction. Samples for sensory evaluations were stored in dark conditions at 4 °C in air-tight bags.

2.4. Preparation of Extracts

Baked or chipped potato samples (10 g) or anthocyanin-rich fruits (5 g) were homogenized with 25 ml acidified ethanol (80%, with 0.1% v/v formic acid). Homogenates were poured into chloroform-resistant tubes and vortexed every 15 minutes for an hour. Then 15 ml chloroform was added to the tubes and they were vortexed every 10 minutes for 30 minutes. The tubes were then centrifuged at 4000 rpm for 10 minutes and stored overnight to allow layer separation. Supernatants were collected and stored at -20 °C until further analyses. Data were corrected for moisture loss due to processing (baking or chipping) and storage. To minimize intra-clonal variability, eight randomized samples (biological replicates) were taken and extracted separately for each time point and processing method.

2.5. Determination of Total Phenolic Content

Total phenolic content of the potato extracts was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al. 1999). In a 96-well microplate, 150 µl of 0.2 M Folin-Ciocalteu reagent was added to 35 µl of potato extract and held for 5 minutes. Then, 115 µl of sodium carbonate solution (7.5% w/v) was added and the mixture was allowed to react for 30 minutes at 45 °C and cooled to room temperature for one hour. The absorbance was read at 765 nm using a microplate reader (Synergy-2, BioTek Instruments Inc., Winooski, VT), and expressed as milligrams of gallic acid equivalents *per* 100 g of fresh potato sample (mg GAE/100 gfw).

2.6. Determination of Total Monomeric Anthocyanin Content

The total monomeric anthocyanin content was determined by pH differential method (Wrolstad 1976). In a 96-well microplate, 290 µl of buffers (pH 1.0 and pH 4.5)

were added separately to 10 µl of dilute potato extract. Absorbance (A) was obtained using the equation below.

$$A = (A_{525} - A_{700})_{pH1.0} - (A_{525} - A_{700})_{pH4.5}$$

Monomeric anthocyanin concentration (MAC) was calculated in terms of cyanidin-3-glucoside, using an extinction coefficient (Ɛ) of 26,900 l/cm/mol and molecular weight (MW) of 449.2 g/mol, and standard path length of 1 cm and a dilution factor (DF) of 10 using the formula below.

$$MAC\ (mg/l) = \frac{(A\ x\ MW\ x\ DF\ x\ 1000)}{(Ɛx\ 1)}$$

Anthocyanin content was reported as mg cyanidin-3-glucoside *per* 100 g of fresh potato sample (mg C-3-G equivalents/100 gfw).

2.7. Antioxidant Activity Analysis

Antioxidant activity was measured using modified 2,2-diphenyl-1-picryhydrazyl radical (DPPH) assay (Blois 1958) and modified 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay (Awika et al. 2003; Reddivari et al. 2007a). For the DPPH assay, freshly prepared 285 µl of diluted DPPH solution (240 µg/ml) were added to 15 µl of potato extracts in a 96-well microplate, and allowed to react for thirty minutes. The absorbance was measured at 517 nm using a microplate reader (BioTek Instruments Inc., Winooski, VT) and compared with trolox standards. The antioxidant activity was calculated as mg trolox equivalent *per* 100 g of fresh potato sample (mg TE/100 gfw).

For the ABTS assay, equal volumes of 3 mM ABTS radical and 8 mM potassium persulfate were allowed to react in the dark for at least 16 hours at room temperature to form the mother solution. Then 5 ml of this mother solution was mixed with 145 ml of phosphate buffer (pH 7.4) to make the working solution. In a 96-well microplate, 290 µl

of the ABTS working solution was added to 10 µl of potato extracts and allowed to react for thirty minutes. The absorbance was measured at 734 nm using a microplate reader (BioTek Instruments Inc., Winooski, VT). The antioxidant activity of the samples was expressed as mg trolox equivalent *per* 100 g of fresh potato sample (mg TE/100 gfw).

2.8. Ultra Performance Liquid Chromatography (UPLC) and Mass Spectrometry

Potato extracts (2 µl) were injected in a Waters Acquity UPLC system (Waters Corporation, Milford, MA) using a HSS T3 column (1.8 µm, 1.0 x 100 mm), and a gradient from solvent A (100% water, 0.1% formic acid) to solvent B (95% methanol, 5% water, 0.1% formic acid). Injections were made in 100% A, which was held for 2 minutes, followed by a 13 minute linear gradient to 100% B, followed by a 2.0 minute hold at 100% B. The column was returned to starting condition over 0.1 minutes, and allowed to reequilibrate for 2.9 minutes. Flow rate was kept constant at 140 µl/min for the duration of the run. The column and the auto sampler were held at 50 °C and 5 °C, respectively.

Column eluent was infused into a Micromass Q-Tof Micro mass spectrometer (Waters Corporation, Milford, MA) fitted with an electrospray source. Data were collected in positive ion full scan mode, scanning from m/z 50-1200 at a rate of 2 scans *per* second with an interscan delay of 0.1 second. Calibration was performed prior to sample analysis via infusion of sodium formate solution, with mass accuracy within 3 ppm. The capillary voltage was held at 2200 V, the source temperature at 130 °C, and the desolvation temperature at 300 °C with a nitrogen desolvation gas flow rate of 300 l/hr. The quadrupole was held at collision energy of 7 V. Peak detection was performed using MarkerLynx software (Waters MassLynx, v 4.1, Milford, MA).

2.9. Human Colon Cancer Cell Lines

HCT-116, p53+/+ cells were a generous gift from Dr. Bert Vogelstein and HT-29 cells were purchased from ATCC (Manassas, VA). The cells were maintained at 37 °C in a humidified 5% CO_2 incubator in McCoy's 5A medium supplemented with sodium bicarbonate (2.2 g/l), fetal bovine serum (100 ml/l), and streptomycin/penicillin mix (10 ml/l).

2.10. Cell Proliferation and Apoptosis

Cell proliferation was assessed via BrdU assay (Cell Signaling Technology, MA) and cell counting using an automated cell counter (Nexcelom Bioscience, Lawrence, MA). Briefly, HCT-116 or HT-29 cells were grown in 96 well plates at 4000 cells *per* well in Dulbecco's modified Eagle's medium F-12 (DMEM). After 24 hours, the cells were treated with potato extracts diluted in DMEM having final phenolic concentrations of 10, 20 and 30 µg GAE/ml. The treatments were added in triplicates at the volume of 1ml *per* well and then allowed to incubate for 24 hours. At the end of the incubation period, cell viability was assessed by quantifying the amount of 5-bromo-2'-deoxyuridine (BrdU) incorporated into cellular DNA of proliferating cells using an anti-BrdU antibody. For cell counting, cells were plated at 50,000 cells *per* well in a 12-well plate and treated as above and reported as *per* cent reduction with respect to control. Apoptosis was measured using the Caspase-Glo 3/7 assay (Promega Corporation, Madison, WI). After 24 hour incubation with the extracts, HCT-116 and HT-29 cells were counted and 15,000 cells were added *per* well to a 96-well microplate and the volume was made up to 200 µl using DMEM. Caspase-Glo 3/7 reagent (100 µl) was added to each well and the plate was placed on a shaker at 300 rpm for 5 minutes. The plate was incubated in the dark at

room temperature and luminescence was measured after 30 minutes. Cells undergoing apoptosis have a higher caspase-3 and caspase-7 activity, which result in a stronger luminescence signal. To compare the effects of baking and chipping on the anti-cancer properties of potatoes, the reduction in cell proliferation and apoptotic index were compared at a single concentration, 30 µg GAE/ml, which was the highest concentration in the study.

2.11. Sensory Evaluation

Sensory evaluation of the baked and chipped samples from all seven potato clones was carried out with 116 and 114 untrained panelists, respectively. Evaluations were carried out using samples at day 30 and day 90 to study the effect of storage on the sensory attributes. The baked potato samples were not stored and were served fresh while the chipped samples were tested after two months of storage to mimic market conditions wherein bagged chips may sit on shelves up to three months. The panelists were asked to judge the samples based on their appearance, taste, color, texture and overall acceptability on a 9-point hedonic scale (1 = disliked extremely; 9 = liked extremely) and were asked to rank the samples based on their preference (1 = liked most; 7 = disliked most). Each sample was assigned a three-digit random code and served in a two-ounce portion cup in random order. The panelists were asked to rinse their mouths with distilled water and bite into an unsalted cracker between samples to clean their palates. The consumer panelists were informed that 'purple-colored potatoes are a rich source of anthocyanins which are antioxidants and potential anti-inflammatory agents' through their sensory evaluation forms and asked if they would prefer colored-fleshed potatoes over conventional white-fleshed cultivars given these potential health benefits of

colored-fleshed potatoes. Consumer willingness to pay more for colored-flesh potato chips was also assessed through a simple question. Panelists were asked how much more they would be willing to pay if a 10.5 ounce bag of chips cost $ 3.20.

2.12. Statistical Analysis

Fisher's protected t-test using the Least Square Means test, which was used for comparing group differences with $p < 0.05$ being considered as statistically significant, and Pearson correlation coefficients were calculated using SAS Statistical Analysis System, v.9.2 (SAS Institute Inc., Cary, NC). All results have been expressed as mean ± standard error. For principal component analysis, peak areas were exported to SIMCA-P+ (Umetrics AB, v12.0, Sweden). Data were scaled to unit variance and mean centered before principal component analysis.

3. Results and discussion

3.1. Total Phenolic Content

Total phenolic content of baked and chipped potato samples was measured for seven potato clones every 30 days for 90 days. Total phenolic content of the baked and chipped potato samples ranged from 11.2 to 307.7 mg GAE/100 gfw and 1.8 to 18.7 mg GAE/100 gfw (Table 4.1.) for all seven clones over the entire storage period, respectively. When compared with uncooked samples at Day 90, depending on the clone, baking decreased or increased the phenolic content. Purple-fleshed clones CO97215-2P/P and CO97227-2P/PW showed increased phenolic content post-baking, while chipping retained only 4-7% in all clones.

Researchers have reported both an increase and a decrease in the phenolic content post-baking (Im et al. 2008; Xu et al. 2009; Blessington et al. 2010; Navarre et al. 2010).

The increase in the phenolic content of the two purple-fleshed clones could be due to release of bound phenolic compounds during baking. Cooking may weaken the matrix thus improving the extractability of phenolic compounds and inactivate enzymes that use phenolic compounds as substrate (Ezekiel et al. 2011). Effects of processing cannot be generalized for all potato clones as they differ depending on potato genotype. It was seen that microwaving or baking significantly affects the total phenolic content of Dakota Pearl cultivar but not of the Nordonna cultivar. The total phenolic content of Red Norland cultivar is affected approximately equally by boiling, baking or microwaving (Xu et al. 2009). In this study, chipping and frying resulted in greater losses in phenolic content (Tudela et al. 2002; Im et al. 2008). Chipping/slicing increases the surface area, which might be responsible for the greater degradation of bioactive compounds.

With storage, an increase ($p < 0.05$) was observed in the phenolic content of baked samples of all clones. Baked purple-fleshed potatoes had higher phenolic content ($p < 0.05$) as compared with their white- and yellow-fleshed counterparts throughout the storage duration. For baked Purple Majesty samples, the phenolic content increased at 60 days of storage and then did not change over the next 30 days. Storage may make the matrix weaker leading to a greater release of bound phenolic compounds of stored potatoes after baking.

As seen for the baked samples, CO97227-2P/PW chipped samples had the highest amount of total phenolics while the lowest was in Atlantic chipped samples. However, storage did not seem to influence the phenolic content of the chipped samples with the exception of CO97227-2P/PW. Hence, it is important to consider the effects of genotype

Table 4.1. Total phenolic content of potatoes after storage and processing.

Potato clones	Baked			Chipped				Uncooked
	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90	Day 90
Atlantic	11.2±0.6	17.9±1.0	22.5±0.9*	2.51±0.03	2.61±0.05	2.18±0.02	1.83±0.01	25.1±0.4
Yukon Gold	12.7±0.6	12.8±0.8	25.0±0.8*	3.04±0.03	3.51±0.04	2.65±0.01	2.27±0.01	29.1±0.6
Purple Majesty	69.6±1.5	125.7±7.2*	117.3±3.4*	10.2±0.21	8.34±0.33	7.92±0.31	9.06±0.29	118.1±3.8
AC97521-1R/Y	20.7±0.6	26.7±1.2	38.1±1.3*	3.54±0.04	3.72±0.05	2.96±0.03	2.55±0.02	38.3±0.6
CO97232-2R/Y	31.9±1.1	35.1±2.4	50.2±1.4*	3.11±0.04	3.74±0.03	2.82±0.02	2.33±0.01	44.6±0.5
CO97215-2P/P	143.9±4.5	148.3±2.4	191.7±9.6*	13.14±0.30	13.28±0.23	13.46±0.28	10.86±0.21	117.3±5.0
CO97227-2P/PW	180.1±4.7	213.5±8.5*	307.7±8.0*	18.72±0.50	18.02±0.31	14.98±0.23	13.34±0.32*	205.4±5.5

The letters (P/P, P/PW and R/Y) after some of the advanced selections denote skin/flesh color. P: purple-fleshed; PW: purple-fleshed with white-fleshed patches; R: red skin; Y: yellow-fleshed. *Indicates significant differences ($p < 0.05$) in the phenolic content compared with the initial time point (Day 30 for baked and Day 0 for chipped). Results are presented as mean ± SE of eight replicates for each time point and expressed as mg gallic acid equivalents/100 gfw.

and farm-to-fork operations such as storage and processing while selecting clones for breeding potatoes with greater health-benefiting compounds.

To compare the phenolic content of baked purple-fleshed clones with traditional anthocyanin-rich fruits, the same extraction procedure and analysis were used. Blueberries, grapes, raspberries and strawberries had 323.3 ± 4.1, 199.5 ± 5.0, 170.0 ± 3.1, and 113.7 ± 2.8 mg GAE/100 gfw respectively; whereas, the phenolic content of baked purple-fleshed clones ranged from 117.0 ± 3.4 to 307.7 ± 8.0 mg GAE/100 gfw after 90 days of storage. Thus, the phenolic content of purple-fleshed potatoes was comparable to that of berries making them an affordable choice for most people.

3.2. Anthocyanin Content

The anthocyanin content of the baked and chipped potatoes was calculated only for the purple-fleshed clones. For the baked samples, the anthocyanin content ranged from 13.4 to 81.3 mg C-3-G equivalents/100 gfw while for the chipped samples ranged from 0.8 to 3.2 mg C-3-G equivalents/100 gfw (Table 4.2.). We report for the first time that baking either increases or fully retains the anthocyanin content of the purple-fleshed potatoes, with the exception of Purple Majesty, which retained only 63% anthocyanin content, while chipping resulted in ~97% losses as compared with uncooked samples at Day 90. Purple Majesty baked samples had the lowest anthocyanin content throughout the duration of storage while the CO97227-2P/PW baked samples consistently had the highest anthocyanin content. The anthocyanin content of most baked potato cultivars increased ($p < 0.05$) at the end of the storage. Purple Majesty baked samples showed a peak at 60 days of storage followed by a decrease in the anthocyanin content at 90 days of storage. The anthocyanin trend observed was similar to that of the total phenolic

content which suggests the role of anthocyanins in contributing to the total phenolic content.

CO97227-2P/PW and Purple Majesty chipped samples consistently had the highest and the lowest anthocyanin content, respectively. The chipped samples showed a gradual decrease in the anthocyanin content and at 90 days of storage, however, the differences were not significant.

The anthocyanin content of the baked samples was comparable to that of blueberries (81.8 ± 3.0 mg C-3-G equivalents/100 gfw), raspberries (50.0 ± 0.7 mg C-3-G equivalents/100 gfw), grapes (45.5 ± 0.8 mg C-3-G equivalents/100 gfw) and strawberries (41.9 ± 1.7 mg C-3-G equivalents/100 gfw). Thus, as seen with the total phenolic content, purple-fleshed potatoes can act as a rich source of anthocyanins in the diet.

3.3. Antioxidant Activity

Antioxidant activity of the baked samples ranged from 7.9 to 1270.0 mg TE/100 gfw as measured by the DPPH assay (Table 4.3.) and 28.3 to 1113.0 mg TE/100 gfw as measured by the ABTS assay (Table 4.4.). For the chipped samples, the antioxidant activity ranged from 2.1 to 38.3 mg TE/100 gfw (DPPH) and 2.3 to 33.8 mg TE/100 gfw (ABTS). When compared with uncooked Day 90 samples, baking led to 10-81% loss as assessed by the DPPH assay and up to 26% losses when tested by the ABTS assay. Chipping resulted in losses greater than 97% when measured by DPPH and ABTS assays when compared with uncooked Day 90 samples.

The antioxidant activity of the purple-fleshed samples was significantly higher than the white- and yellow-fleshed samples. Both assays confirmed an increase ($p < 0.05$)

in the antioxidant activity of baked samples with storage. Similar DPPH values for baked white-fleshed potatoes have been reported to range from 11.3 to 21.2 mg TE/100 gfw (Xu et al. 2009). The chipped samples showed different trends for the DPPH and ABTS assays in terms of antioxidant activity with storage (Tables 4.3 and 4.4). Chipped samples from most clones showed no change in the antioxidant activity over the entire period of the storage when measured by the DPPH or ABTS assays.

The slight differences between the antioxidant values of the ABTS and DPPH assays could be due to differential reactivity of bioactive compounds towards the ABTS and DPPH radicals due to steric hindrance (Prior et al. 2005). Although correlations between the assays might be strong, reflecting the general trend, the values can be different (Thaipong et al. 2006; Dudonné et al. 2009).

The antioxidant values of the baked potatoes were comparable to that of anthocyanin-rich fruits. The DPPH values for blueberries, strawberries, raspberries and grapes were 918.2 ± 16.2, 570.7 ± 9.1, 640.1 ± 6.5, and 367.1 ± 10.3 mg TE/100 gfw, respectively, while the ABTS values were 542.9 ± 7.8, 413.3 ± 5.5, 380.8 ± 6.2 and, 236.8 ± 6.8 mg TE/100 gfw, respectively. Thus, baked purple-fleshed potatoes can also serve as a rich source of antioxidants.

3.4. UPLC-MS profile of Phenolic Compounds

Based on the data from the spectrophotometric assays, processed samples from four clones were selected for screening of their metabolite profiles. Three of the four selected were commercially available cultivars representative of their color – Atlantic (white-fleshed), Yukon Gold (yellow-fleshed) and Purple Majesty (purple-fleshed) – and

Table 4.2. Anthocyanin content of potatoes after storage and processing.

Potato clones	Baked			Chipped				Uncooked
	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90	Day 90
Purple Majesty	13.4±0.2	25.7±1.6*	18.6±0.9	1.24±0.07	1.13±0.03	0.93±0.05	0.81±0.05	29.6±0.9
CO97215-2P/P	31.3±0.8	36.0±1.9	44.1±1.6*	1.57±0.09	1.29±0.06	1.12±0.05	1.07±0.03	32.1±2.2
CO97227-2P/PW	51.2±1.7	61.1±2.7*	81.3±2.6*	3.24±0.14	3.25±0.22	2.79±0.09	2.61±0.17	82.9±2.4

The letters (P/P, and P/PW) after some of the advanced selections denote skin/flesh color. P: purple-fleshed; PW: purple-fleshed with white-fleshed patches. *Indicates significant differences ($p < 0.05$) in the phenolic content compared with the initial time point (Day 30 for baked and Day 0 for chipped). Results are presented as mean ± SE of eight replicates for each time point and expressed as mg cyanidin-3-glucoside equivalents/100 gfw.

Table 4.3. Antioxidant activity (DPPH) of potatoes after storage and processing.

Potato clones	Baked			Chipped				Uncooked
	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90	Day 90
Atlantic	8.4±0.8	22.7±0.9*	20.4±1.0*	2.84±0.02	2.36±0.02	2.38±0.03	2.07±0.02	107.8±0.5
Yukon Gold	7.9±0.8	14.0±0.9*	22.0±0.9*	3.33±0.02	3.03±0.02	3.33±0.02	3.55±0.01	115.2±0.5
Purple Majesty	678±14	982±26.3*	1071±18*	34.0±0.04	32.7±0.06	37.5±0.05	38.3±0.12	1401±5.8
AC97521-1R/Y	20.4±1.0	32.9±1.4*	40.9±1.2*	3.32±0.01	3.24±0.01	3.60±0.01	3.72±0.01	117.1±0.5
CO97232-2R/Y	28.6±1.3	31.4±2.0	52.9±2.0*	3.27±0.02	3.13±0.02	3.44±0.02	3.54±0.02	112.6±0.3
CO97215-2P/P	855±16	946±29*	1087±26*	33.0±0.06	31.8±0.09	35.8±0.14	37.5±0.2	1314±13.7
CO97227-2P/PW	1096±20	1228±8*	1270±7*	31.3±0.2	30.7±0.2	35.1±0.2	36.0±0.4	1412.2±1.3

The letters (P/P, P/PW and R/Y) after some of the advanced selections denote skin/flesh color. P: purple-fleshed; PW: purple-fleshed with white-fleshed patches; R: red skin; Y: yellow-fleshed. *Indicates significant differences ($p < 0.05$) in the phenolic content compared with the initial time point (Day 30 for baked and Day 0 for chipped). Results are presented as mean ± SE of eight replicates for each time point and expressed as mg trolox equivalents/100 gfw.

Table 4.4. Antioxidant activity (ABTS) of potatoes after storage and processing.

Potato clones	Baked			Chipped				Uncooked
	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90	Day 90
Atlantic	28.3±3.1	101.9±4.8*	136.4±5.1*	2.28±0.06	2.67±0.09	3.66±0.36	2.75±0.41	144.4±4.9
Yukon Gold	57.5±5.6	78.8±3.5*	146.9±3.9*	3.01±0.10	3.45±0.05	7.11±0.60*	5.58±0.43	164.7±4.1
Purple Majesty	559±32	649±28*	670±27*	15.77±0.92	15.94±0.70	21.11±1.89*	17.41±0.78	908.3±20.1
AC97521-1R/Y	93.8±4.6	153.8±5.04*	184.5±4.5*	4.61±0.07	4.45±0.12	9.02±0.70*	7.92±0.84*	198.2±3.4
CO97232-2R/Y	180.2±5.0	173.10±10.3	211.7±4.1*	4.25±0.07	4.24±0.10	6.36±0.28	6.61±0.45	226.0±4.1
CO97215-2P/P	585±31	722.6±38.6*	783±40*	17.99±1.08	20.16±1.02	26.34±3.03*	20.60±1.68	1058.4±23.4
CO97227-2P/PW	661±50	865.6±20.4*	1113±22*	21.49±0.86	30.30±1.96*	33.85±1.80*	27.18±2.25	1285.4±25.1

The letters (P/P, P/PW and R/Y) after some of the advanced selections denote skin/flesh color. P: purple-fleshed; PW: purple-fleshed with white-fleshed patches; R: red skin; Y: yellow-fleshed. *Indicates significant differences ($p < 0.05$) in the phenolic content compared with the initial time point (Day 30 for baked and Day 0 for chipped). Results are presented as mean ± SE of eight replicates for each time point and expressed as mg trolox equivalents/100 gfw.

the fourth was a purple-white-fleshed advanced selection, CO97227-2P/PW, which had the highest phenolic content, antioxidant activity and anthocyanin content.

Chlorogenic acid is the most abundant phenolic acid found in potatoes (Dao and Friedman 1992). In the baked white- and yellow-fleshed cultivars, chlorogenic acid degraded to negligible amounts (Table 4.5) as compared with uncooked white- and yellow-fleshed samples seen in Table 3.1. However, a 13 – 100% increase was seen in the chlorogenic acid content of the purple-fleshed clones that were baked after 90 days of storage. Degradation may be due to the susceptibility of chlorogenic acid to heat and, similar to our results, reports suggest a 100% loss after baking in an oven at 212°C for 45 minutes (Dao and Friedman 1992). Conversely, some studies show that chlorogenic acid is reduced but not completely destroyed after baking. However, the baking was done at 178°C for 40 minutes and the study claims that the peels could act as barriers against the loss of chlorogenic acid (Xu et al. 2009). In our present study, the baking was done at 204°C for 75 minutes which could explain the loss of chlorogenic acid in baked Atlantic and Yukon Gold potatoes. Chipped samples showed a decrease in the phenolic acids (Table 4.6.) as compared with uncooked samples (Table 3.1.). Storage led to an increase in the chlorogenic acid content. Low temperature, strong light, wounding, pathogen attack and other environmental stresses during storage can lead to the synthesis of phenolic compounds via the phenylpropanoid pathway (Dixon and Paiva 1995). Thus, storage at different temperatures might alter the levels of phenolic acids in processed potato products.

Baked purple-fleshed samples contained anthocyanins in their glycosylated form such as petunidin-3-rutinoside-5-glucoside (Pet-3-rut-5-glc) and malvidin-3-rutinoside-5-

glucoside (Mal-3-rut-5-glc). Many anthocyanins were acylated with para-coumaric acid such as peonidin-3-(p-coumaroyl)-rutinoside-5-glucoside (peo-3-coum-rut-5-glc), petunidin-3-(p-coumaroyl)-rutinoside-5-glucoside (pet-3-coum-rut-5-glc), pelargonidin-3-(p-coumaroyl)-rutinoside-5-glucoside (pel-3-coum-rut-5-glc) and malvidin-3-(p-coumaroyl)-rutinoside-5-glucoside (mal-3-coum-rut-5-glc) (Table 4.5.). CO97227-2P/PW cultivar had approximately a 5-fold higher amount of mal-3-coum-rut-5-glc as compared with Purple Majesty. Pet-3-coum-rut-5-glc was the most abundant anthocyanin, followed by peo-3-coum-rut-5-glc. Storage increased most individual anthocyanins in both purple-fleshed clones. It is known that low temperature storage can lead to biosynthesis of phenolic compounds including anthocyanins through the activation of phenyl ammonia-lyase (PAL), a key enzyme in the phenylpropanoid pathway(Jiang and Joyce 2003) This could explain the observed increase in the monomeric anthocyanin content from day 0 to day 90. Chipped samples also contained glycosylated anthocyanins and pet-3-coum-rut-5-glc was the most abundant anthocyanin followed by peo-3-coum-rut-5-glc and mal-3-coum-rut-5-glc (Table 4.6.).

Principal component analysis of approximately 1600 peaks obtained through UPLC-MS revealed overall differences in the metabolite profiles of the baked (Figure 4.1.) and chipped (Figure 4.2.) potato samples. Baked Atlantic and Yukon Gold samples had similar metabolite profiles (Figure 4.1.). Storage altered the metabolite profile of the baked samples of both these cultivars. The similarity among the profiles of these cultivars also reduced with storage. Baked samples of the purple-fleshed clones were different from the non-purple-fleshed cultivars and also among themselves indicating that different

Table 4.5. Phenolic compound profile of baked potato samples by UPLC-MS.

Compound	Molecular ion M^+ (m/z)	Retention time (mins)	Atlantic		Yukon Gold		Purple Majesty		CO97227-2P/PW	
			Day 30	Day 90	Day 30	Day 90	Day 30	Day 90	Day 30	Day 90
Chlorogenic acid	355	6.11	0.05± <0.01	1.50±0.19	0.05± 0.02	1.40± 0.28	16.76± 0.20	27.49± 0.86	37.97± 0.78	52.33± 0.68
Caffeic acid	181	6.15	0.35± 0.06	0.75±0.07	0.34± 0.10	0.63± 0.07	6.19± 0.78	9.97± 0.78	11.53± 0.50	13.28± 0.35
Ferulic acid	177	7.56	-	0.08± <0.01	-	0.07± <0.01	-	-	-	-
Sinapic acid	207	7.76	-	-	-	0.02± <0.01	-	-	-	-
Pet-3-rut-5-glc	787	5.79	-	-	-	-	323	653	881	1265
Mal-3-rut-5-glc	801	6.19	-	-	-	-	43	61	181	215
Peo-3-coum-rut-5-glc isomer	917	7.7	-	-	-	-	-	-	1443	1449
Pet-3-coum-rut-5-glc	933	7.92	-	-	-	-	7171	8937	10889	12053
Pel-3-coum-rut-5-glc	887	8.11	-	-	-	-	190	-	1454	1469
Peo-3-coum-rut-5-glc	917	8.21	-	-	-	-	1273	290	8288	9164
Mal-3-coum-rut-5-glc	947	8.31	-	-	-	-	462	527	1866	2613

The phenolic acid values are expressed as mg/100 gfw potato. The anthocyanins have been reported as area under the curve. Values are presented as mean ± SE of six replicates

Table 4.6. Phenolic compound profile of chipped potato samples by UPLC-MS.

Compound	Molecular ion M^+ (m/z)	Retention time (mins)	Atlantic		Yukon Gold		Purple Majesty		CO97227-2P/PW	
			Day 0	Day 90	Day 0	Day 90	Day 0	Day 90	Day 0	Day 90
Chlorogenic acid	355	6.11	0.21± <0.01	0.13± <0.01	0.22± 0.01	0.20±0.01	1.11± 0.02	1.00± 0.04	1.10± 0.03	1.10± 0.04
Caffeic acid	181	6.15	0.06± <0.01	0.05± <0.01	0.05± 0.01	0.06± <0.01	0.36± 0.02	0.31± 0.02	0.36± 0.01	0.38± 0.03
Ferulic acid	177	7.56	-	-	-	-	-	-	-	-
Sinapic acid	207	7.76	-	-	-	-	-	-	-	-
Pet-3-rut-5-glc	787	5.79	-	-	-	-	623	548	927	952
Mal-3-rut-5-glc	801	6.19	-	-	-	-	87	81	241	243
Peo-3-coum-rut-5-glc isomer	917	7.7	-	-	-	-	-	-	987	915
Pet-3-coum-rut-5-glc	933	7.92	-	-	-	-	11497	10176	12113	10286
Pel-3-coum-rut-5-glc	887	8.11	-	-	-	-	24	-	1707	1758
Peo-3-coum-rut-5-glc	917	8.21	-	-	-	-	716	343	9410	9666
Mal-3-coum-rut-5-glc	947	8.31	-	-	-	-	1075	672	3178	3310

The phenolic acid values are expressed as mg/100 gfw potato. The anthocyanins have been reported as area under the curve. Values are presented as mean ± SE of six replicates

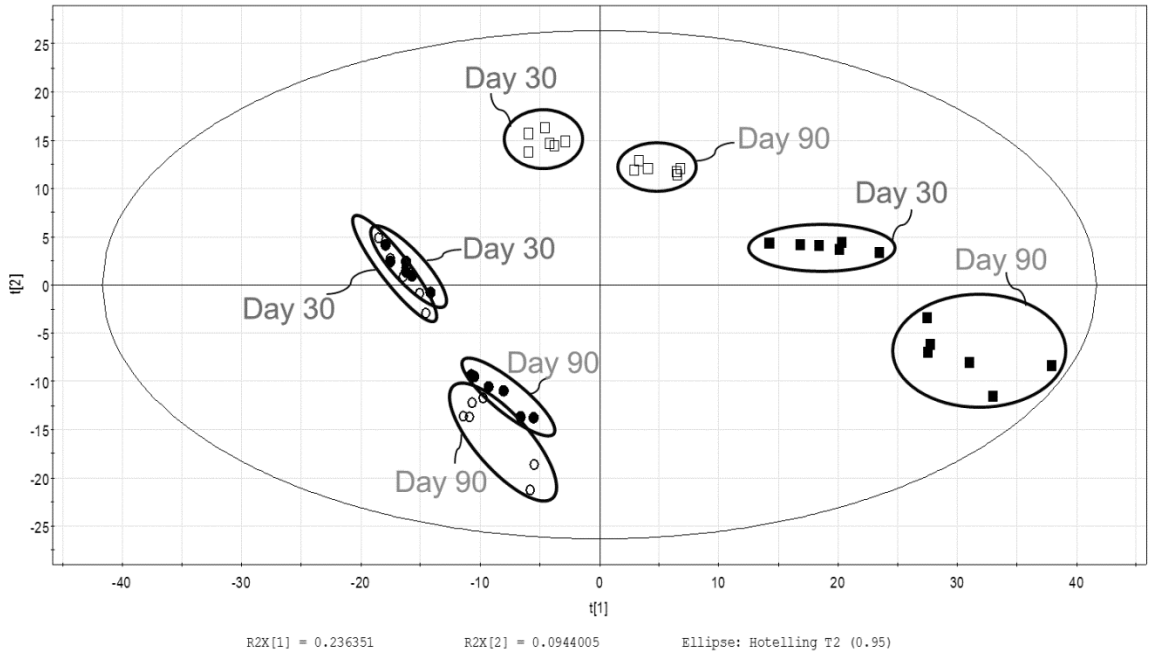


Figure 4.1. Metabolite profiles of baked potatoes during storage - principal component analysis. The bubbles point out difference in phenolic profiles due to storage. Plot shows all the individual data points. Data are represented for Atlantic (●), Purple Majesty (□), Yukon Gold (○) and CO97227-2P/PW (■) cultivars.

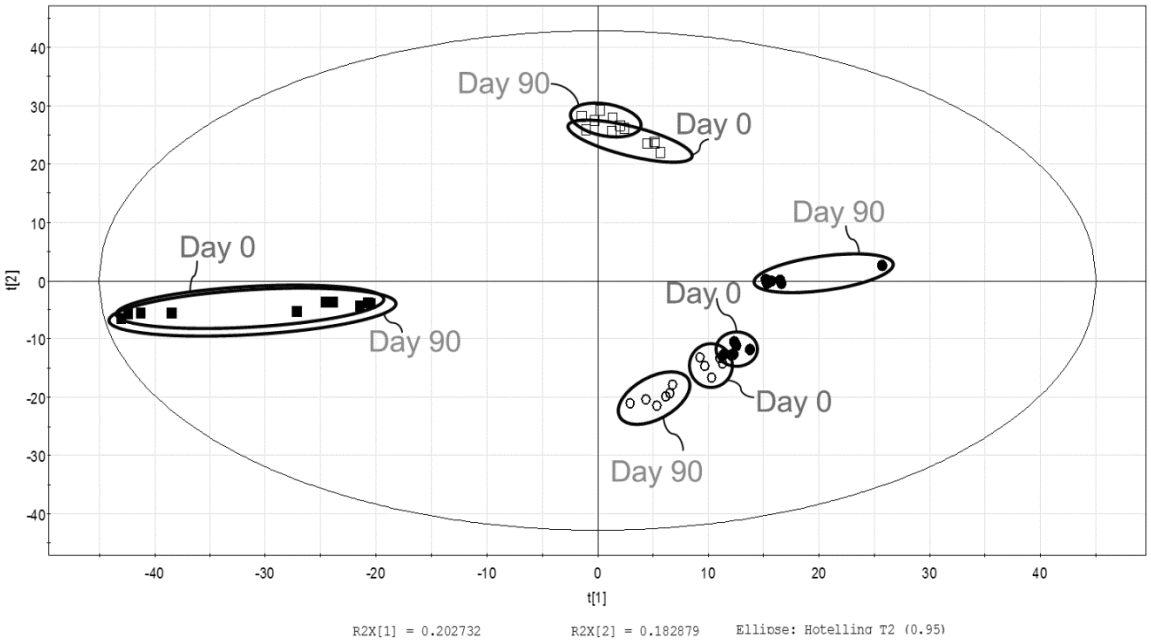


Figure 4.2. Metabolite profiles of chipped potatoes during storage – principal component analysis. Storage caused slight modification of the profiles as pointed out by the bubbles. Plot shows all the individual data points. Data are represented for Atlantic (●), Purple Majesty (□), Yukon Gold (○) and CO97227-2P/PW (■) cultivars.

purple-fleshed potato clones might differ in their metabolite profiles and hence their bioactivity. Storage caused a change in the metabolite profiles of the purple-fleshed potato clones as well. Chipped samples of Atlantic and Yukon Gold had similar metabolite profiles at Day 0. However, at Day 90, a large variation in the profiles was observed (Figure 4.2.). The metabolite profiles of the chips of purple-fleshed clones were different from each other. It was observed that storage did not cause a large change in their metabolite profiles even after 90 days.

3.5. Human Colon Cancer Cell Proliferation and Apoptosis Studies

The anti-cancer activity of baked and chipped potato samples from four potato clones (Atlantic, Yukon Gold, Purple Majesty and CO97227-2P/PW), as described in section 3.4., before and after storage, was tested against early stage (HCT-116, p53 +/+) and advanced stage (HT-29) human colon cancer cell lines. Compared to uncooked samples, bioactivity of the baked potato samples against cell proliferation reduced for most clones while ability to induce apoptosis was comparable at 30 μg GAE/ml (Figures 4.3. and 4.4.). In spite of dosing at same phenolic concentration, chipping significantly suppressed the anti-proliferative and pro-apoptotic properties of potatoes against HCT-116 cells as compared with uncooked samples. This might be due to a change in the phenolic composition of the samples due to chipping. In general, storage suppressed the anti-proliferative properties of potatoes, but some clones were superior in retaining their bioactivity as seen by cell counting (Figures 4.5. and 4.6.) and the BrdU assays (Figures 4.7. and 4.8.). Baked and chipped CO97227-2P/PW samples had more potent anti-proliferative properties against HCT-116 cell lines compared with the other three

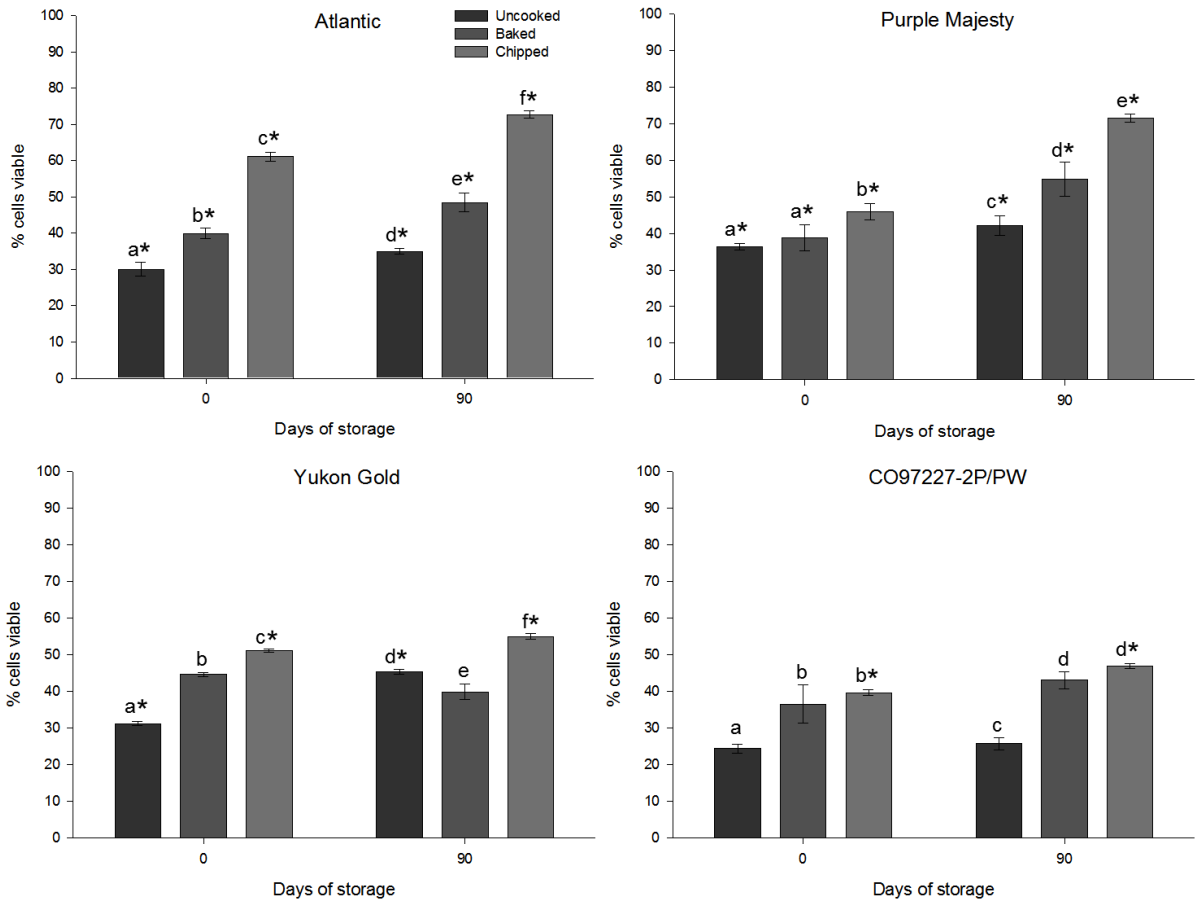


Figure 4.3. Baking and chipping suppressed the anti-proliferative activity in most potato clones at 30 µg GAE/ml concentration. Values with different letters in each graph indicate significant difference ($p < 0.05$) between % cells viable for different processing methods at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given processing method. Results are presented as mean ± SE of four replicates for each time point.

cultivars; with approximately 50% reduction observed in cell proliferation at 30 µg GAE/ml. A similar response was observed in the elevation of apoptosis of HCT-116 cells by baked (Figure 4.9.) and chipped (Figure 4.10.) potatoes. Baked and chipped potatoes showed a dose-dependent increase in the induction of apoptosis with respect to the control (solvent control in media). Purple-fleshed potatoes caused a greater increase in apoptosis as compared to white-and yellow-fleshed cultivars.

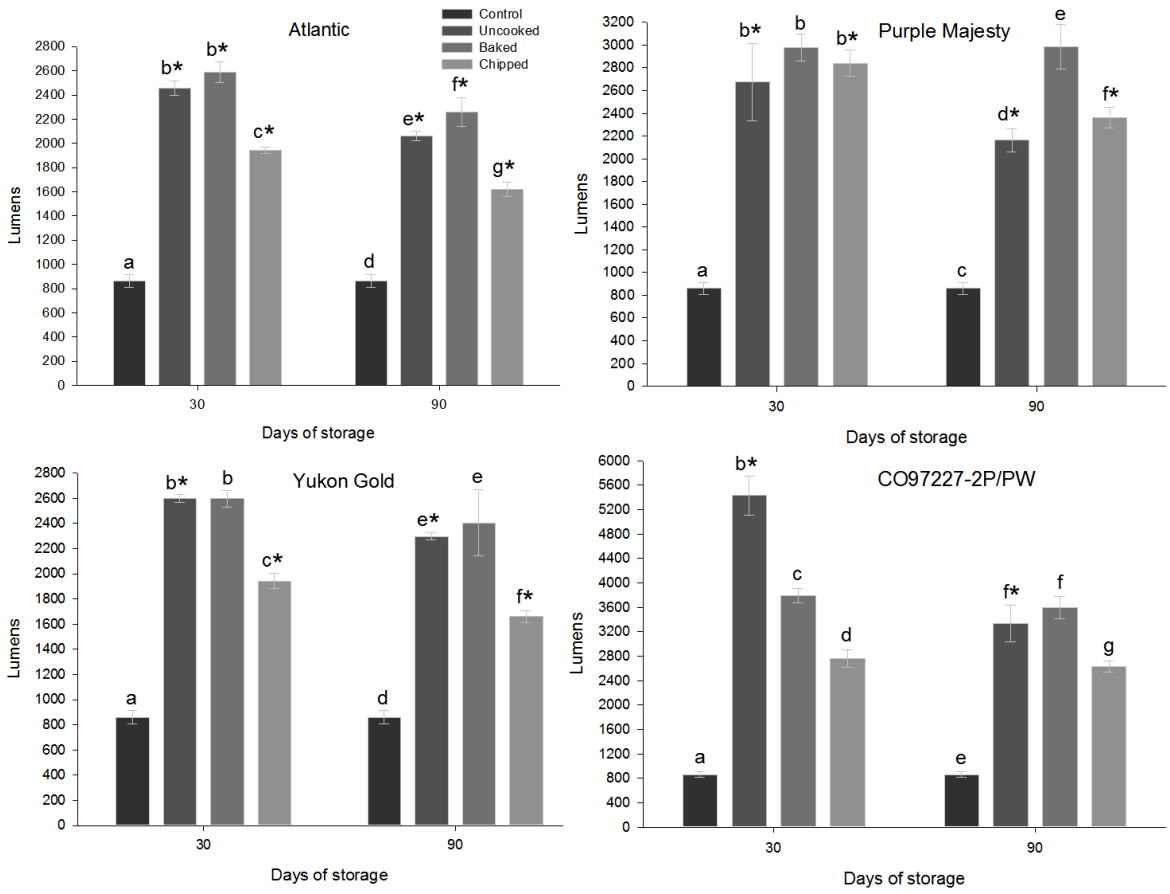


Figure 4.4. Baking and chipping suppressed the pro-apoptotic activity of most potato clones at 30 µg GAE/ml concentration. Values with different letters in each graph indicate significant difference ($p < 0.05$) between apoptotic cells for different processing methods at a given time point. *Indicates a significant difference ($p < 0.05$) between apoptotic cells at two different time points for a given processing method. Results are presented as mean ± SE of four replicates for each time point.

A dose-dependency was observed for the anti-proliferative effect of baked samples against HT-29 cell lines (Figures 4.11. and 4.12.). However, the effect was suppressed as compared to HCT-116 cell lines. In general, storage did not alter the activity of the extracts. A similar trend was observed in the induction of apoptosis (Figure 4.13.). However, the effect of storage in suppressing the proapoptotic activity was more pronounced. The bioactivity of the chipped samples at the tested concentrations did not

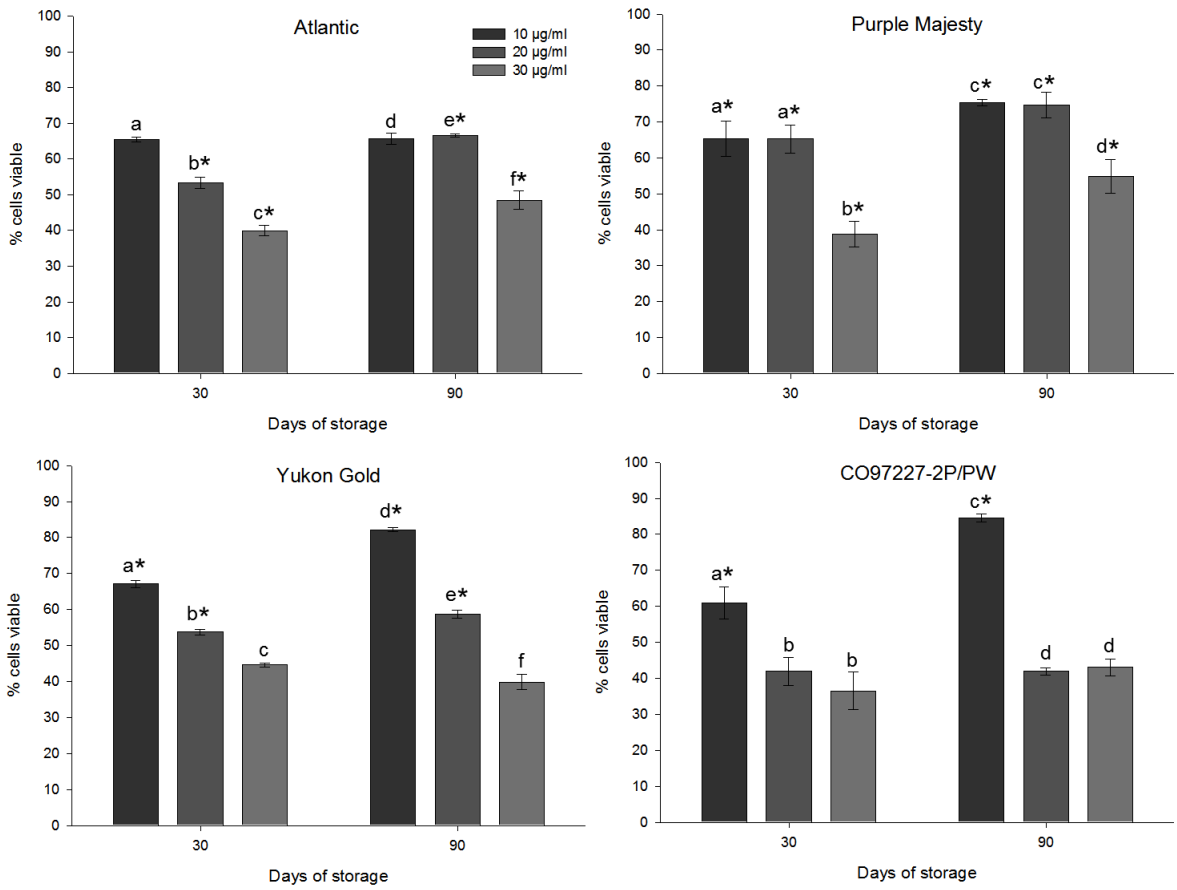


Figure 4.5. Baked potato extracts suppressed proliferation of HCT-116 cells in a dose-dependent manner. Cell number was measured using a cellometer as described in Materials and Methods and represented as % cells viable. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

produce any general trend when tested against advanced stage HT-29 cell lines. This was consistent for both, cell proliferation (Figures 4.14. & 4.15.) and apoptosis assays (Figure 4.16.). These results indicate that chipping and deep frying suppress or completely destroys the anti-cancer properties of potatoes against human colon cancer cell lines. Baked samples were more effective in elevating apoptosis as compared with chipped samples suggesting that baking retains greater bioactivity compared with chipping.

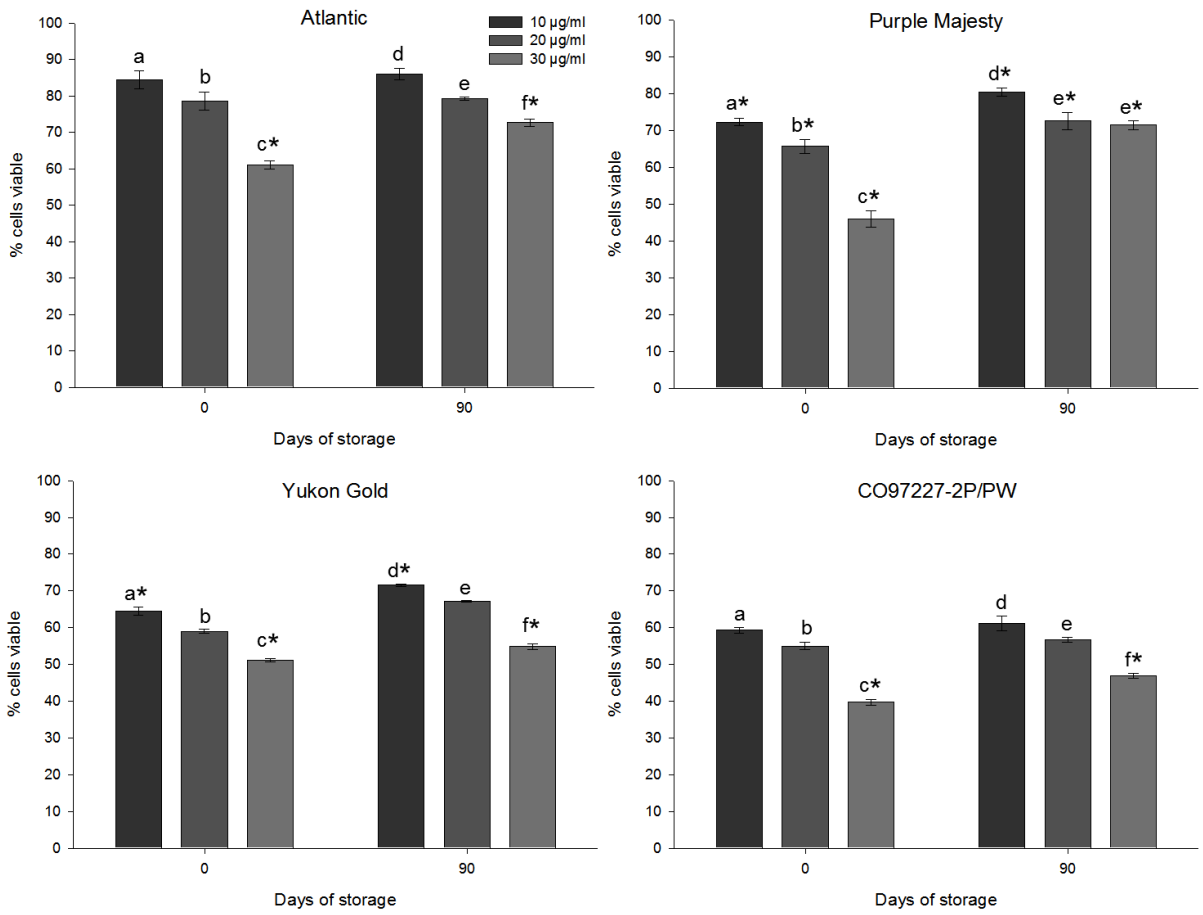


Figure 4.6. Chipped potato extracts suppressed proliferation of HCT-116 cells in a dose-dependent manner. Cell number was measured using a cellometer as described in Materials and Methods and represented as % cells viable. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

CO97227-2P/PW was more potent in inducing apoptosis possibly due to the presence of petunidin and malvidin anthocyanins which have been reported to be pro-apoptotic and anti-proliferative (Stushnoff et al. 2008), respectively as compared to Purple Majesty in which these anthocyanins were at undetectable or very low levels.

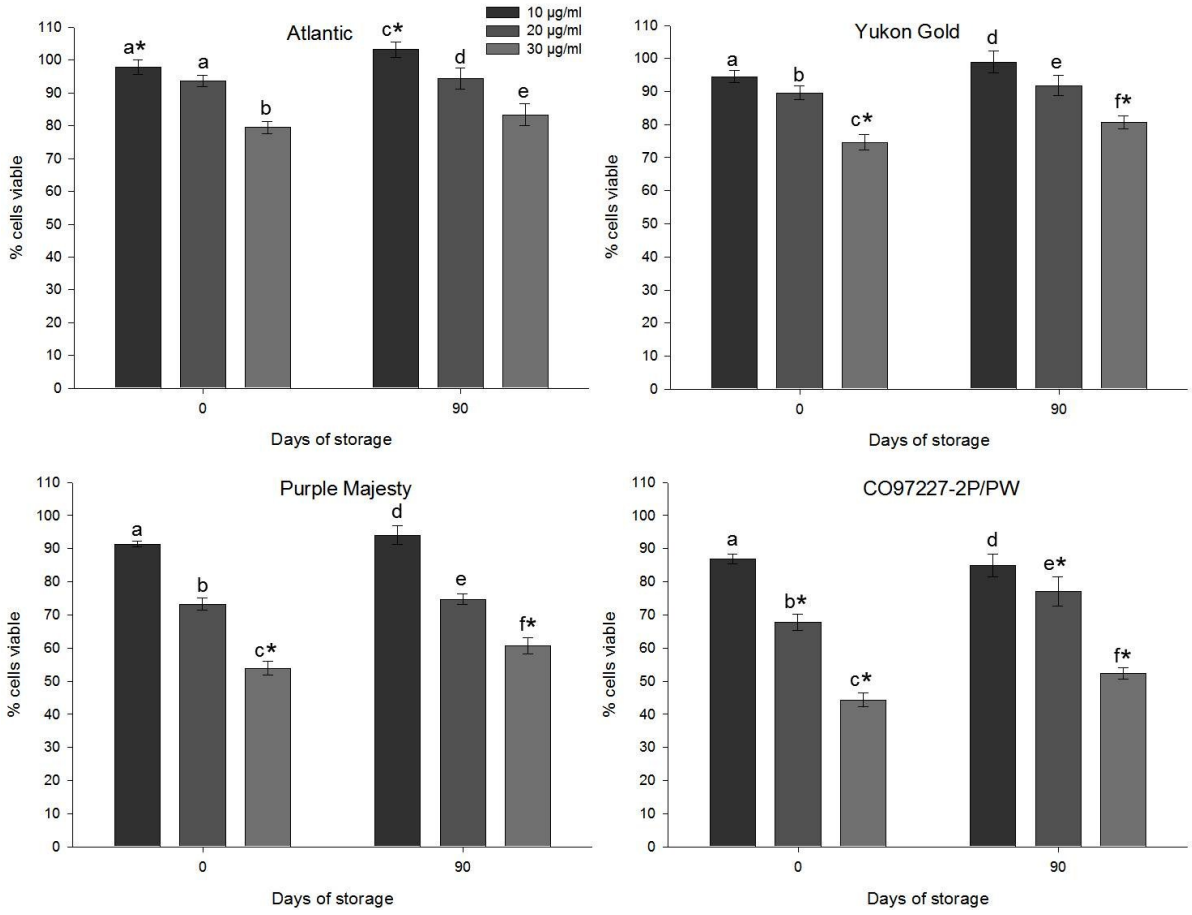


Figure 4.7. Baked potato extracts suppressed proliferation of HCT-116 cells in a dose-dependent manner. Cell viability was measured using the BrdU assay as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

3.6. Sensory Analysis

Sensory evaluations were performed to compare the acceptance of purple-fleshed potatoes with traditional white- and yellow-fleshed varieties. The evaluation of sensory parameters has been shown in the form of spider-charts (Figure 4.17.) and the overall

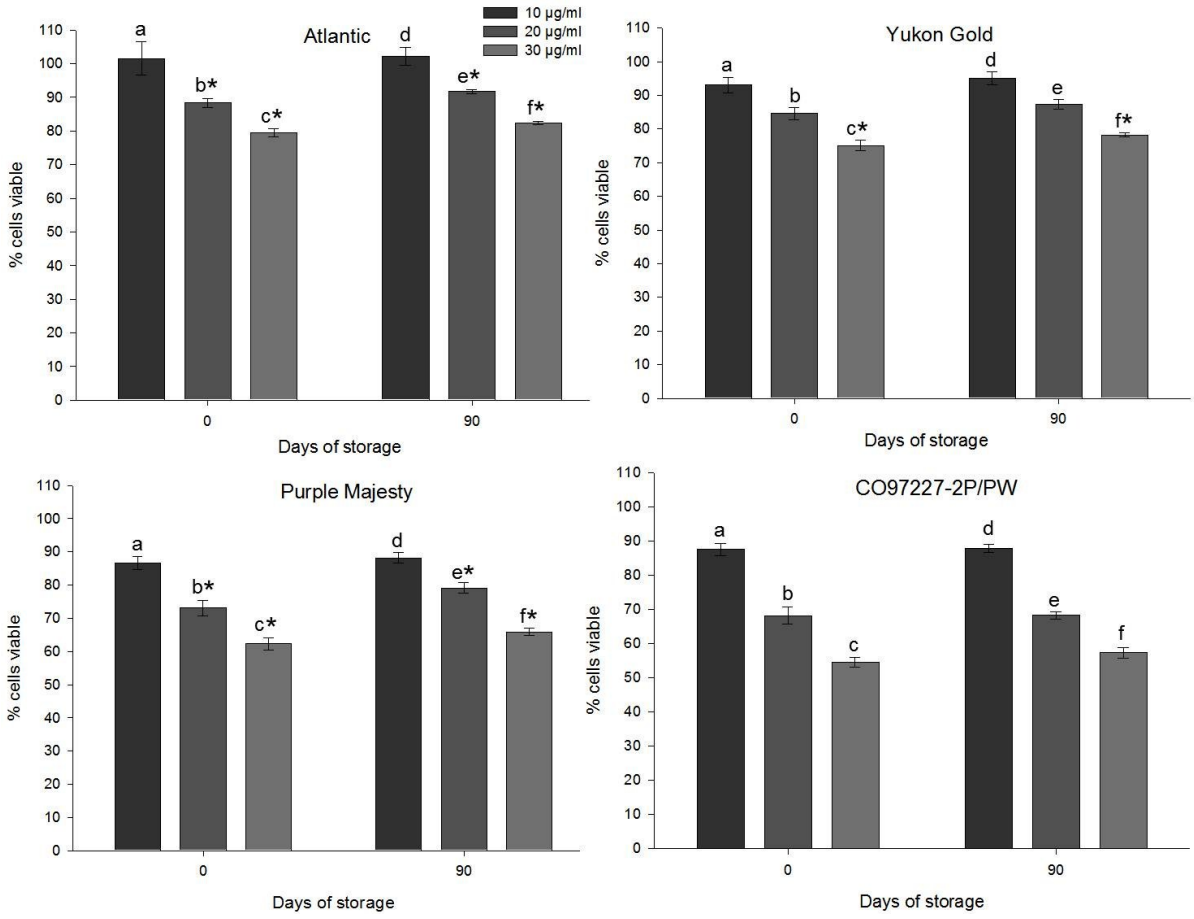


Figure 4.8. Chipped potato extracts suppressed proliferation of HCT-116 cells in a dose-dependent manner. Cell viability was measured using the BrdU assay as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

ranking is listed in Table 4.7. Yukon Gold potatoes, a common baking cultivar, were used as the standard for baked potatoes. For the Day 30 sensory evaluation, Yukon Gold baked potatoes received a mean score of 6.79 from 116 consumer panelists for overall acceptability. Only AC97521-1R/Y received a higher score (6.91) than the standard, although not significantly different. Yukon Gold potatoes received a mean score of 5.82

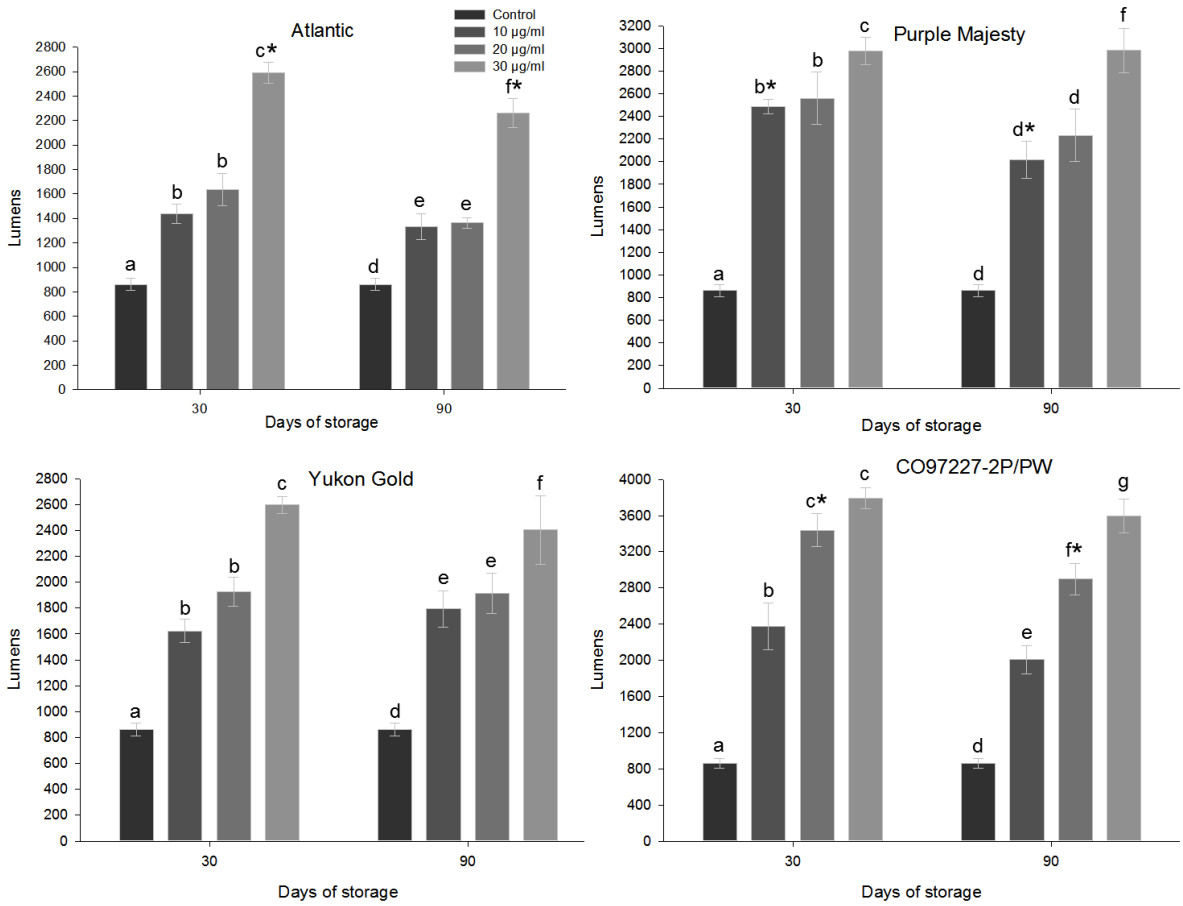


Figure 4.9. Baked potato extracts elevated dose-dependent apoptosis in HCT-116 colon cancer cells. Apoptosis was measured using Caspase-Glo 3/7 assay as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between apoptotic cells at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between apoptotic cells at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

in the Day 90 evaluation. In the Day 30 evaluation, Yukon Gold baked potatoes received a mean rank value of 3.24 and only CO97232-2R/Y received a slightly better mean rank score (2.89) which was not significant from the standard. All other potato clones were ranked lower than these two clones for baking. In Day 90 evaluations, baked Yukon Gold potato ranking mean score was 4.36. AC97521-1R/Y (mean rank = 3.05), Purple Majesty

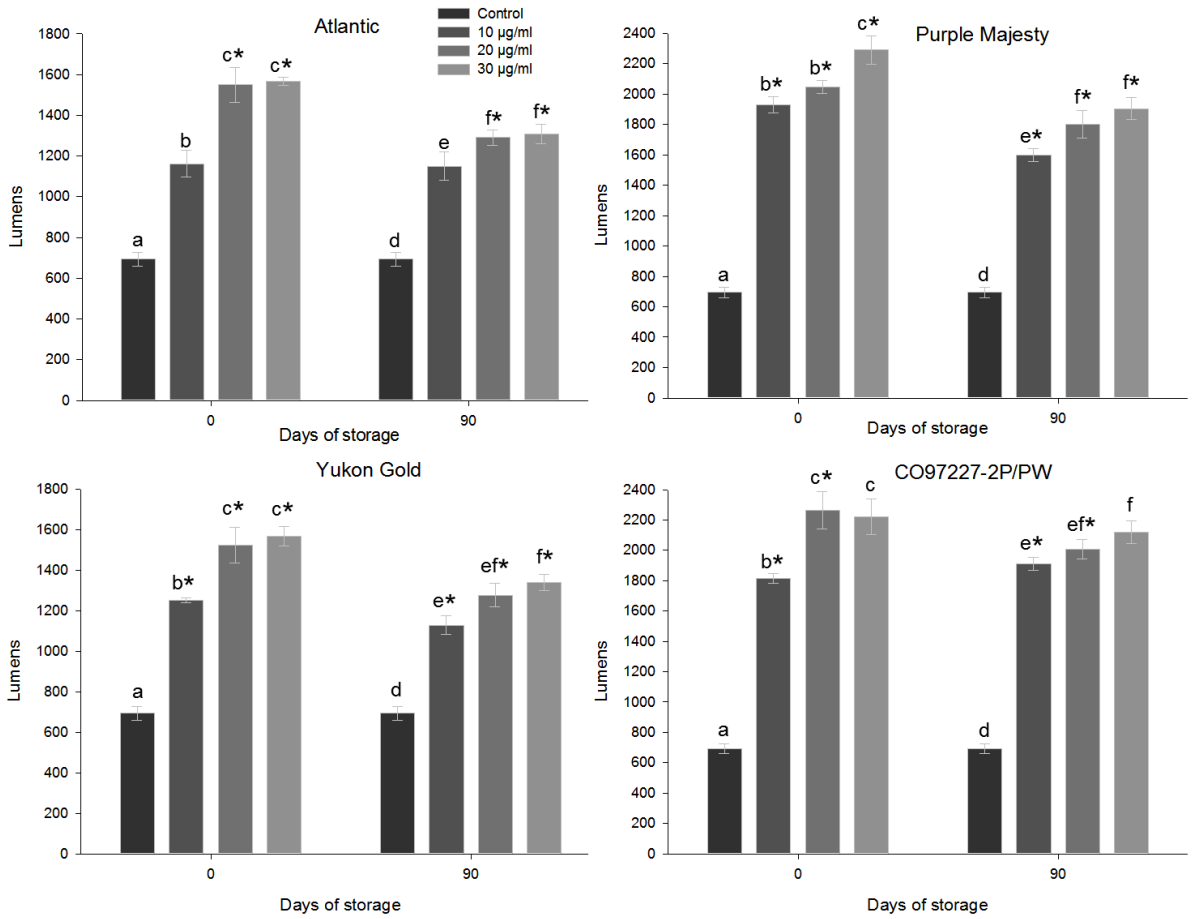


Figure 4.10. Chipped potato extracts elevated dose-dependent apoptosis in HCT-116 colon cancer cells. Apoptosis was measured using Caspase-Glo 3/7 assay as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between apoptotic cells at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between apoptotic cells at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

(mean rank = 3.54), and CO97232-2R/Y (mean rank = 3.57) were liked significantly lower than the standard. Atlantic potatoes, a popular chipping cultivar, were used as the standard for potato chips. In the Day 30 study, the Atlantic chips received a mean score of 7.15 from 114 consumer panelists for overall acceptability. No other potato clone was statistically higher than Atlantic. Again in the Day 90 study, Atlantic chips had the

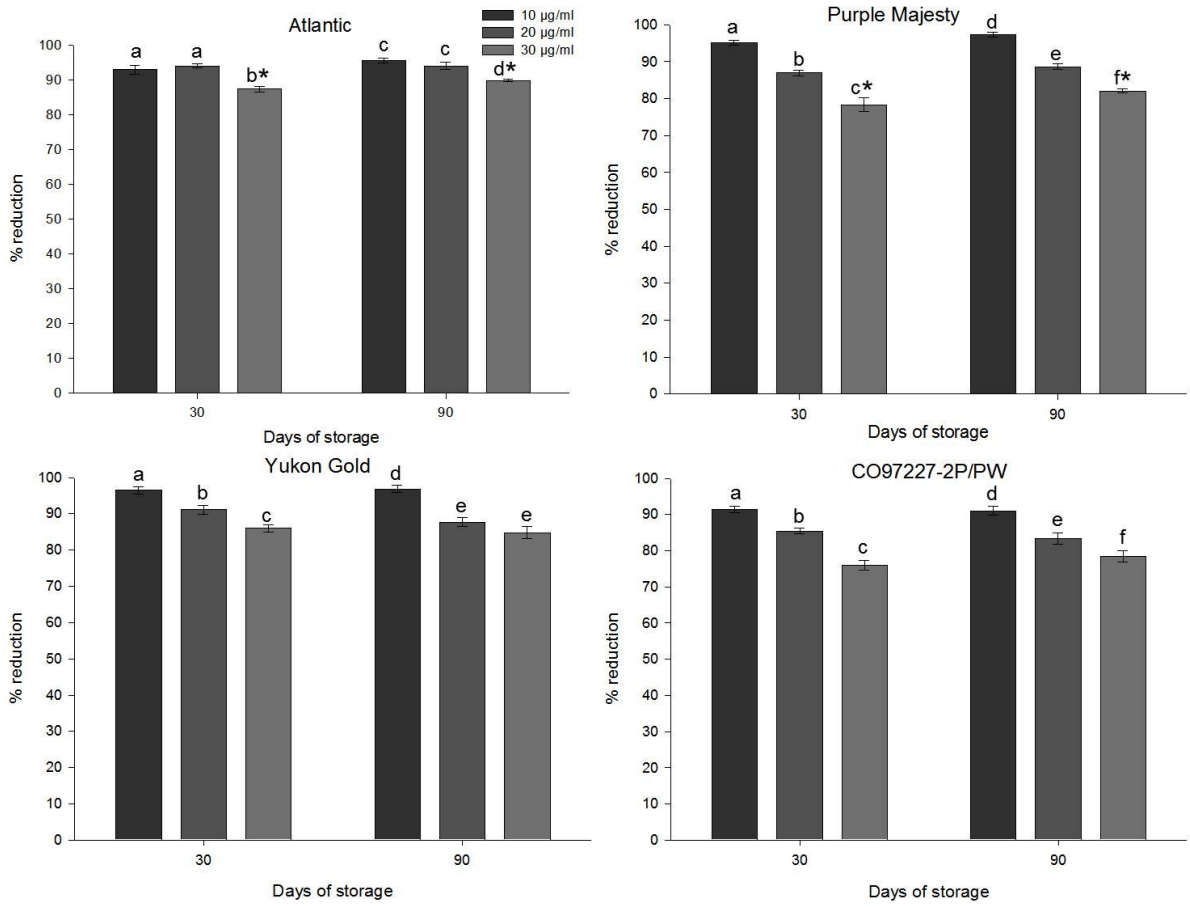


Figure 4.11. Baked potato extracts suppressed proliferation of HT-29 cells in a dose-dependent manner. Cell number was measured using a cellometer as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

highest mean score for overall acceptability (7.01). Overall acceptability for Purple Majesty (6.83) and other potato clones was not higher than the standard. Atlantic chips received a rank mean score of 2.56 after 30 days of storage and 2.91 after 90 days of storage. No potato clones were ranked "liked more" than the standard Atlantic. Yukon Gold potato chips were ranked slightly lower than the standard at Day 30 evaluation

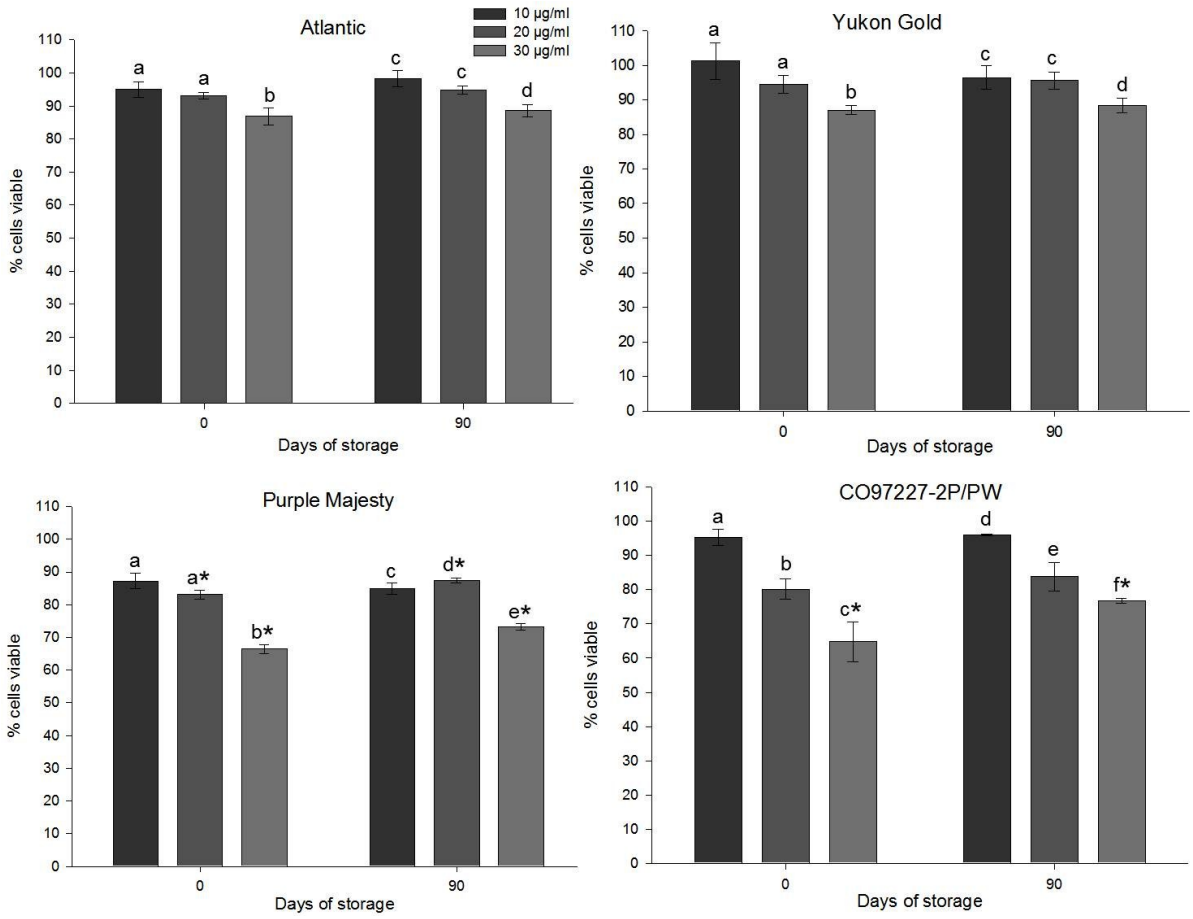


Figure 4.12. Baked potato extracts suppressed proliferation of HT-29 cells in a dose-dependent manner. Cell viability was measured using the BrdU assay as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

(3.26), while Purple Majesty received a similar mean rank (2.96). Thus, some purple-fleshed clones were comparable with traditional cultivars in terms of their sensory scores.

Eighty four per cent (n = 94) of the panelists responded that they would prefer colored-flesh potatoes over traditional white-fleshed potatoes if they knew purple-fleshed potatoes had potential health benefits. When asked if they would be willing to pay more

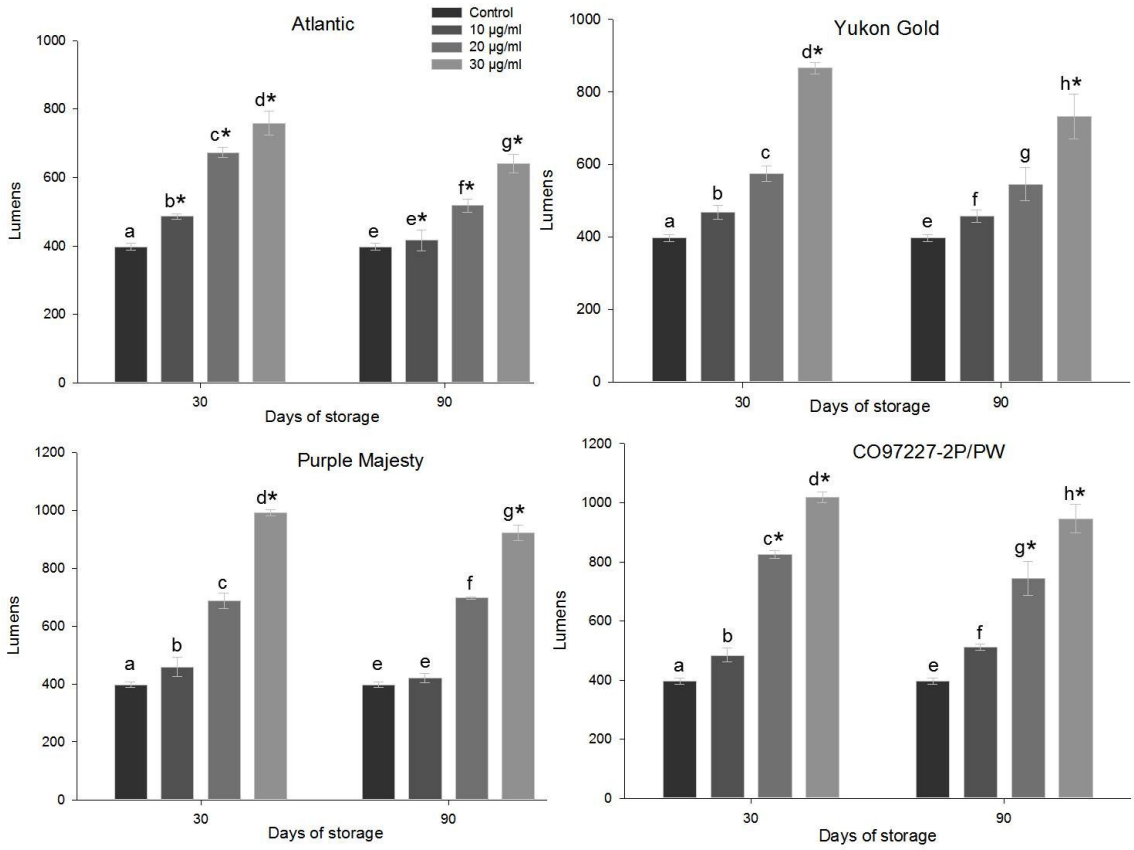


Figure 4.13. Baked potato extracts induced dose-dependent apoptosis in HT-29 colon cancer cells. Apoptosis was measured using Caspase-Glo 3/7 assay as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between apoptotic cells at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between apoptotic cells at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

for colored-flesh potato products, 55% (n=61) were willing while 45% (n=50) were not. Panelists were asked how much more they would be willing to pay if a 10.5-ounce bag of chips cost $3.20. They were willing to pay an average of $0.83 more *per* bag of chips made from colored-flesh potatoes.

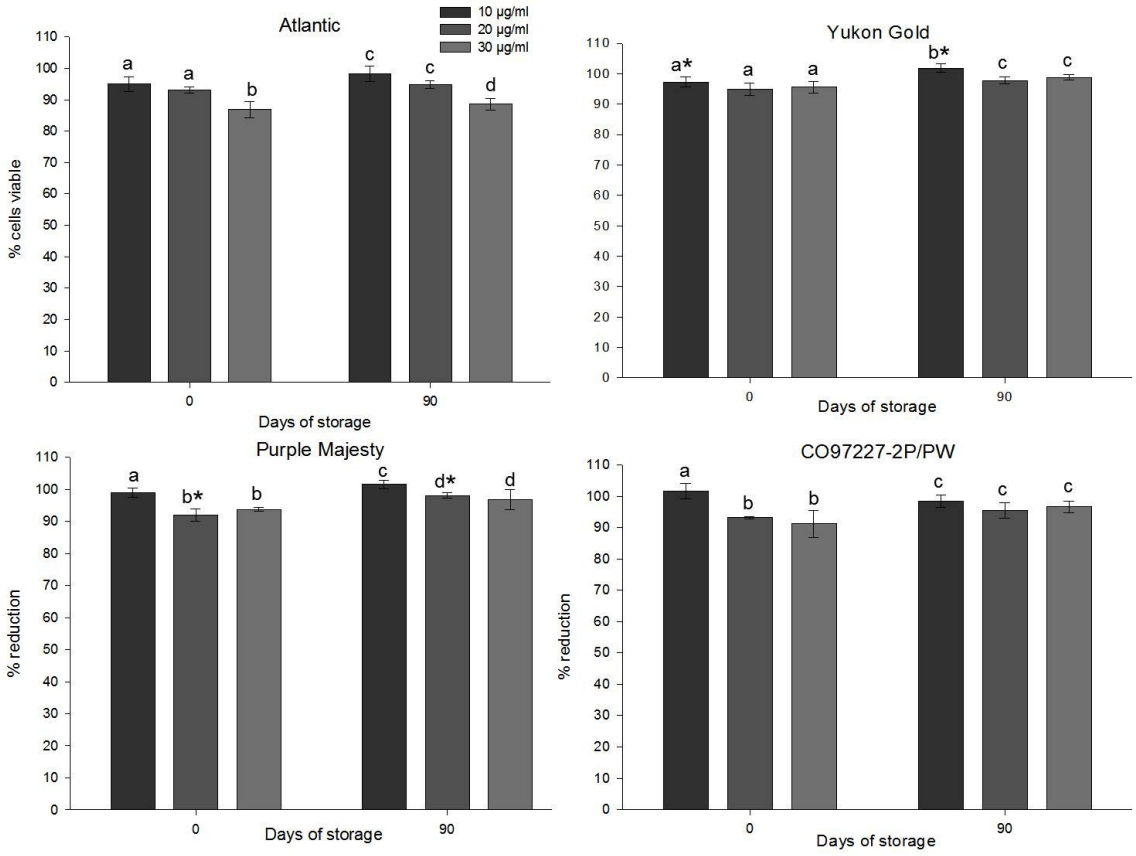


Figure 4.14. Chipped potato extracts did not suppress proliferation of HT-29 cells in a dose-dependent manner. Cell number was measured using a cellometer as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

3.7. Correlations

Phenolic content and antioxidant capacity had positive correlations in baked ($r = 0.92$, $p < 0.0001$) and chipped ($r = 0.81$, $p < 0.0001$) samples. Phenolic content also showed a strong correlation with anthocyanin content ($r = 0.95$, $p < 0.0001$ for baked and $r = 0.87$, $p < 0.0001$ for chipped samples). This is similar to a strong positive correlation

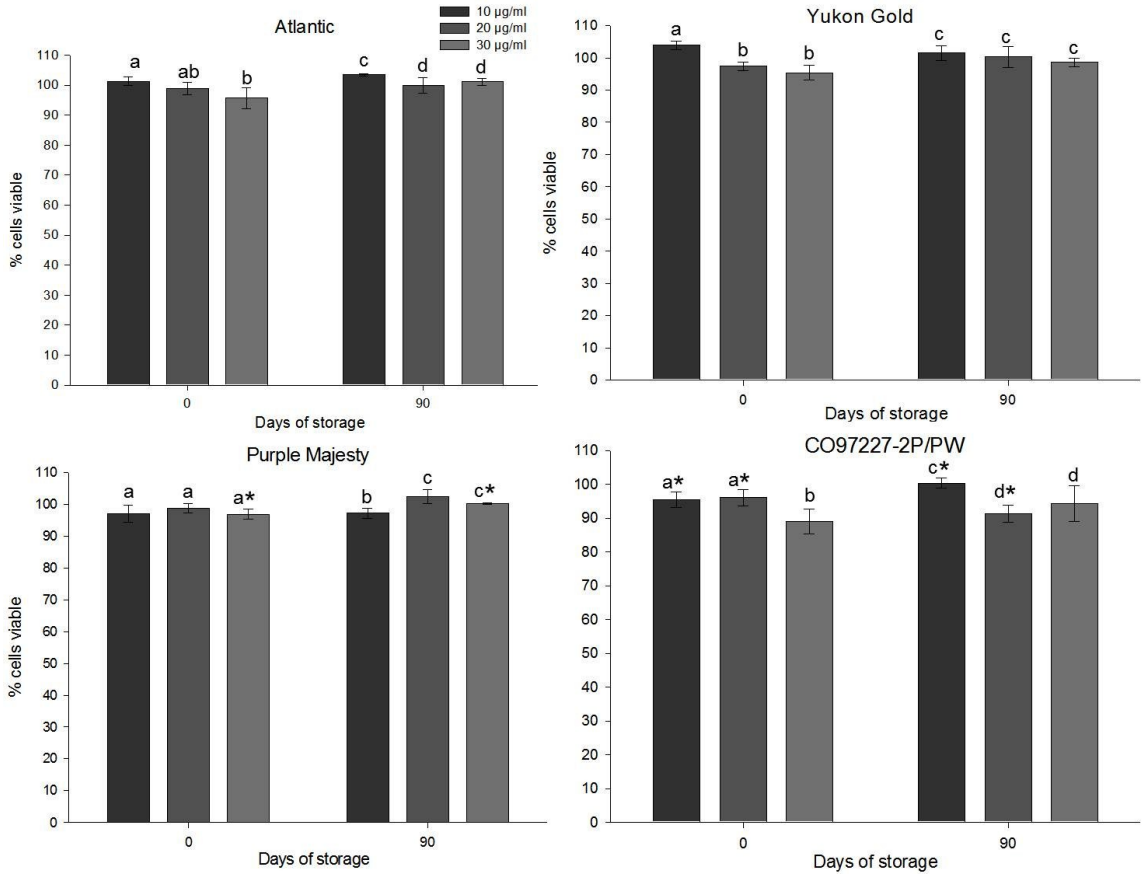


Figure 4.15. Chipped potato extracts did not suppress proliferation of HT-29 cells in a dose-dependent manner. Cell viability was measured using the BrdU assay as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between percentage reduction at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between percentage reduction at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

between the total phenolic and anthocyanin content ($r = 0.91$) as reported by Reyes, et al. (2005) for uncooked potatoes indicating that baking and chipping did not alter the functionality of anthocyanins as phenolic compounds or their antioxidant capacities. Antioxidant activity measured using DPPH assay showed a strong positive correlation with ABTS assay antioxidant activity ($r = 0.96$, $p < 0.0001$ for baked and $r = 0.83$, $p <$

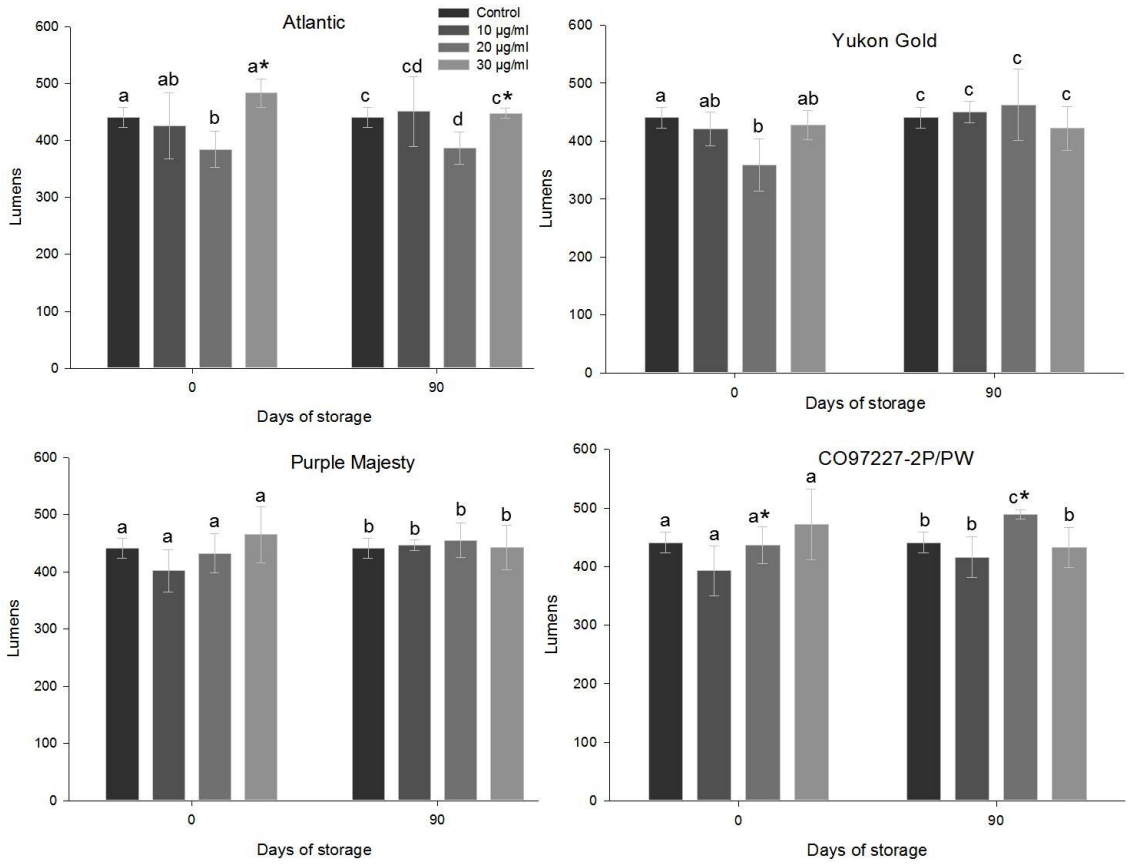


Figure 4.16. Chipped potato extracts did not induce dose-dependent apoptosis in HT-29 colon cancer cells. Apoptosis was measured using Caspase-Glo 3/7 assay as described in Materials and Methods. Values with different letters in each graph indicate significant difference ($p < 0.05$) between apoptotic cells at two different concentrations at a given time point. *Indicates a significant difference ($p < 0.05$) between apoptotic cells at two different time points for a given concentration. Results are presented as mean ± SE of four replicates for each time point.

0.0001 for chipped samples) (Thaipong et al. 2006; Reddivari et al. 2007a; Dudonné et al. 2009).

Though storage duration was strongly correlated with antioxidant activity ($r = 0.87$, $p < 0.004$) of most genotypes for the baked samples, the percentage of viable cancer cells was moderately to strongly correlated for Atlantic ($r = 0.79$, $p < 0.06$), Purple Majesty ($r = 0.87$, $p < 0.02$) and CO97227-2P/PW ($r = 0.63$, $p < 0.2$) which

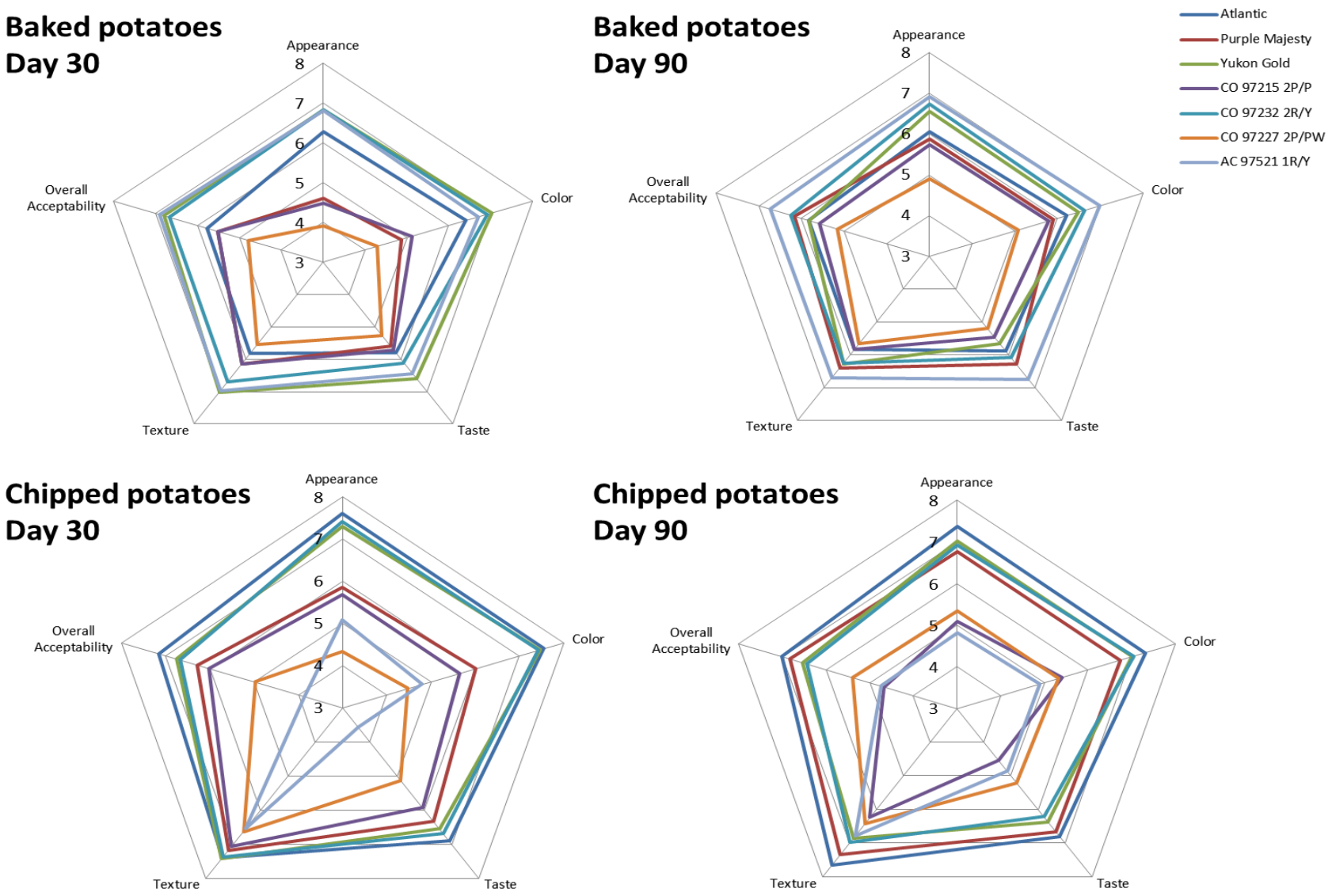


Figure 4.17. Mean hedonic scores from consumer panelists for stored baked and chipped potatoes. Sensory analysis was performed on 116 and 114 untrained panelists for the baked and chipped samples respectively. (1 = disliked extremely; 9 = liked extremely)

Table 4.7. Average sensory rank of baked and chipped samples.

Potato Clones	Baked		Chipped	
	Day 30	Day 90	Day 30	Day 90
Atlantic	4.42^{b} (4)	4.02^{ab} (4)	2.56^{a} (1)	2.91^{a} (1)
Yukon Gold	3.24^{a} (2)	4.36^{b} (5)	3.26^{a} (2)	3.29^{a} (3)
Purple Majesty	4.53^{b} (6)	3.54^{a} (2)	3.58^{ab} (4)	2.97^{a} (2)
AC97521-1R/Y	3.25^{a} (3)	3.05^{a} (1)	6.00^{c} (7)	5.42^{c} (7)
CO97232-2R/Y	2.89^{a} (1)	3.57^{a} (3)	3.47^{ab} (3)	3.43^{a} (4)
CO97215-2P/P	4.48^{b} (5)	4.42^{b} (6)	3.98^{b} (5)	5.29^{bc} (6)
CO97227-2P/PW	5.16^{c} (7)	4.96^{b} (7)	5.19^{c} (6)	4.76^{b} (5)

Values in the bracket indicate the ranking order. Different letters indicate significant differences ($p < 0.05$) between the clone rankings for a given time point.

indicates a suppression of anti-proliferative properties. Chipped samples also showed a strong correlation between storage duration and percentage of viable cancer cells ($r = 0.87$, $p < 0.005$) indicating that storage suppressed the anti-proliferative activity of the potatoes post-chipping. Apoptosis induction did not exhibit a correlation with storage duration for the baked samples. For the chipped samples, a strong negative correlation was observed ($r = -0.85$, $p < 0.003$) indicating suppression of anti-proliferative properties

with storage. However, for CO97227-2P/PW, the correlation was not strong ($r = -0.34$, $p < 0.5$) which could indicate that storage duration did not suppress its anti-cancer properties. Thus, it was observed that storage duration influenced the apoptosis of the baked potato extracts. However, in the chipped samples, both proliferation and apoptosis were dependent on storage duration for most genotypes.

Correlation coefficients were calculated for overall acceptability sensory scores and total phenolic content. For the baked potatoes after 30 days of storage, there was a negative correlation between the two parameters ($r = -0.87$; $p < 0.01$). Similar results were observed between mean rank and total phenolic content ($r = 0.80$; $p < 0.03$). No other correlations were significant.

CHAPTER V

CONCLUSIONS

Potatoes are receiving much attention lately for their role in promoting obesity and diabetes. However, the consumption of specialty/colored potatoes has increased by 17% due to their putative health benefits. Colored-flesh potatoes are usually stored before consumption, so it is important to understand the effect of storage on the potato bioactive compounds. These results, for the first time, show that storage alters the phenolic compound profiles in potatoes and elevates total phenolic content but suppresses biological activity. Thus, it is important to optimize the storage conditions in order to retain the biological activity of potato bioactive compounds. It was also observed that colored-flesh potatoes, containing anthocyanins, had higher bioactivity as compared with the white- and yellow-fleshed ones. Hence, breeders can utilize these colored-flesh potato cultivars as parental material in the breeding programs to develop cultivars with potent health-benefits. Results also warrant the use of *in vitro* and *in vivo* biological assays in conjunction with quantitative analytical techniques in assessing the genotype, storage and processing effects on health benefits of fruits and vegetables.

This study also demonstrated the effect of post-storage processing on the bioactive compounds found in white-, yellow- and purple-fleshed potato clones. There are growing concerns regarding caloric intake due to potato consumption. However, purple-fleshed potatoes can deliver health-benefiting polyphenolic compounds in levels comparable to blueberries and grapes with fewer calories being consumed with respect to traditional cultivars. We observed that half of a baked purple-fleshed potato (~ 100 g) has total phenolic content equivalent to three and a half yellow-fleshed Yukon Gold potatoes

or 45 blueberries or 25 grapes. This study for the first time showed that potato compounds retained bioactivity against colon cancer cells even after post-storage processing. Storage caused a shift in the metabolite profile of the potato samples which could possibly explain the suppression of anti-cancer properties of processed potato samples post-storage. However, some clones retained their anti-cancer properties better than others. Hence, these clones could be utilized as parent material for breeding programs to develop genotypes that retain their bioactive properties post-storage and processing. As purple-fleshed potato clones differed in the content and composition of bioactive compounds and their anti-cancer properties, flesh color alone may not be a good indicator of health-benefiting properties.

Regardless of the health-benefits, the sensory attributes and consumer acceptance of these new cultivars should not be discounted. Sensory analysis revealed consumers' readiness to accept colored-flesh potatoes provided they are educated on the health benefits. Hence, purple-fleshed potatoes can be a healthier choice as they possess greater levels of bioactive compounds and anti-cancer properties even after processing as compared with their white- and yellow-fleshed counterparts. We are currently confirming these *in vitro* results by evaluating the baked and chipped colored-flesh potatoes for their anti-inflammatory properties using obese pigs, a highly relevant model for human paradigm. Farm-to-fork operations need to be systematically studied and optimized so that potatoes and other fruits and vegetables can retain their health-benefiting properties and, thus, act as a popular delivery mechanism for bioactive compounds.

REFERENCES

Afaq, F., M. Saleem, C. G. Krueger, J. D. Reed and H. Mukhtar (2005). "Anthocyanin- and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-kappaB pathways and inhibits skin tumorigenesis in CD-1 mice." International Journal of Cancer **113**(3): 423-433.

Al-Saikhan, M. S., L. R. Howard and J. C. Miller (1995). "Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum* L.)." Journal of Food Science **60**(2): 341-343.

André, C. M., M. Ghislain, P. Bertin, M. Oufir, M. del Rosario Herrera, L. Hoffmann, et al. (2006). "Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients." Journal of Agricultural and Food Chemistry **55**(2): 366-378.

André, C. M., M. Oufir, L. Hoffmann, J.-F. Hausman, H. Rogez, Y. Larondelle, et al. (2009). "Influence of environment and genotype on polyphenol compounds and *in vitro* antioxidant capacity of native Andean potatoes (*Solanum tuberosum* L.)." Journal of Food Composition and Analysis **22**(6): 517-524.

Anonymous. (2010). "United States Potato Board: NET Potato Consumption Trends." Retrieved 15th November, 2011, from http://www.perishablesgroup.com/dnn/LinkClick.aspx?fileticket=bcjkRS_XZqY%3D&tabid=5327.

Anonymous. (2011). "Colorado Potatoes." Retrieved 15th November, 2011, from http://www.coloradopotato.org/.

Awika, J., L. Rooney, X. Wu, R. Prior and L. Cisneros-Zevallos (2003). "Screening methods to measure antioxidant activity of sorghum (Sorghum bicolor) and sorghum products." Journal of Agricultural and Food Chemistry **51**(23): 6657-6662.

Bagchi, D., C. K. Sen, M. Bagchi and M. Atalay (2004). "Anti-angiogenic, antioxidant, and anti-carcinogenic properties of a novel anthocyanin-rich berry extract formula." Biochemistry (Mosc) **69**(1): 75-80, 71 p preceding 75.

Barba, A. A., A. Calabretti, M. d'Amore, A. L. Piccinelli and L. Rastrelli (2008). "Phenolic constituents levels in cv. Agria potato under microwave processing." LWT - Food Science and Technology **41**(10): 1919-1926.

Bassoli, B. K., P. Cassolla, G. R. Borba-Murad, J. Constantin, C. L. Salgueiro-Pagadigorria, R. B. Bazotte, et al. (2008). "Chlorogenic acid reduces the plasma glucose peak in the oral glucose tolerance test: effects on hepatic glucose release and glycaemia." Cell Biochemistry and Function **26**(3): 320-328.

Bellows, A. C., B. Onyango, A. Diamond and W. K. Hallman (2008). "Understanding consumer interest in organics: production values vs. purchasing behavior." Journal of Agricultural & Food Industrial Organization **6**(1): 2.

Blessington, T., M. Nzaramba, D. Scheuring, A. Hale, L. Reddivari and J. Miller (2010). "Cooking methods and storage treatments of potato: Effects on carotenoids, antioxidant activity, and phenolics." American Journal of Potato Research **87**(6): 479-491.

Blois, M. S. (1958). "Antioxidant determinations by the use of a stable free radical." Nature **181**(4617): 1199-1200.

Bravo, L. (1998). "Phenolic phytochemicals: Chemistry, dietary sources, metabolism, and nutritional significance." Nutrition Reviews **56**: 317–333.

Breithaupt, D. E. and A. Bamedi (2002). "Carotenoids and carotenoid esters in potatoes (*Solanum tuberosum* L.): new insights into an ancient vegetable." Journal of Agricultural and Food Chemistry **50**(24): 7175-7181.

Brown, C. (2004). Nutrient Status of Potato: Assessment of Future Trends. Proceedings 43rd Washington State Potato Conference.

Brown, C., D. Culley, C. P. Yang, R. Durst and R. Wrolstad (2005). "Variation of anthocyanin and carotenoid contents and associated antioxidant values in potato breeding lines." Journal of the American Society for Horticultural Science **130**(2): 174-180.

Brown, C., R. Durst, R. Wrolstad and W. De Jong (2008). "Variability of phytonutrient content of potato in relation to growing location and cooking method." Potato Research **51**(3): 259-270.

Burlingame, B., B. Mouillé and R. Charrondière (2009). "Nutrients, bioactive non-nutrients and anti-nutrients in potatoes." Journal of Food Composition and Analysis **22**(6): 494-502.

Chu, Y.-F., J. Sun, X. Wu and R. H. Liu (2002). "Antioxidant and antiproliferative activities of common vegetables." Journal of Agricultural and Food Chemistry **50**(23): 6910-6916.

Chun, O., D. Kim, N. Smith, D. Schroeder, J. Han and C. Lee (2005). "Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables

in the American diet." Journal of the Science of Food and Agriculture **85**(10): 1715-1724.

Dao, L. and M. Friedman (1992). "Chlorogenic acid content of fresh and processed potatoes determined by ultraviolet spectrophotometry." Journal of Agricultural and Food Chemistry **40**(11): 2152-2156.

Dewanto, V., X. Wu, K. Adom and R. Liu (2002). "Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity." Journal of Agricultural and Food Chemistry **50**(10): 3010-3014.

Dixon, R. A. and N. L. Paiva (1995). "Stress-induced phenylpropanoid metabolism." Plant Cell **7**(7): 1085-1097.

Dudonné, S. p., X. Vitrac, P. Coutière, M. Woillez and J.-M. Mérillon (2009). "Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays." Journal of Agricultural and Food Chemistry **57**(5): 1768-1774.

Eichhorn, S. and P. Winterhalter (2005). "Anthocyanins from pigmented potato (*Solanum tuberosum* L.) varieties." Food Research International **38**(8-9): 943-948.

Ezekiel, R., B. Singh, D. Kumar and A. Kumar (2008). "Effect of growing location on the processing quality of two potato cultivars stored at low temperature." Indian Journal of Horticulture **65**: 52-59.

Ezekiel, R., N. Singh, S. Sharma and A. Kaur (2011). "Beneficial phytochemicals in potato -- a review." Food Research International **In Press, Corrected Proof**.

Faller, A. L. K. and E. Fialho (2009). "The antioxidant capacity and polyphenol content of organic and conventional retail vegetables after domestic cooking." Food Research International **42**(1): 210-215.

FAO. (2008). "The potato: International Year of the potato 2008." Retrieved 27th January, 2011, 2011, from http://www.potato2008.org/.

FAO. (2009). "FAO Statistical Databases." Retrieved 25th January 2011, from http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor.

Feng, R., Y. Lu, L. L. Bowman, Y. Qian, V. Castranova and M. Ding (2005). "Inhibition of activator protein-1, NF-kappaB, and MAPKs and induction of phase 2 detoxifying enzyme activity by chlorogenic acid." Journal of Biological Chemistry **280**(30): 27888-27895.

Friedman, M. (1997). "Chemistry, Biochemistry, and Dietary Role of Potato Polyphenols. A Review." Journal of Agricultural and Food Chemistry **45**(5): 1523-1540.

Giusti, M. M. and R. E. Wrolstad (2003). "Acylated anthocyanins from edible sources and their applications in food systems." Biochemical Engineering Journal **14**(3): 217-225.

Gliszczynska-Swiglo, A. (2006). "Antioxidant activity of water soluble vitamins in the TEAC (trolox equivalent antioxidant capacity) and the FRAP (ferric reducing antioxidant power) assays." Food Chemistry **96**(1): 131-136.

Gollop, R., S. Even, V. Colova-Tsolova and A. Perl (2002). "Expression of the grape dihydroflavonol reductase gene and analysis of its promoter region." Journal of Experimental Botany **53**(373): 1397-1409.

Gollop, R., S. Farhi and A. Perl (2001). "Regulation of the leucoanthocyanidin dioxygenase gene expression in *Vitis vinifera*." Plant Science **161**(3): 579-588.

Häkkinen, S. (2000). "Flavonols and phenolic acids in berries and berry products." Kuopio University Publications D. Medical Sciences 221: 17-18.

Hayashi, K., H. Hibasami, T. Murakami, N. Terahara, M. Mori and A. Tsukui (2006). "Induction of apoptosis in cultured human stomach cancer cells by potato anthocyanins and its inhibitory effects on growth of stomach cancer in mice." Food Science and Technology Research **12**(1): 22-26.

Herrman, T., S. Love, B. Shafii and R. Dwelle (1996). "Chipping performance of three processing potato cultivars during long-term storage at two temperature regimes." American Journal of Potato Research **73**(9): 411-425.

Hidalgo, M., S. Martin-Santamaria, I. Recio, C. Sanchez-Moreno, B. de Pascual-Teresa, G. Rimbach, et al. (2012). "Potential anti-inflammatory, anti-adhesive, anti/estrogenic, and angiotensin-converting enzyme inhibitory activities of anthocyanins and their gut metabolites." Genes & Nutrition: 1-12.

Ieri, F., M. Innocenti, L. Andrenelli, V. Vecchio and N. Mulinacci (2011). "Rapid HPLC/DAD/MS method to determine phenolic acids, glycoalkaloids and anthocyanins in pigmented potatoes (*Solanum tuberosum* L.) and correlations with variety and geographical origin." Food Chemistry **125**(2): 750-759.

Im, H., B. Suh, S. Lee, N. Kozukue, M. Ohnisi-Kameyama, C. Levin, et al. (2008). "Analysis of phenolic compounds by high-performance liquid chromatography and liquid chromatography/mass spectrometry in potato plant flowers, leaves,

stems, and tubers and in home-processed potatoes." Journal of Agricultural and Food Chemistry **56**(9): 3341-3349.

Isherwood, F. A. (1976). "Mechanism of starch-sugar interconversion in *Solanum tuberosum*." Phytochemistry **15**(1): 33-41.

Jansen, G. and W. Flamme (2006). "Coloured potatoes (*Solanum tuberosum* L.) – Anthocyanin content and tuber quality." Genetic Resources and Crop Evolution **53**(7): 1321-1331.

Jiang, Y. and D. C. Joyce (2003). "ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit." Plant Growth Regulation **39**(2): 171-174.

Jin, U. H., J. Y. Lee, S. K. Kang, J. K. Kim, W. H. Park, J. G. Kim, et al. (2005). "A phenolic compound, 5-caffeoylquinic acid (chlorogenic acid), is a new type and strong matrix metalloproteinase-9 inhibitor: isolation and identification from methanol extract of *Euonymus alatus*." Life Sciences **77**(22): 2760-2769.

Jing, P., J. A. Bomser, S. J. Schwartz, J. He, B. A. Magnuson and M. M. Giusti (2008). "Structure-function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of colon cancer cell growth." Journal of Agricultural and Food Chemistry **56**(20): 9391-9398.

Kaspar, K. L., J. S. Park, C. R. Brown, B. D. Mathison, D. A. Navarre and B. P. Chew (2011). "Pigmented potato consumption alters oxidative stress and inflammatory damage in men." Journal of Nutrition **141**(1): 108-111.

Kotíková, Z., A. Hejtmánková, J. Lachman, K. Hamouz, E. Trnková and P. Dvorak (2007). "Effect of selected factors on total carotenoid content in potato tubers (*Solanum tuberosum* L.)." Plant Soil and Environment **53**(8): 355.

Lachman, J., K. Hamouz, M. Šulc, M. Orsák, V. Pivec, A. Hejtmánková, et al. (2009). "Cultivar differences of total anthocyanins and anthocyanidins in red and purple-fleshed potatoes and their relation to antioxidant activity." Food Chemistry **114**(3): 836-843.

Lee, W. J. and B. T. Zhu (2006). "Inhibition of DNA methylation by caffeic acid and chlorogenic acid, two common catechol-containing coffee polyphenols." Carcinogenesis **27**(2): 269.

Legrand, D. and A. J. Scheen (2007). "Does coffee protect against type 2 diabetes?" Revue Medicale de Liege **62**(9): 554-559.

Leszczyński, G. L. s. a. W. (1989). Potato Science and Technology. New York, Elsevier Applied Science.

Lewis, C. E., J. R. L. Walker and J. E. Lancaster (1999). "Changes in anthocyanin, flavonoid and phenolic acid concentrations during development and storage of coloured potato (*Solanum tuberosum* L.) tubers." Journal of the Science of Food and Agriculture **79**(2): 311-316.

Lewis, C. E., J. R. L. Walker, J. E. Lancaster and K. H. Sutton (1998). "Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: Coloured cultivars of *Solanum tuberosum* L." Journal of the Science of Food and Agriculture **77**(1): 45-57.

Liu, R. H. (2004). "Potential synergy of phytochemicals in cancer prevention: mechanism of action." Journal of Nutrition **134**(12 Suppl): 3479S-3485S.

Lutaladio, N. and L. Castaldi (2009). "Potato: The hidden treasure." Journal of Food Composition and Analysis **22**(6): 491-493.

Mattila, P. and J. Hellström (2007). "Phenolic acids in potatoes, vegetables, and some of their products." Journal of Food Composition and Analysis **20**(3-4): 152-160.

Mazza, G. and E. Miniati (1993). Anthocyanins in fruits, vegetables, and grains. Boca Raton, CRC Press.

Meyers, K. J., C. B. Watkins, M. P. Pritts and R. H. Liu (2003). "Antioxidant and antiproliferative activities of strawberries." Journal of Agricultural and Food Chemistry **51**(23): 6887-6892.

Mizuno, M., M. Kamei and H. Tsuchida (1998). "Ascorbate peroxidase and catalase cooperate for protection against hydrogen peroxide generated in potato tubers during low-temperature storage." Biochemistry and Molecular Biology International **44**(4): 717-726.

Morris, W., L. Ducreux, D. Griffiths, D. Stewart, H. Davies and M. Taylor (2004). "Carotenogenesis during tuber development and storage in potato." Journal of Experimental Botany **55**(399): 975.

Mqndy, N. I., S. B. Geddedahl and E. O. Mobley (1966). "Effect of storage temperature on the cytochrome oxidase and polyphenol oxidase activities and phenolic content of potatoes." Journal of Food Science **31**(1): 32-37.

Mulinacci, N., F. Ieri, C. Giaccherini, M. Innocenti, L. Andrenelli, G. Canova, et al. (2008). "Effect of Cooking on the Anthocyanins, Phenolic Acids, Glycoalkaloids,

and Resistant Starch Content in Two Pigmented Cultivars of *Solanum tuberosum* L." Journal of Agricultural and Food Chemistry **56**(24): 11830-11837.

Naito, K., Y. Umemura, M. Mori, T. Sumida, T. Okada, N. Takamatsu, et al. (1998). "Acylated pelargonidin glycosides from a red potato." Phytochemistry **47**(1): 109-112.

Navarre, D., R. Shakya, J. Holden and S. Kumar (2010). "The Effect of Different Cooking Methods on Phenolics and Vitamin C in Developmentally Young Potato Tubers." American Journal of Potato Research **87**(4): 350-359.

Navarre, D. A., A. Goyer and R. Shakya (2009). Nutritional Value of Potatoes: Vitamin, Phytonutrient, and Mineral Content. Advances in Potato Chemistry and Technology. S. Jaspreet and K. Lovedeep. San Diego, Academic Press**:** 395-424.

Navarre, D. A., S. S. Pillai, R. Shakya and M. J. Holden (2011). "HPLC profiling of phenolics in diverse potato genotypes." Food Chemistry **127**(1): 34-41.

Nicoli, M., M. Anese and M. Parpinel (1999). "Influence of processing on the antioxidant properties of fruit and vegetables." Trends in Food Science & Technology **10**(3): 94-100.

Nzaramba, M., L. Reddivari, J. Bamberg and J. Miller Jr (2009). "Antiproliferative activity and cytotoxicity of *Solanum jamesii* tuber extracts on human colon and prostate cancer cells *in vitro*." Journal of Agricultural and Food Chemistry **57**(18): 8308-8315.

Olsson, M. E., K. E. Gustavsson, S. Andersson, A. Nilsson and R. D. Duan (2004). "Inhibition of cancer cell proliferation *in vitro* by fruit and berry extracts and

correlations with antioxidant levels." Journal of Agricultural and Food Chemistry **52**(24): 7264-7271.

Onyeneho, S. N. and N. S. Hettiarachchy (1993). "Antioxidant activity, fatty acids and phenolic acids compositions of potato peels." Journal of the Science of Food and Agriculture **62**(4): 345-350.

Percival, G. C. and L. Baird (2000). "Influence of storage upon light-induced chlorogenic acid accumulation in potato tubers (*Solanum tuberosum* L.)." Journal of Agricultural and Food Chemistry **48**(6): 2476-2482.

Price, K., J. Bacon and M. Rhodes (1997). "Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (*Allium cepa*)." Journal of Agricultural and Food Chemistry **45**(3): 938-942.

Prior, R. L., X. Wu and K. Schaich (2005). "Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements." Journal of Agricultural and Food Chemistry **53**(10): 4290-4302.

Reddivari, L., A. Hale and J. Miller (2007a). "Determination of phenolic content, composition and their contribution to antioxidant activity in specialty potato selections." American Journal of Potato Research **84**(4): 275-282.

Reddivari, L., A. Hale and J. Miller (2007b). "Genotype, location, and year influence antioxidant activity, carotenoid content, phenolic content, and composition in specialty potatoes." Journal of Agricultural and Food Chemistry **55**(20): 8073-8079.

Reddivari, L., J. Vanamala, S. Chintharlapalli, S. H. Safe and J. Miller (2007c). "Anthocyanin fraction from potato extracts is cytotoxic to prostate cancer cells

through activation of caspase-dependent and caspase-independent pathways." Carcinogenesis **28**(10): 2227-2235.

Rehman, Z., F. Habib and W. Shah (2004). "Utilization of potato peels extract as a natural antioxidant in soy bean oil." Food Chemistry **85**(2): 215-220.

Reyes, L., J. Miller and L. Cisneros-Zevallos (2004). "Environmental conditions influence the content and yield of anthocyanins and total phenolics in purple- and red-flesh potatoes during tuber development." American Journal of Potato Research **81**(3): 187-193.

Reyes, L., J. Miller and L. Cisneros-Zevallos (2005). "Antioxidant capacity, anthocyanins and total phenolics in purple-and red-fleshed potato (*Solanum tuberosum* L.) genotypes." American Journal of Potato Research **82**(4): 271-277.

Reyes, L., J. Villarreal and L. Cisneros-Zevallos (2007). "The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue." Food Chemistry **101**(3): 1254-1262.

Rhodes, M. J. C. and L. S. C. Wooltorton (1978). "Changes in the activity of hydroxycinnamyl CoA:quinate hydroxycinnamyl transferase and in the levels of chlorogenic acid in potatoes and sweet potatoes stored at various temperatures." Phytochemistry **17**(8): 1225-1229.

Rosenthal, S. and S. Jansky (2008). "Effect of production site and storage on antioxidant levels in specialty potato (*Solanum tuberosum* L.) tubers." Journal of the Science of Food and Agriculture **88**(12): 2087-2092.

Rumbaoa, R., D. Cornago and I. Geronimo (2009). "Phenolic content and antioxidant capacity of Philippine potato (*Solanum tuberosum*) tubers." Journal of Food Composition and Analysis **22**(6): 546-550.

Russo, G. L. (2007). "Ins and outs of dietary phytochemicals in cancer chemoprevention." Biochemical Pharmacology **74**(4): 533-544.

Shakya, R. and D. A. Navarre (2006). "Rapid screening of ascorbic acid, glycoalkaloids, and phenolics in potato using high-performance liquid chromatography." Journal of Agricultural and Food Chemistry **54**(15): 5253-5260.

Shenkin, A. (2006). "The key role of micronutrients." Clinical Nutrition **25**(1): 1-13.

Shin, D. Y., W. S. Lee, J. N. Lu, M. H. Kang, C. H. Ryu, G. Y. Kim, et al. (2009). "Induction of apoptosis in human colon cancer HCT-116 cells by anthocyanins through suppression of Akt and activation of p38-MAPK." International Journal of Oncology **35**(6): 1499-1504.

Singh, N., V. Kamath, K. Narasimhamurthy and P. S. Rajini (2008). "Protective effect of potato peel extract against carbon tetrachloride-induced liver injury in rats." Environmental Toxicology and Pharmacology **26**(2): 241-246.

Singleton, V. L., R. Orthofer and R. M. Lamuela-Raventós (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology. P. Lester, Academic Press. **299:** 152-178.

Smith, B. and P. Rubery (1981). "The effects of infection by *Phytophthora infestans* on the control of phenylpropanoid metabolism in wounded potato tissue." Planta **151**(6): 535-540.

Solfanelli, C., A. Poggi, E. Loreti, A. Alpi and P. Perata (2006). "Sucrose-specific induction of the anthocyanin biosynthetic pathway in Arabidopsis." Plant Physiology **140**(2): 637-646.

Spanos, G., R. Wrolstad and D. Heatherbell (1990). "Influence of processing and storage on the phenolic composition of apple juice." Journal of Agricultural and Food Chemistry **38**(7): 1572-1579.

Spooner, D. M., K. McLean, G. Ramsay, R. Waugh and G. J. Bryan (2005). "A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping." Proceedings of the National Academy of Sciences of the United States of America **102**(41): 14694-14699.

Spychalla, J. P. and S. L. Desborough (1990). "Superoxide dismutase, catalase, and α-tocopherol content of stored potato tubers." Plant Physiology **94**(3): 1214-1218.

Stushnoff, C., D. Holm, M. Thompson, W. Jiang, H. Thompson, N. Joyce, et al. (2008). "Antioxidant properties of cultivars and selections from the Colorado Potato Breeding Program." American Journal of Potato Research **85**(4): 267-276.

Sukhotu, T. and K. Hosaka (2006). "Origin and evolution of Andigena potatoes revealed by chloroplast and nuclear DNA markers." Genome **49**(6): 636-647.

Thaipong, K., U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D. Hawkins Byrne (2006). "Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts." Journal of Food Composition and Analysis **19**(6-7): 669-675.

Thompson, M. D., H. J. Thompson, J. N. McGinley, E. S. Neil, D. K. Rush, D. G. Holm, et al. (2009). "Functional food characteristics of potato cultivars (*Solanum*

tuberosum L.): Phytochemical composition and inhibition of 1-methyl-1-nitrosourea induced breast cancer in rats." Journal of Food Composition and Analysis **22**(6): 571-576.

Tsuda, T. (2012). "Dietary anthocyanin-rich plants: Biochemical basis and recent progress in health benefits studies." Molecular Nutrition & Food Research **56**(1): 159-170.

Tudela, J., E. Cantos, J. Espín, F. Tomás-Barberán and M. Gil (2002). "Induction of antioxidant flavonol biosynthesis in fresh-cut potatoes. Effect of domestic cooking." Journal of Agricultural and Food Chemistry **50**(21): 5925-5931.

USDA-NASS (2010). "Potatoes 2009 Summary." USDA National Agricultural Statistics Service.

USPB. (2011). "Potato History, United States Potato Board." Retrieved 25th January 2011, from http://www.potatogoodness.com/AboutPotatoes/Potato_History.php.

Vanamala, J., T. Leonardi, B. S. Patil, S. S. Taddeo, M. E. Murphy, L. M. Pike, et al. (2006). "Suppression of colon carcinogenesis by bioactive compounds in grapefruit." Carcinogenesis **27**(6): 1257-1265.

Verde Méndez, C. M., M. Rodríguez Delgado, E. Rodríguez Rodríguez and C. Díaz Romero (2004). "Content of free phenolic compounds in cultivars of potatoes harvested in Tenerife (Canary Islands)." Journal of Agricultural and Food Chemistry **52**(5): 1323-1327.

Vinson, J. A., J. Jang, Y. A. Dabbagh, M. M. Serry and S. Cai (1995). "Plant polyphenols exhibit lipoprotein-bound antioxidant activity using an *in vitro* oxidation model

for heart disease." Journal of Agricultural and Food Chemistry **43**(11): 2798-2799.

Vitrac, X., F. Larronde, S. Krisa, A. Decendit, G. Deffieux and J.-M. Mérillon (2000). "Sugar sensing and Ca^{2+}-calmodulin requirement in *Vitis vinifera* cells producing anthocyanins." Phytochemistry **53**(6): 659-665.

von Elbe, J. H. and S. J. Schwartz (1996). Colorants. Food Chemistry. O. R. Fennema. New York, Marcel Dekker**:** 651−722.

Wang, L.-S., S. Carmella, R. Keyes, C.-T. Kuo, Y.-W. Huang, S. S. Hecht, et al. (2012). Anthocyanins and Cancer Prevention

Nutraceuticals and Cancer. F. H. Sarkar, Springer Netherlands**:** 201-229.

Winch, T. (2007). Root Crops. Growing food: a guide to food production, Springer**:** 208-212.

Wrolstad, R. E. (1976). "Color and pigment analyses in fruit products." Oregon Agricultural Experiment Station Bulletin **624**(Oregon St. Univ., Corvallis, OR.).

Xu, X., W. Li, Z. Lu, T. Beta and A. Hydamaka (2009). "Phenolic content, composition, antioxidant activity, and their changes during domestic cooking of potatoes." Journal of Agricultural and Food Chemistry **57**(21): 10231-10238.

You, Q., B. Wang, F. Chen, Z. Huang, X. Wang and P. G. Luo (2010). "Comparison of anthocyanins and phenolics in organically and conventionally grown blueberries in selected cultivars." Food Chemistry **125**(1): 201-208.

Zheng, W. and S. Y. Wang (2002). "Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries." Journal of Agricultural and Food Chemistry **51**(2): 502-509.

Zheng, Y., S. Y. Wang, C. Y. Wang and W. Zheng (2007). "Changes in strawberry phenolics, anthocyanins, and antioxidant capacity in response to high oxygen treatments." LWT-Food Science and Technology **40**(1): 49-57.