DISSERTATION

EFFECT OF FARM TO FORK OPERATIONS ON BIOACTIVE COMPOUNDS IN WHITE-FLESHED AND COLOR-FLESHED POTATOES

Submitted by

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ABSTRACT

EFFECT OF FARM TO FORK OPERATIONS ON BIOACTIVE COMPOUNDS IN WHITE-FLESHED AND COLOR-FLESHED POTATOES

The potato, *Solanum tuberosum* L., is one of the most commonly consumed food crops worldwide, and is the leading vegetable crop in the United States with 69% of per capita consumption as processed potatoes. In addition to micro- and macro-nutrients, color-fleshed potatoes are one of the richest plant sources for health promoting components such as resistant starch, polyphenols, and carotenoids. In contrast, potatoes are well known to contain naturally occurring glycoalkaloids (GA; α-chaconine and α-solanine) and processing-induced acrylamide (AL). Potatoes can be stored up to one year before being processed/consumed and the effect of genotype, storage (4^{o}C or 10^{o}C; 3 or 6 months) and processing (baking and frying) on both toxic and health beneficial compounds remains unknown. We hypothesized that cultivar, storage and processing alters bioactive content in potato tuber and potato products. To test this hypothesis, raw, baked, and chipped of white-, yellow-, red-, and purple-fleshed potatoes from initial (fresh) and stored tubers were evaluated for AL/vitamin C and GAs using Ultra Performance Liquid Chromatography (UPLC) and High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD/UPLC-DAD), respectively. Total phenolic content (Folin - Ciocalteu reducing), anthocyanin content (pH differential method), antioxidant activity (DPPH and ABTS assay) were also determined. Raw potatoes were analyzed for reducing sugars (glucose and fructose) using a spectrophotometer. Sensory attributes (9-point hedonic scale) of baked and potato chips were assessed using untrained consumer panelists (n= 94 – 114). The content of GA/AL increased

with storage, dependent on cultivar. Reducing sugar content in raw potatoes increased with storage, thereby, AL content in potato chips positively correlated with reducing sugars. Purple-fleshed potatoes had higher ($p \leq 0.05$) total phenolic content, anthocyanin content, and antioxidant activity than red-fleshed potatoes and white-fleshed potatoes. The interaction effect of storage time and temperature on total phenolic content, anthocyanin content, and antioxidant activity was genotype-dependent. Baking led to a significant ($p \leq 0.05$) increase in total phenolic content, anthocyanin content, and antioxidant activity; whereas chipping led to significant losses in total phenolic content, anthocyanin content, and antioxidant activity. However, red- and purple-fleshed potatoes could serve as potential sources of non-nutrient health-benefiting compounds in the human diet even after storage and processing. Vitamin C content in potato tubers and processed potatoes was genotype-dependent. Vitamin C content rapidly declined with storage after six months of storage irrespective of storage temperature (4°C or 10°C). Chipping and frying resulted in significantly reduced vitamin C levels compared to baked potatoes and unprocessed potatoes among all tested cultivars. An increase in GA and AL content, bioactive compounds, and antioxidant activity found with storage was cultivar dependent. However, vitamin C decreased with storage. Thus, it is critical to measure GA and AL content not only in the fresh tubers but also in the final potato products such as baked and chipped potatoes. It is critical to adjust food systems processes to consistently deliver lower GA and AL content, while retaining the beneficial bioactives, vitamin C, and sensory attributes of the final potato products. Adjusting farm-to-fork operations to retain the health-benefiting compounds in food crops while reducing natural and process-induced toxicants will aid in countering growing epidemic of chronic diseases globally.

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DEDICATION

This dissertation is dedicated to my mother, pure spirit, Mrs. Fatima A. Amer and my father Mr. Saleh Massoud Amer, whose unconditional love and support made this possible. I also dedicate this work to each member of my family and friends in Libya and the USA for their constant words of encouragement.

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CHAPTER ONE: INTRODUCTION

The potato (*Solanum tuberosum* L.) is the most important vegetable crop around the world. It is ranked the 4th most available food crop after wheat, rice, and maize with 314 million tons fresh-weight (fw) produced in 2006 (Food and Agriculture Organization, 2008). Potatoes are the leading vegetable crop in the United States with a per capita consumption of about 112 lbs annually, with 35 lbs fresh market and 77 lbs as processed potatoes (Osteen et al., 2012). Potatoes provide from 5 to 15% of dietary calories (carbohydrate and starch) for different cultures across the world (American Institute for cancer research, 2007), especially for the poorest and most undernourished nations. This is because potatoes are inexpensive and easy to grow under various conditions. Potatoes also provide protein, important vitamins (B-complex and C), and minerals such as potassium, phosphorus, calcium, and magnesium (Burlingame et al., 2009). Moreover, unpeeled potatoes are a good source for dietary fiber and the micro-nutrients iron and zinc. In addition, fresh potatoes are free of fat and cholesterol (United Sates Potato Board, 2007). Besides having vitamins and minerals, potatoes also contain different health benefiting compounds such as phenolics, carotenoids, and flavonoids with strong free-radical scavenging, antiproliferative, and antimicrobial activities which relate to the synergistic effects of potato phytochemicals (Dick Vreugdenhil, 2011). Scavenging of free radicals leads to protection against chronic illnesses by reducing oxidative stress and thus DNA, proteins, and lipid damage (Gomes et al., 2003).

Color-fleshed potatoes are one of the richest plant sources for bioactive polyphenols. The most important phytochemicals are antioxidants such as vitamin C (Brown et al., 2007), and E; mainly α-tocopherol (Ahrne et al., 2007), chlorogenic acid, and carotenoids (Griffiths et al.,

2007). Additionally, in color-fleshed potatoes anthocyanins can be found. The concentration of antioxidants and their antioxidant activity in potatoes are also dependent on genotype, post-harvest storage, and processing parameters (Blessington et al., 2010).

Sixty nine percent of potatoes sold in most cultures are primarily in the form of processed potatoes (Lucier and Ali, 2006). Processing methods leads to a loss of nutrients. Industrial and home-processing methods such as microwave and conventional cooking methods resulted in significant losses in total phenolics content. However, total antioxidant activity was increased or remained unchanged by cooking in pepper, peas, and broccoli (Turkmen and Velioglu, 2005). This can be explained due to improvement of antioxidant properties of bioactive compounds or generation of novel compounds such as the Maillard reaction products (Nicoli et al., 1999). Total anthocyanins content in a cultivar of pigmented potatoes (Purple Majesty) increased by different cooking methods (boiling, microwaving, and steaming) except baking. However, total phenolic compounds decreased significantly in the cooked potatoes of the Purple Majesty cultivar (Lemos, 2013). Madiwale et al. (2012) found that the baking and chipping of potatoes led to significant losses in the phenolic, anthocyanin content, and antioxidant activity. Peeling led to less loss of nutrients (e.g. 13% loss of ascorbic acid) during boiling of unpeeled potatoes compared to 41% loss of ascorbic acid in peeled potatoes (Weber, 1998). The phenolic acid content decreased by 80% after peeling the blue-fleshed potatoes and by 60% in the yellow cultivar (Rytel et al., 2014).

Even after processing, potatoes and potato products can still act as "delivery system" for bioactive compounds, particularly antioxidants in a human diet. However, they also contain some undesirable compounds, which are either produced by heating at a high temperature such as acrylamide or naturally occurring such as glycoalkaloids. The presence of these toxic

compounds has begun a global concern of food safety. Most of the studies have focused on either glycoalkaloids or acrylamide and have not focused on both of them together. Little information is available on the effect of genotype, storage, and processing on the concentration of glycoalkaloids, acrylamide, vitamin C, bioactive compounds, and sensory attributes. Therefore, it is critical to identify suitable cultivars, storage conditions, and processing methods that minimize potato toxic compounds, maintain vitamin C, and bioactive compounds with high quality sensory attributes for consumers.

Objectives:

Preliminary study:

1- Study the effect of genotype, storage time (90 days), and storage temperature (4ºC) on glycoalkaloids and acrylamide content in potato chips of white- and color-fleshed potatoes using Ultra-Performance Liquid Chromatography (UPLC) and High-Performance Liquid Chromatography (HPLC), respectively, and correlate glycoalkaloids and acrylamide content with sensory attributes of potato chips.

Main study:

1- Determine the effect of genotype, storage time (three or six months), storage temperature (4ºC or 10ºC), and processing (baking and chipping) on the total phenolic content, antioxidant activity, and total monomeric anthocyanin content of white- and color-fleshed potatoes using spectrometric methods.

2- Evaluate the effect of genotype, storage time (three or six months), storage temperature (4ºC or 10ºC), and processing (baking and chipping) on acrylamide content of chipped and baked white- and color-fleshed potatoes using UPLC.

3- Quantify reducing sugars content (glucose and fructose) in raw potato tubers.

4- Study the effect of genotype, storage time (three or six months), and storage temperature (4ºC or 10ºC) on sensory attributes of potato chips and baked potatoes and correlate the sensory attributes with acrylamide content.

5- Study the effect of genotype, storage time (three or six months), storage temperature (4ºC or 10ºC), and processing (baking and chipping) on vitamin C content of chipped and baked white- and color-fleshed potatoes using UPLC.

CHAPTER TWO: REVIEW OF LITERATURE

1. Introduction to the potato

The potato (*Solanum tuberosum* L.) is one of the most widely consumed food crops around the world and is surpassed only by rice, wheat, and maize in production (Burlingame et al., 2009). Over half of the world's potato production (159 million tons) is in Asia, Africa, and Latin America where the poor and undernourished people depend on potatoes as a major source of carbohydrates in their diets. Potatoes are also the leading vegetable crop in the United States with per capita consumption of about 112 lbs annually including 35 lbs as fresh market (Osteen, 2012) and 77 lbs as processed potatoes (Lucier and Ali, 2006). Potatoes provide significant amounts of protein, potassium, phosphorus, calcium, magnesium, iron, zinc, vitamins C, B_1, B_6, B_9, and its peels are high in dietary fiber. Since 2008 called the "The International Year of the Potato" http://www.fao.org/potato-2008/en, the societies and growers are focusing on potatoes due to their role in human health and alleviating poverty. This is due to the fact that potatoes are inexpensive and rich in health-modulating bioactive compounds such as anthocyanins and other polyphenols (Liu, 2004). Potatoes are also known to contain undesirable compounds occurring either naturally (glycoalkaloids) or produced during processing of potatoes at high temperatures such as acrylamide (Friedman and Levin, 2008).

1.1. The history of the potato

The potato is a member of the *Solanaceae* family. Potatoes are cultivated originally around the Lake Titacaca, Andes region of South America, specifically Peru and Bolivia. Potatoes were brought to Spain and Portugal and then were dispersed to different parts of Europe in the late 15th century during the search for gold in Peru. Potatoes are a very good source of vitamin C, but at

that time, potatoes were primarily used to prevent starvation in early European sailors (Spooner et al., 2005; Pringle, 2009). During the 16th century, potatoes arrived in North America as a gift sent from Nathaniel Butler (the governor of Bermuda) to Francis Wyatt (the governor of Virginia). The first potato cultivation was done in New Hampshire by early Scottish-Irish immigrants and then spread across the United States. Potatoes are still grown and are consumed currently worldwide as a snack food.

1.2. Potato growth

There are two common methods for potato propagation: true potato seed or vegetative propagation. The advantages of using true potato seed are: prevention of disease transmission, reduction of acreage used for seed production and storage, and shipment convenience (Ross, 1986). Despite the inherent advantages of this method it is not typically used in practice. Potatoes are mainly propagated by vegetative methods (cloning) due to the inherent genetic stability. Potato tubers have eyes from which the new growth begins to form stems (sprouts), which then give rise to the new plant. Vegetative seed can be either a whole tuber or cut tuber. In general, growth of potato tuber appears in several stages: planted seed tuber; vegetative growth; tuber initiation; tuber bulking (Figure 2.1). The stages of growth depend on many factors such as environmental factors (e.g. soil type, elevation, temperature, cultivars selected, availability of moisture, and geographic location) and management practices (Stephen et al., 2003). The first phase of potato growth is planting in well-drained soil to allow the tuber to develop roots and a shoot. In the second phase, the shoot begins developing leaves and branches. The next phase is the initiation of tuber development followed by the fourth stage of tuber bulking and the tuber shaping. During growth, photosynthesis provides the potato plant energy that is stored as starch in potato tubers.

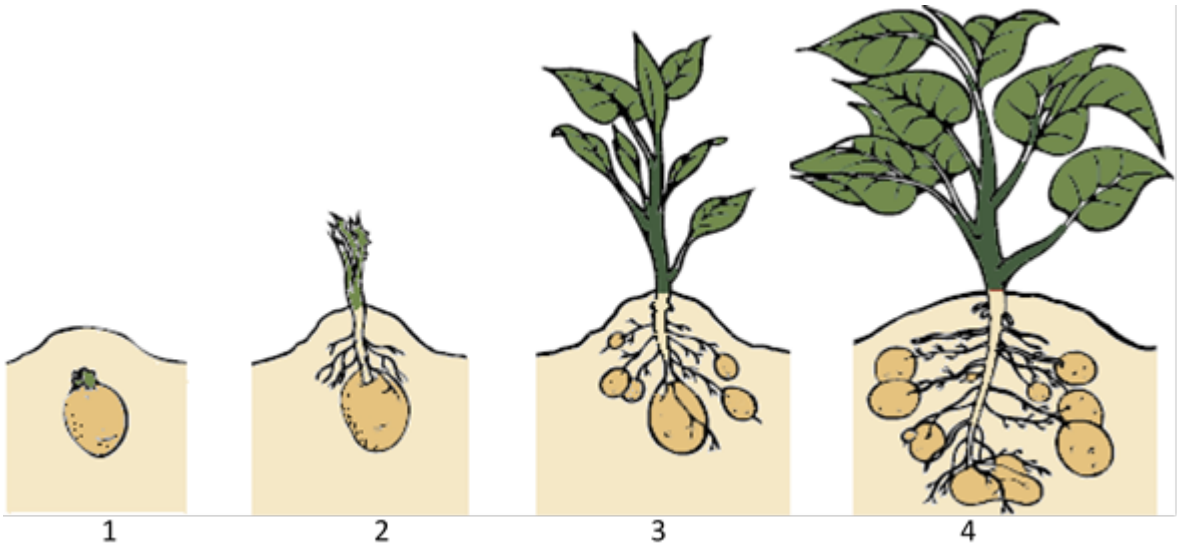

Figure 2.1. Stages of potato plant development (FAO, 2008). 1) Planted seed tuber; 2) Vegetative growth; 3) Tuber initiation; 4) Tuber bulking.

At the end of the growth season, the potato plant dies and detaches from the tubers. Potato tubers are collected by harvesters who collect the plant and surrounding soil and separate the potato plant from soil debris. Potatoes are a cool-season plant. The maximum yield is reached when average daytime temperatures are around 21ºC. Low temperatures at night are very important for the accumulation of carbohydrates and the dry matter in the tubers. Moreover, cooler night temperatures slow respiration rates, resulting in a larger tuber from less dry mater loss and more starch storage. The best temperature for initiating tubers is between 16°C and 19°C. At 21ºC, tuber development declines and then stops when soil temperatures reach 30°C or above (Stephen et al., 2003).

1.3 Potato physiology

The potato belongs to the *Solanaceae* family that includes pepper, eggplant, and tomato. *Solanum tuberosum* L. is distinguished into two subspecies: *andigena* and *tuberosum. Andigena* is cultivated in the Andes (South American) and is adapted to short day conditions, whereas

tuberosum is a widely cultivated species and has adapted to long day conditions (Sukhotu and Hosaka, 2006). In addition to being a cool-season plant, potatoes need fertile, well-drained, and slightly acidic soil (pH 5.8-6.5). Potatoes form tubers four to six inches below the soil surface. During growing, potatoes exposed directly to sunlight turn green, causing the flesh to taste bitter and lead to an increase in the level of toxic compounds such as glycoalkaloids. Furthermore, the potato plant is very sensitive to drought, especially during the flowering phase since this is the peak time for tuber formation. Potato plants grow either white flowers or colored flowers linked to either white or pink skinned tubers, respectively (Winch, 2007). Potato starch is produced from the plant leaves during growth, but is transferred to its underground stems to form the tubers. Usually, potatoes are harvested between two and three weeks after the plant flowers. The number and size of tubers is influenced by the tuber initiation phase, environmental factors, and physiological factors. Environmental factors include planting date, temperatures of soil, nutrition, and water management. Physiological factors include cultivar/selection, seed size, and the number of stems produced by seed tubers.

1.4 Potato cultivars

Potato genotypes differ in type, color, shape, taste, texture, and cooking characteristics (Food and Agriculture Organization, 2008). There are around 5,000 genotypes known and cultivated around the world (Lutaladio and Castaidi, 2009). Potatoes are classified into waxy, starchy, and all-purpose cultivars (Nonnecke, 1989). The best category for boiling is waxy while the starchy types are best for baking. The all-purpose cultivar falls in between these two types. Matured potatoes have less sugar content and lower moisture compared to baby potatoes. Matured potatoes also have thick skins and can be kept in cold storage for up to a year before being used. In the United States, the most-grown potato cultivars are russets, long whites round red, round whites, and Yukon Gold (United States Potato Borad, 2007). Russet Burbank is the

most common cultivar for baking featuring sturdy brown skins with mesh-like netting on the surface, and starchy flesh. Matured long whites are starchy while the newly harvested tubers are thin-skinned and waxy. Yukon Gold is a lightly yellow-fleshed cultivar and was developed in 1989 at the University of Guelph, Ontario, Canada. This cultivar is good for boiling, baking, and chipping (Nonnecke, 1989). Atlantic is a chipping potato cultivar developed by Agricultural Research Service at Beltsville in 1978. Color-fleshed potatoes have a flesh that ranges from red to dark blue and dark purple (Stelljes, 2001).

2. Toxic compounds found in potatoes

2.1. The post-processing toxic compound: Acrylamide (AL)

2.1.1. Acrylamide formation

The Swedish National Food Authority and the University of Stockholm discovered Acrylamide (AL) in food in April 2002 (Tareke et al., 2002). AL is formed from the reaction of reducing sugars (free glucose, fructose, and hydrolyzed starch) with asparagine, an amino acid via the Maillard reaction. AL formation occurs during processing at temperature above 120°C (248ºF) as shown in Figure 2.2 (Tareke et al., 2002). The Maillard reaction creates flavor and changes the color in cooked foods (Mottram et al., 2002; Stadler et al., 2003). Heated potato products such as French fries and potato chips contain a high concentration of AL ranging from 424 µg/kg to 1,739 µg/kg (Pedreschi, 2009). This is due to the presence of high amounts of the reactants asparagine, 94 mg/100g (Martin and Ames, 2001) and reducing sugars, 0.1-3.0 g/kg after harvesting. Reducing sugars content may increase to 20 g/kg with nonoptimal storage conditions such as low temperature (Amrein et al., 2004). Formation of AL can be reduced in the presence of cations by preventing the formation of schiff base. However, this leads to change the

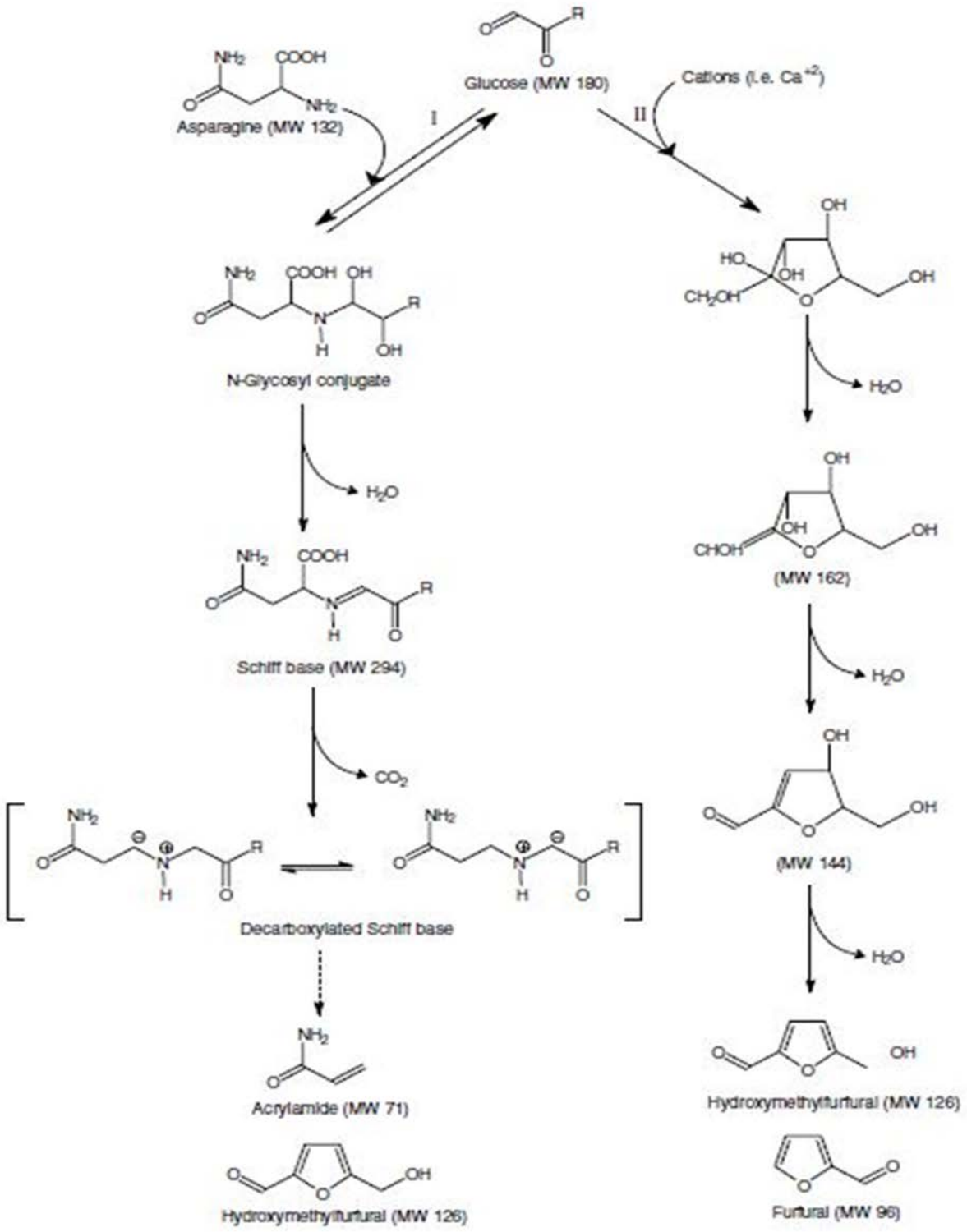

Figure 2.2. Formation of acrylamide during the pyrolysis of asparagine with glucose (Gokmen and Senyuva, 2007b).

pathway toward the dehydration of glucose forming a furfural and hydroxymethylfurfural during processing of potatoes (Gokmen and Senyuva, 2007b). Furfural is irritating to the eyes, respiratory tract, and skin and reported to be a central nervous system poison in large doses in some animals, causing hyper-reflexia convulsions (United States Department of Health and Human Services, 1978).

2.1.2. Factors that affect acrylamide content in potato products

The variation in AL content is not only due to the variability of the reactant levels present in selected cultivars, but AL is also affected by post-harvest handling, differences in cooking methods, processing conditions (e.g., frying, cooking time, and temperature), moisture level, pH, the presence of additives, and storage conditions (Williams, 2005). Low temperature storage (below 8°C) is not appropriate for potatoes intended for the processing market. Therefore, storage at 8°C or above will reduce the potential for the formation of AL in the potato product upon baking or frying. However, potatoes cannot be stored for long-term at 8°C without the use of sprout suppressing agents (Kumar et al., 2004). Non-cold storage can only be achieved by chemical treatments of potatoes with sprout suppressing agents. The consumer due to health and safety concerns does not desire chemical treatments. In order to lower AL formation, potatoes used for processing should contain reducing sugar levels below 3 g/kgfw (United States Department of Health and Human Services, 2007). The reducing sugar accumulation is dependent upon the initial levels and storage conditions such as storage temperature. Interestingly, selecting potato cultivars with low reducing sugars also results in the production of golden colored potato chips that are desirable to consumers. This can be explained due to low sugar levels lead to less browning and thus less AL formation. Climatic conditions affect the AL level in potato products by affecting the reducing sugar content in raw materials. For example,

dry summers produce potato tubers with lower reducing sugar content. Santerre et al. (1986) reported that sucrose content in potato tubers tend to increase after a large rainfall due to the fact that soil moisture affects soil temperature. The temperature affects sugar content of tubers whereas, temperature higher than 25°C result in elevated reducing sugars levels. High temperatures contribute to a reduction of the transferring of sugars from leaves to tubers and a reduction in the rate of incorporation of those sugars into the tuber tissue resulting in a lower specific gravity. Moreover, an increase in the sugar content of potato tuber exposed to temperatures below 8-12°C was observed by Arreguinlozano and Bonner (1949). Higher temperatures during growth have negative effect on the rate of starch synthesis by enhancing the respiration process (Davies et al., 1989).

Another factor that affects AL levels is nitrogen fertilization. Production of potato tubers with adequate nitrogen fertilization during growth produces a product with a lower reducing sugar concentration at harvest and less accumulation during storage (De Wilde et al., 2006). Tuber maturity also affects sugar content of potato tubers. The immature and young tubers generally have more reducing sugars, thereby, increased AL formation in the final products (De Wilde et al., 2006). However, when potato tubers reach maturity, the ratio of sucrose to reducing sugars reaches a minimum value and thus the high levels of reducing sugars can make the potatoes unacceptable for high temperature processing.

2.1.3. Acrylamide and health

AL is a neurotoxicant and carcinogen in animal models (Rice, 2005; Friedman and Levin, 2008), thus is potentially a human carcinogen (U.S Department of Health and Human Services, 2007). AL has been reported to increase the risk for ovarian, endometrial, and renal cell cancers in humans (Virk-Baker et al., 2014). AL is biotransformed in the liver by the action of

cytochrome P4502E1 to reactive epoxide or glycidamide, which seems to be responsible for its genotoxic effects. AL is also converted to glycidamide via a detoxification reaction by reacting with glutathione via the action of glutathione-S-transferase enzyme. Glycidamide, but not AL, reacts with DNA via the Michael addition reaction (Doerge et al., 2007; Doroshyenko et al., 2009; Kopp and Dekant, 2009), formed DNA adducts and mutation (Besaratinia and Pfeifer, 2004). Glycidamide that does not react with DNA is then hydrolyzed to the nontoxic 2, 3-dihydroxypropanamide acid (glyceramide) and dihydroxypropionic acid and is excreted as urinary metabolites (Sumner et al., 2003).

2.1.4. Effect of processing on acrylamide in potato products

Processing of potatoes can improve taste, nutrition, and quality, but can occasionally lead to the formation of AL. This compound is not present in raw potatoes or formed during boiling, but it can be formed at the higher temperatures associated with frying and oven-baking (Ahn et al., 2002). Processing parameters could be controlled during production of potatoes products. Processing of raw potato tubers to a specific surface volume ratio (SVR) impacts the AL content in the final products. Researchers suggest that cutting potato tubers with intermediate or high SVR, higher temperatures and longer processing times resulted in reduced AL levels. Low SVR resulted in increased AL levels in potato products with the longer time and higher temperature of frying (Taubert et al., 2004). This can be explained due to a heat transfer to the core of potato slice with low SVR takes very less time, thus the threshold temperature in the core may be sufficient for AL formation. During frying, the formation of AL is dependent on the heating temperature and duration of heating (Williams, 2005). A decrease of AL concentration at temperatures above 170°C was reported by Mottram et al. (2002). Contradicted results were also observed by Kita et al. (2004) and Pedreschi et al. (2005), who found that decreasing the frying

temperature will result in reduction of AL formation in potato chips by approximately 60% and 75% when the frying temperature is reduced from 190°C to 170°C and from 185°C to 160°C, respectively. AL is formed during the final frying stage and a non-linear rate of AL formation with increased temperature according to Taeymans et al. (2004). Therefore, at lower frying temperatures and longer frying times, AL concentrations were reduced. There is, however, lower temperature limit of 150°C due to affected quality, higher fat uptake, and poor texture (soft). Therefore, it is recommended that the frying temperature should be between 170°C and 175°C for optimum potato product quality and less AL. Accumulating evidence from studies on bread suggests that cooking and frying time has the greatest influence on the level of AL formation in bread. AL content increased from about 300 ppb to 1200 ppb when bread was baked at 270°C for 18 to 32 minutes (Surdyk et al., 2004). The formation of AL in potato products is highly dependent on frying time reflecting that the AL formation is dependent on the temperature and time of frying (Williams, 2005). However, the approach of AL minimization should be to search for a time/temperature combination at which AL levels may be reduced without affecting antioxidant content. Optimization of processing parameters plays an important role in health and AL reduction (Ou et al., 2010; Li et al., 2012) as well as the quality of the final product. However, it is important to note that, AL formation is coupled to product quality by generation of flavor and color compounds, which are part of the product's characteristics. These compounds cannot be obtained without minimal AL formation. The other important factor besides temperature and time known to have an effect on the formation of AL is water activity. Only free water in potatoes can participate in the Maillard reaction. Therefore, at higher water activity, the reaction rate decreases because of a dilution of the reactants by water. The AL formation only begins when high temperatures and moisture content is lowered (below 20 g/100g) have been

reached especially on the surface of fried potatoes (Baumann and Escher, 1995). In a study on plantains, AL was decreased by increasing initial water activity (Miao et al., 2014). Water activity could also influence the formation of 5-hydroxymethylfurfural, a cytotoxic compound (Miao et al., 2014). Based on the above-mentioned the water activity is a key factor in the Maillard reaction and therby in AL formation. Water activity can be controlled by such as addition of salt and sugar interacting with water through dipole-dipole, ionic and hydrogen bonds, addition of starches, and proteins through interacting with water via dipole-dipole forces and ionic bonds. Frying temperatures also important since water activity is temperature-dependent and results in changes in water binding.

The major strategy to reduce the AL in potato products is to reduce the precursor levels (reducing sugars and asparagine) in raw potato materials by blanching or by addition of exogenous additives. Soaking or blanching of raw potato slices in water has also been proposed to decrease oil absorption and enhance post-frying quality by creating a surface barrier and leaching out the reducing sugars (May et al., 2006). Thereby, the decrease in the reducing sugar content from 62% to 51% of original levels results in the reduction of AL by up to 25%. Blanching can be even more effective by the addition of additives. (i) Presence of organic acids in blanching water (such as citric or acetic acid), mitigate AL up to 90% (Jung et al., 2003; Kita et al., 2004). However, this approach can cause the souring of flavor along with the addition that limiting the generation of aroma compounds during thermal processing (Gokmen and Senyuva, 2007a). (ii) Another possible approach is to use amino acids (e.g., glycine or glutamine) during soaking or blanching to compete with asparagine during the Maillard reaction. This approach can reduce AL levels up to 30%, but it also affects the sensory properties of thermally processed potato products by changing the profile of aroma compounds (Gokmen and Senyuva, 2007b).

(iii) The use of salts such as NaCl and $CaCl_2$ during soaking or blanching has been shown to reduce the level of AL formed during frying up to 50% or 95%, respectively. The side effect of this approach results in a change of the Maillard reaction and at the same time leads to the formation of 5-hydroxylmethylfurfural, a toxic compound, as shown in a fructose-asparagine model potato system at 150°C and 180°C (Gokmen and Senyuva, 2007b ; Capuano and Fogliano, 2011). (iv) Another possible approach to lowering asparagine concentrations before frying by using asparaginase enzyme to convert asparagine to aspartic acid and ammonia. Therefore, the use of asparaginase is effective in interrupting the interaction of asparagine with reducing sugars, without altering the appearance or taste of the final product. Novel methods that could be applied to produce the desired low AL potato products were recently published by Pedreschi et al. (2008 ; 2011). According to these methods (i, ii, iii, iv), potato chips and French fries contain 75% to 80% lower levels of AL. Reduction of AL can be further increased up to 90% by the combination of soaking in asparaginase solution with the conventional blanching in hot water (Pedreschi et al., 2011). This can be explained by the fact that blanching helps to leach out reducing sugars and asparagine from inside and the enzyme reduces asparagine in the external layers of the potato. Recently (v) a novel strategy of AL reduction proposed by Kalita and Jayanty (2013) is to soak potatoes in vanadium salt solution. This approach resulted in 90% reduction of AL content in potato chips by the inhibition of Schiff base formation through the binding of a vanadyl-asparagine complex. This resulted in significant reduction of AL formation in fried potato products. In comparison to the first three approaches (i, ii, iii), which have side effects, the last two approaches (iv, v) do not seem to have side effects and may be better options to reduce AL levels in potato products.

2.2. The pre-processing toxic compound: Glycoalkaloids (GAs)

Potatoes and potato products are also known to contain toxic compounds called GAs (α-solanine and α-chaconine) are believed to have a role in pest resistance and are important in potato flavor (Sinden et al., 1976) and increased consumer acceptance. Potatoes with GA levels greater than 140 mg/kgfw have a bitter taste whereas small quantities of GAs below 140 mg/kgfw improve taste (Sinden et al., 1976). Potatoes have been consumed regularly by many people worldwide without any side effect, which suggests that low levels of GAs are found in properly stored and handled potatoes. However, accumulation of GAs in potatoes affects potato quality and could be toxic to humans if they are consumed in high levels.

2.2.1. Glycoalkaloids pathway

GAs are formed by steroidal glycoalkaloid biosynthetic pathway (SGA; Ginzberg et al., 2012). Acetate reacts with coenzyme A to form the intermediates of mevalonic acid, squalene, lanosterol, and cycloartenol. Cycloartenol metabolism leads to the synthesis of plant sterols and specifically cholesterol. Cholesterol is also formed by the mevalonic acid pathway and is the starting point for the GAs biosynthesis in the potato tuber. Cholesterol is converted to the aglycone moiety of GAs. The next step of GAs synthesis is that the solanidine aglycone is glycosylated by activity of glycosyltransferase enzymes (a glucosyltransferase and a galactosyltransferase), by adding the sugar side chain (glucose, galactose or rhamnose) to the 3-hydroxy position of the aglycone (Figure 2.3). Glycosylatation of the aglycone solanidine yields γ-chaconine and γ-solanine. β-forms of chaconine and solanine are formed by action of glycosyltransferase (SGT_2). Lastly, rhamnosyltranferase catalyzes the formation of α-chaconine and α-solanine from β-forms.

Potato glycoalkaloids consist of the same aglycone solanidine, but they differ in the carbohydrate side chain attached to the 3-OH group of the aglycone. α-chaconine is usually present at a slightly higher concentration than α-solanine. However, the difference in toxicity has been attributed to their differing in concentration and carbohydrate-side chains. Potato glycoalkaloids are present in potato tubers at differing levels and consumption of potatoes results in the ingestion of both glycoalkaloids. Thus, in combination, they have synergistic effects resulting in increased toxicity even at low level when compared to α-chaconine or α-solanine alone (Rayburn et al., 1995).

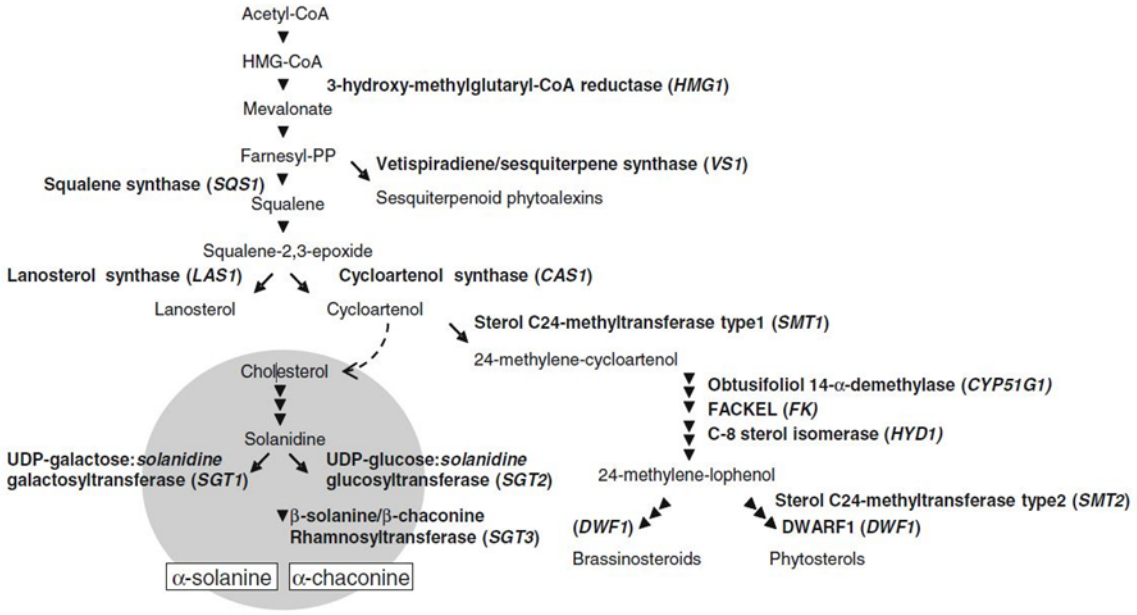

Figure 2.3. Schematic representation of proposed SGA biosynthetic pathway. Arrowheads represent enzymatic steps (Ginzberg et al., 2012).

2.2.2. Glycoalkaloids and health

GAs serve as natural defenses against pests and insects, but they are toxic for humans. The maximum level of GAs is permitted 200 mg/kg per whole fresh tuber (Friedman and McDonald, 1997); as levels above could be toxic to humans. The toxicity of GAs is due to their membrane disruptive properties and inhibition of acetylcholinsterase and butyrylcholinesterase

activity (Krasowski et al., 1997). Higher concentrations of GAs disrupt cholesterol-containing membranes in the intestinal epithelium, thus promoting colonic inflammation and colon cancer (Iablokov et al., 2010). Interestingly, within acceptable GAs ranges, they have been known to have anticancer, anticholesterol, and anti-inflammatory properties.

2.2.3. Factors that affect GAs content in potatoes

2.2.3.1. Effect of genotype and post-harvest factors on glycoalkaloids

There are many pre- and post-harvest factors that elevate the levels of GAs to an unsafe level. These factors include genotype, poor growing conditions, sprouting, mechanical injury, fungal attack, exposure to light, and sub-optimal storage conditions (Friedman and McDonald, 1997). In some cases, it is easy to see the signs of physical change or damage (e.g., sprouting and greening). Along with efforts to identify new potato selections to elevate health-benefiting compounds content in potato tubers, new cultivars should be screened to study the behavior of potato GAs during storage to ensure GA levels in tubers remain below established limits of 200 mg/kgfw. Considerable variations of total GA content have been reported in literature. According to Valcarcel et al. (2014), who assessed the GA content in 60 cultivars of potatoes planted in two different locations, potato GA content ranged from 4 to 957 mg/kg of dry weight in the flesh and from 150 to 8133 mg/kg in the potato skin. Another study by Deusser et al. (2012) demonstrated that total GA contents ranged from 585 to 5342 mg/kg in dry peel and from 7 to 466 mg/kg in dry flesh. Friedman et al. (2003) reported that the values of potato GAs ranged from 84 to 2226 mg/kg in dry peel and from 5 to 592 mg/kg in dry flesh. The difference in GA content in dry peel in above mentioned-study may relate to the variation between tested genotypes or to the environmental factors. The most influential factor on GA production is the weather, especially a combination of cold temperature excessive rain and a lack of adequate

sunshine (Bomer and Mattis, 1924). Moreover, harvesting potatoes at later maturity stages maximized yields while minimizing the total GAs content according to a study by Reyes et al. (2004). Exposure of harvested potato tubers to light, whether incandescent, fluorescent, or natural can significantly increase the level of GAs in potato tubers. Increased GA levels due to the exposure to light leads to a greener color in the potato tubers but the synthesis of GAs in cold-stored potatoes tubers is preceded by the chlorophyll formation (Ramaswamy and Nair, 1984). Storage at low (below 5ºC) or high temperatures (above 10°C) induces an increase of GA levels in potato tubers (Haase, 2010). The best storage temperatures for potato tubers to be used for chips and French fry production are intermediate temperatures (7°C to 10°C). Storage time has the same effect as light and temperature; the longer the storage time, the higher the level of GAs in potato tubers (Sengul et al., 2004). Sprouting also increases the level of GAs in potato tubers; therefore, using sprout inhibitors can reduce GAs in potato tubers (Friedman and McDonald, 1997).

2.2.3.2. Effect of processing on glycoalkaloids

Heating temperatures utilized during the production of potato products have small effect on the content of potato GAs due to the thermo-stable nature of GAs (Finotti et al., 2006) and the distribution in the tuber; thus, they remain at high levels even after being cooked at the desired frying and baking temperature. The concentration of GAs is mainly located in the periderm of the tuber. In general, all plant parts have GAs except the pith. The concentration and accumulation in potatoes are related to the genotype and environmental factors during growth, harvest, and storage. Potato GAs concentrate in a small 1.5 mm layer under the skin, therefore peeling will remove 60% - 90% of GAs present but if the potato tubers are very high in GAs, the peeling will remove only up to 35%, because of diffusion of GAs into the deeper tissues (Mader

et al., 2009). Thus, potato GAs can be reduced by one or more pre-treatments of raw potato such as peeling and blanching (Friedman et al., 2003; Rytel et al., 2005). Partial food processing such as peeling is only one of stress factors affecting potato GAs. The effective way to reduce the GA content is to remove the peels, but the peel also has high amounts of phenolic compounds, which have health benefits. GA content was shown to decrease significantly during peeling (30%), cutting, washing, and blanching (28%), but only a slight reduction was observed during frying (Takagi et al., 1990; Rytel et al., 2005; Elzbieta, 2012). In the Blaue St Galler cultivar, 57% of the original level of GAs remained after peeling whereas, in the Agria cultivar it was only 5% (Lachman et al., 2013). According to Tajner-Czopek et al. (2012), the frying of potato chips resulted in a decrease of GA content up to 83% which conflicts with previous findings (Takagi et al., 1990; Rytel et al., 2005; Elzbieta, 2012). Respectively, GA content reduced to 8% and 39% in cooked un-peeled and peeled potatoes (Tajner-Czopek et al., 2012). Potato GAs are stress metabolites and genetically controlled. Thereby, the most effective way of obtaining products with low levels of GAs is to select cultivars that are initially very low in GAs. However, levels of potato GAs are not affected by boiling, freeze-drying, or dehydration, but high-temperature processing, such as deep-frying at or above 170°C has significant reduction of GAs in final products.

3. Potato bioactive compounds

Potatoes contain a small amount of bioactive compounds or phytochemicals, which are considered secondary plant metabolites. Potato bioactive compounds have strong antioxidant and antiproliferative activities, which relate to the combination of phytochemicals. These compounds can be divided into 5 major groups: polyphenols, carotenoids, alkaloids, nitrogen-containing, and organo-sulfur compounds (Figure 2.4; Liu, 2004). Polyphenols are the most abundant

phytochemicals in the human diet. More than 8,000 polyphenols have been identified and classified into subgroups: phenolic acids, tannins, stilbenes, coumarins, and flavonoids; and anthocyanins (Bravo, 1998 ; Liu, 2004). Recently, the recognition of their antioxidant properties in the prevention of degenerative diseases, such as cancer and cardiovascular diseases has received much attention (Liu, 2013 ; Pistollato et al., 2014). Although potatoes have lower levels of phenolic compounds compared to fruits, such as oranges and apples (Chun et al., 2005), they are the third largest provider in polyphenol content due to the magnitude of potato consumption (112 lbs/person/year) in the American diet (Osteen, 2012).

3.1. Phenolic acids

Phenolic acids are one of the most prominent classes of phytochemicals present in the potato. These compounds are synthesized from phenylalanine, which is the starting point of the shikimate pathway (Figure 2.5; Dixon and Paiva, 1995). There are two sub-groups of phenolic acids, which can be distinguished: hydroxybenzoic acid (e.g., gallic acid, protocatechuic acid, and *p*-hydroxybenzoic acid) and hydroxycinnamic acid (e.g., caffeic, chlorogenic, coumaric, ferulic, and sinapic acid). In general, the hydroxycinnamic acids are more common than hydroxybenzoic acids (Manach et al., 2004). Purple-, red-skinned and fleshed potato tubers are rich in phenolic acids. Chlorogenic acid accounts for more than 90% of the total phenolic acids (Malmberg and Theander, 1985). The most common phenolic acids found in raw, baked, and chipped potatoes are chlorogenic acid and caffeic acid (Reddivari et al., 2007a ; Madiwale et al., 2012). Combined effect of storage for three months at 4°C and processing (baking) resulted in increased chlorogenic acid content in white-fleshed potatoes and ranged from 0.05 mg/100 gfw to 1.5 mg/100 gfw and from 16.8 mg/100 gfw to 52.3 mg/100 gfw in color-fleshed potatoes. Caffeic acid followed the same trend in white-fleshed potatoes and ranged from 0.3 to 0.8

mg/100 gfw and from 6.2 mg/100 gfw to 13.3 mg/100 gfw in color-fleshed potatoes. Hence, it is very important to consider the effect of genotype on potato bioactive compounds. The interaction effects of chipping, frying, and storage resulted in no significant difference in chlorogenic acid and caffeic acid contents. However, the interaction effects of processing (baking) and storage time resulted in a significant increase in chlorogenic acid and caffeic acid contents of baked potatoes compared to unprocessed potatoes, depending on cultivar (Madiwale et al., 2012). Therefore, it is very a critical to consider the genotype and farm-to-fork operations such as storage and processing.

3.2. Anthocyanins

Anthocyanins contribute to color (red, blue, and purple), and appearance of potato tubers. Purple- and red-fleshed potatoes provide a natural source of anthocyanin pigments and they have been associated with beneficial health effects. There are six anthocyanidins: pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin. Potato anthocyanin are either glycosylated, polyhydroxy and/or polymethoxy derivatives of the 2-phenylbenzopyrylium (flavylium) salts (Mazza and Miniati, 1993). Red- and purple-fleshed potatoes have acylated glucosides of pelargonidin. Along with acylated glucosides of pelargonidin, purple-fleshed potatoes also have acylated glucosides of malvidin, petunidin, peonidin, and delphinidin (Brown et al., 2005 ; Lachman and Hamouz, 2005). Acylated anthocyanins have been reported to show high pH stability and thermostability compared to non acylated ones (Terahara, 2006). Color-fleshed potatoes are rich in anthocyanins with a range of 5.5 to 35 mg/100 gfw (Brown et al., 2007). The purple-fleshed potatoes have higher anthocyanins when compared to the red-fleshed potatoes. According to Madiwale et al. (2012), purple-fleshed potatoes had different metabolite profiles compared to white-fleshed potatoes, mainly attributed to the presence of anthocyanins.

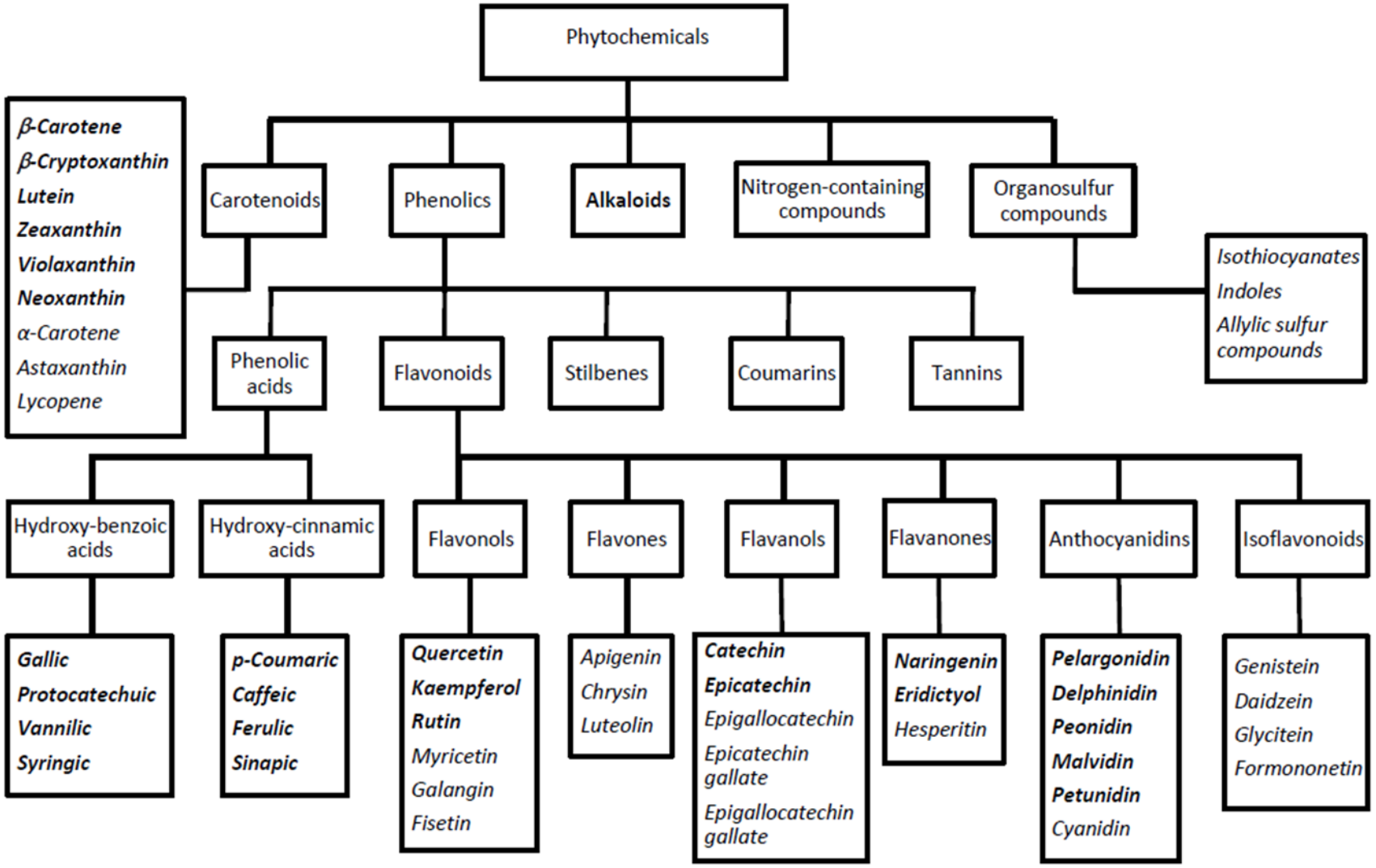

Figure 2.4. Classification of dietary phytochemicals; Phytochemicals commonly found in potatoes in **bold** (Liu, 2004).

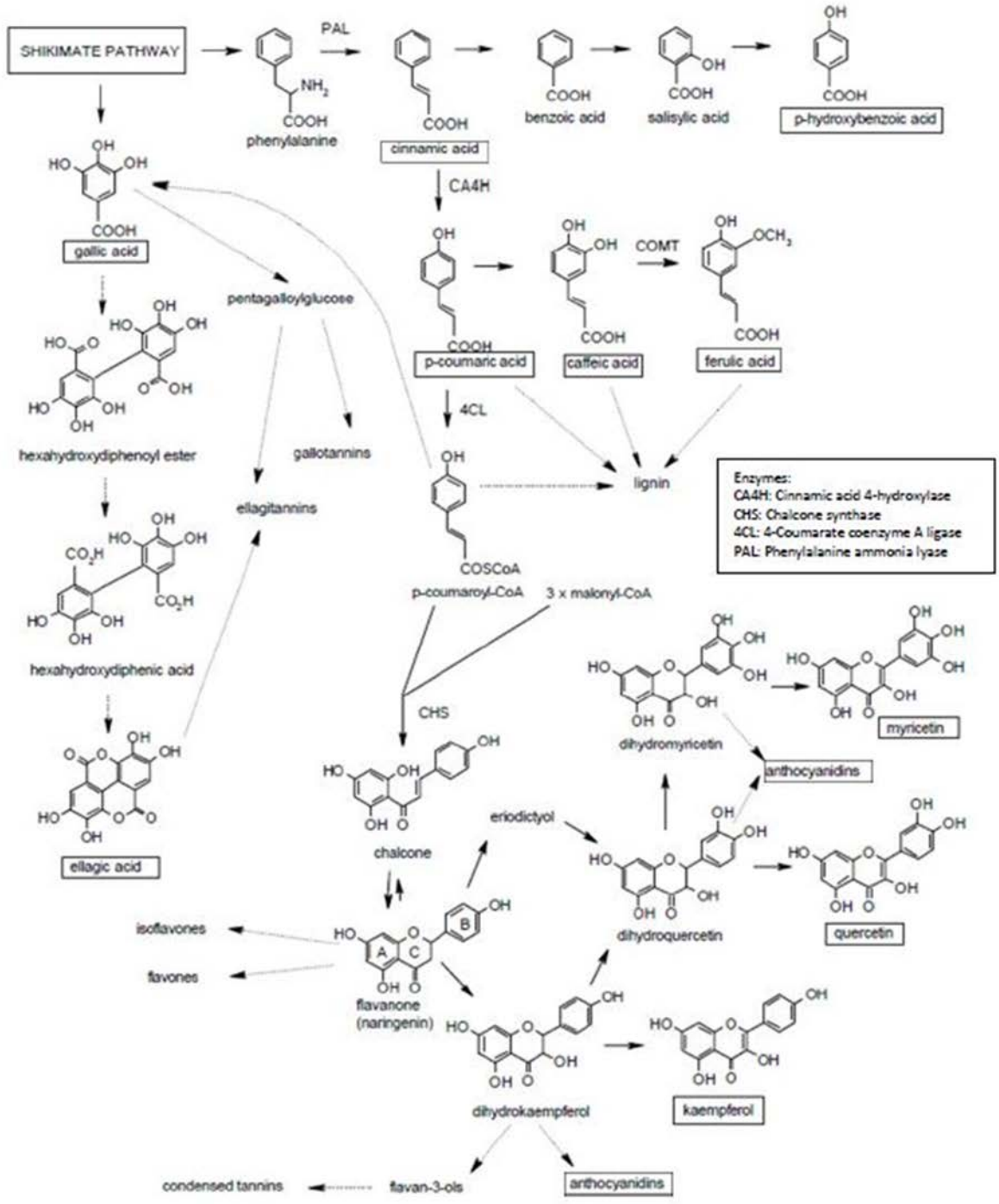

Figure 2.5. Biosynthesis of hydroxycinnamic acids, hydroxybenzoic acids, and flavonoids (Dixon and Paiva, 1995).

The most common method to determine the total monomeric anthocyanins is the pH differential method, which depends on a strctural change of the anthocyanin chromophore between pH 1.0 and 4.5 (Lee et al., 2005).

3.3 Health-benefiting properties

Potatoes contain different compounds with strong antioxidant and antiproliferative activities due to the combination of phytochemicals. Potato bioactive compounds such as polyphenols and carotenoids have received much attention due to their chemopreventive properties. For example, polyphenols have demonstrated anticancer activities in a number of cancer cell lines, such as liver, colon, and prostate. Furthermore, these compounds have been shown to prevent heart disease (Hertog, 1995), type 2 diabetes (Ramdath et al., 2014), and inflammation (Kobuchi et al., 1999). Nzaramba et al. (2009) reported that extracts from *Solanum jamesii* showed cytotoxic and proliferative effects against HT-29 human colon cancer and LNCap human prostate cancer cell lines. Caffeic acid, is known to block the biosynthesis of leukotriene. Leukotriene is a member of eicosanoid inflammatory mediators in leukocytes and its overproduction can cause inflammation in asthma and allergic rhinitis (Koshihara et al., 1984). Caffeic and ferulic acids are known also to detoxify carcinogen metabolites of polycyclic aromatic hydrocarbons (Huang et al., 1996).

Anthocyanins have many health benefits such as scavenging free radicals, reducing cell proliferation, up-regulating/increasing apoptosis, and modulating mitogen-activated protein kinase activities (Afaq et al., 2005; Jing et al., 2008 ; Shin et al., 2009). When comparing a white-fleshed potato diet with an anthocyanin-rich purple-fleshed potato diet, the latter had anti-inflammatory effect and reduced plasma levels of C-reactive protein, 8-hydrodeoxguanosine, and interleukin-6 in healthy men consuming a white and purple-fleshed potato diet (Kaspar et al.,

2011). Anthocyanin fraction from color-flesh potatoes induces apoptosis in LNCaP (androgen dependent) and PC-3 (androgen in-dependent) prostate cancer cells via caspase-dependent and independent pathways (Reddivari et al., 2007). In addition, we have previously shown that extracts from purple-fleshed potatoes had more potent anticancer effects on colon cancer cells compared to white- and yellow-fleshed potatoes likely due to the presence of anthocyanins in purple-fleshed potatoes (Madiwale et al., 2012).

3.4. Factors affecting potato bioactive compounds

Potato bioactive compounds are affected by genotype, environment (soil and nutrient supply, location, climate, and season), and processing (baking, steaming, boiling, chipping, frying, and microwaving). Post-harvest handling and storage conditions such as relative humidity, packaging, temperature, light, and storage time also affect the content of the bioactive compounds in potatoes. Ninety percent of dry weight of the potato tuber consists of starch produced through the photosynthesis. During growth, the potato plant uses this sugar for growth via a process called respiration. The rate of this process is increased dramatically by temperature. Environmental stress (temperature, light, water) affects the growth, tuber production, and chemical composition of potato bioactive compounds indirectly by providing the prerequisites for photosynthesis (Hewett, 2006) and thereby providing energy or precursors of the synthesis of the bioactive compounds. It is believed that environmental stress activate the primary metabolic pathway, which are required for the biosynthesis of secondary metabolites due to it generates the carbon skeletons needed for secondary metabolites production (Jacobo-Velázquez and Cisneros-Zevallos, 2012).

3.4.1. Effect of genotype and environment on potato bioactive compounds

3.4.1.1. Genotype

Field trials from 1995 to 1997 were conducted by Hamouz et al. (1999) to study the effect of environmental conditions of regions with varying altitudes, cultivars (Agria and Karin), year, and production methods on total phenolic content. Hamouz et al. (1999) reported potato tubers from the Czech Republic region with higher altitude (cooler and more humid climate) had higher phenolic content (46.3 mg/100g) when compared with the drier, warmer, and lower altitude regions (43.5 mg/100g). However, the total phenolic acid content was cultivar-dependent rather than climatically influenced. Another study by Stushnoff et al. (2008) demonstrated the effect of genotype on total phenolic acid and antioxidant activity of white- yellow- red-, and purple-fleshed potatoes during five year potato production study. They found that genotype had a significant influence on total phenolic acid and antioxidant activity, whereas genotypes with red or purple skin and flesh (Purple Majesty and Mountain Rose) had higher total phenolic and antioxidant activity than the non-pigmented genotypes (Rio Grande Russet and Yukon Gold). The influence of 25 potato genotypes location (McCook and Dalhart; Texas) and year (2003 and 2005) on total phenolics and antioxidant were studied by Reddivari et al. (2007b). The total phenolic, its composition, and antioxidant activity differed significantly between genotypes and locations. They also observed that genotypic effects were larger than location, year, and interaction effects of genotype, year, and location.

3.4.1.2. Nutrients and soil

Bioactive production in plants is affected by farming practices and post-harvest factors. Environmental factors such as soil type, nutrients, location, and growing season can impact the production of plant secondary metabolites. The main differences between organic and

conventional food are exposure to pesticides and/or herbicides in conventional food whereas organic foods are protected naturally. Many researchers show that organic foods are richer in nutrients; in particular polyphenolic compounds and vitamin C (Vinha et al., 2014). However, two comparison studies by Rosenthal and Jansky (2008) and by Faller and Fialho (2009) demonstrated no significant difference in polyphenol content or antioxidant activity between organic and conventionally grown potatoes. Additionally, polyphenols in conventionally grown potatoes were more resistant to losses during cooking compared to organic potatoes.

3.4.1.3. Location, climate, and season

The effects of location, climate, and season on bioactive compounds in potatoes have been reported. The findings imply there are numerous factors such as temperature, rainfall, soil altitude, and light conditions at a given location influencing the bioactive compounds in the potato. The effect of two different locations (Dalhart, Texas; McCook, Nebraska, USA) was studied by Reddivari et al. (2007b). Their group measured bioactive compounds of 25 genotypes at different altitude, latitude, rainfall, mean annual temperature, and production season. They found that the potato genotypes grown in McCook were higher in total phenolic content and antioxidant activity, but lower in carotenoid content when compared to Dalhart. Another study by Reyes et al. (2004) studied the effect of environmental conditions on the content, yield of anthocyanins, and total phenolics in purple- and red-fleshed potatoes during growth in both Texas and Colorado. They observed that total phenolic content in both locations decreased with tuber growth, maturity, and weight of tuber. Cold temperature and longer days in Colorado contributed to 2.5 and 1.4 times higher total phenolic acids content, respectively, when compared to Texas-grown tubers. Additionally, Brown et al. (2008) found that genotypes grown at higher altitudes were lower in carotenoid content and antioxidant capacity, but higher in anthocyanin

content. Conflicting results of the effect of production year on the potato bioactive compounds have been reported. No influence of year in the anthocyanin content of 23 color-fleshed genotypes was reported by Jansen and Flamme (2006). Kotikova et al. (2007) observed that a significant effects of the year of cultivation on total carotenoid content (5.8 mg/kgfw in 2004 and 20.9 mg/kgfw in 2005). This was supported by Rosenthal and Jansky (2008), who observed that antioxidant activity of fresh tubers at all locations was higher in 2006 than 2005.

3.4.2. Effect of storage on potato bioactive compounds

Maintaining a year-long market supply requires storage of potatoes for up to one year before processing or consumption (Herrman et al., 1996). At harvest, potato tubers are dormant and not sprouted, but after a period of storage, tuber dormancy is broken and sprouting occurs. Low storage temperature can inhibit potato sprouting and extend its shelf-life. Sprouting causes biochemical changes leading to changes in nutritional and/or processing qualities of potato tubers (Suttle, 2004). The preservation method led to an accumulation of sugars by a process called "low-temperature sweetening" (Tareke et al., 2002), causing the accumulation of glucose and fructose in potato tubers at low storage temperature (Jansky and Fajardo, 2014) as discussed earlier, it may relate to the formation of AL.

For bioactive compounds, storing potatoes at low temperatures has been reported to induce the generation of potato polyphenols via the phenylpropanoid pathway (Dixon and Paiva, 1995) by activation of the key regulatory enzyme (phenylalanine ammonialyase), responsible for the biosynthesis of anthocyanins (Rhodes and Wooltorton, 1978). Prolonged storage can cause a decrease or no change in the levels of phenolic acids (Kulen et al., 2013). According to Stushnoff et al. (2008), the phenolic content of some genotypes increased up to 100% while other genotypes had little or no change in total phenolic content when the pigmented and non-

pigmented potato tubers were stored at 5ºC for 263 days. The reconditioning method of potato tubers after storage caused a significant increase in the total phenolic acids when eight potato genotypes were stored at different storage temperatures between 4ºC and 20ºC (Blessington et al., 2010).

Storing potatoes at low temperatures led to starch converting to reducing sugars; mainly glucose and fructose (Isherwood, 1976). This can lead to up-regulation of genes coding for dihydroflavonol reductase (DFR) and anthocyanidin synthase (ANS), key regulatory enzymes involved in anthocyanin biosynthesis pathway (Vitrac et al., 2000; Gollop et al., 2002). Lewis et al. (1998) demonstrated that low storage temperature (4ºC) results in increases in anthocyanin concentration of colored tubers, however, there were no changes in anthocyanin content when the tubers were stored at higher temperatures. Storage of six potato genotypes at 4ºC for 135 days in 86% humidity had no effect in the anthocyanin content in another study (Jansen and Flamme, 2006). The effect of storage for 90 days at 4ºC resulted in increased anthocyanin content of purple-fleshed potatoes, Purple Majesty and CO97227-2P/PW cultivars (Madiwale et al., 2012). Potato bioactive compounds vary with cultivar, growth, location, and storage conditions. Reducing sugars are accumulated rapidly when the potato tubers are subject to storage at low temperature, which contributes to activate DFR and ANS enzymes. Storing potato tubers at low temperature elevates the total phenolic content only in purple-fleshed cultivars. However, low storage temperature increases the antioxidant activity of all cultivars.

3.4.3. Effect of processing on potato bioactive compounds

Processing of potatoes can improve taste, nutrition, and quality, but can also lead to changes in its physical and chemical composition (Spanos and Wrolstad, 1990; Dewanto et al., 2002) of potato bioactive compounds. In most cultures, 68% of sold potatoes are consumed in

some processed form. Potatoes are primarily boiled, fried, mashed, chips, or consumed as French fries (Lucier and Ali, 2006). Potato tubers occupy a remarkable position in the American diet, with per capita of 112 lbs annually, with 35 lbs as fresh market and 77 lbs as processed (Osteen, 2012). Thermal processing provides a high level of food safety, however, contributes to degradation of several bioactive compounds and nutritional attributes.

3.4.3.1. Peeling

Peels of color-fleshed potatoes have the highest anthocyanin content (0.65 g/kgfw) when compared to whole tubers and flesh (0.3 g/kg and 0.2 g/kgfw), respectively (Jansen and Flamme, 2006). Peeling causes a significant loss of bioactive compounds such as phenolic acids and anthocyanins (Dao and Friedman, 1992; Lachman et al., 2013), however, peeling also leads to a significant decrease in the total GAs. Therefore, it is a big challenge for food safety and the potato industry to reduce potato GAs, but retain the bioactive compounds.

3.4.3.2. Chipping and frying

Various frying techniques have an impact on bioactive compounds. Fried potatoes had greater levels of phenolic acids (chlorogenic acid, caffeic acid, para-coumaric acid, and vanillic acid) compared to uncooked and boiled potatoes (Blessington et al., 2010), whereas, chipping led to significant losses in phenolic acids content compared to uncooked potatoes (Madiwale et al., 2012). Another study showed that frying resulted in a 76% and 66% loss in chlorogenic acid content and caffeic acid derivatives, respectively, when potato strips were fried in sunflower oil at 190ºC for four minutes (Tudela et al., 2002). One reason for conflicting results could be that the total phenolic content and antioxidant activity are increased due to increasing the extractability of phenolic compounds with frying. The increase in total phenolic content and antioxidant activity was claimed by Blessington et al. (2010) and Navarre et al. (2010) and it

could be due to the presence of antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and tocopherols that are added to commercial cooking oils to prevent rancidity. Blanching potato slices before frying also contributes to the loss of phenolic compounds due to leaching of compounds in water. The process of blanching inactivates oxidative enzymes, thus preventing greater losses (Takenaka et al., 2006).

The anthocyanin content of baked and chipped purple-fleshed potatoes ranged from 13.4 mg to 81.3 mg and from 0.8 to 3.2 mg of cyanidin-3-glucoside equivalents /100 gfw, respectively, in a study by Madiwale et al. (2012). The variation in anthocyanin content between baked and chipped potatoes is due to the processing effects. Chipping resulted in 97% of anthocyanin loss as compared to unprocessed samples (Madiwale et al., 2012). Thermal processing was reported to cause degradation of anthocyanins and enzymes in the presence of polyphenol oxidase (Patras et al., 2010). Anthocyanins had the highest retainability among tested phytochemicals after cooking treatments (boiling > microwave > baked) in all cultivars in comparison with raw potatoes (Lachman et al., 2013).

Frying also led to an increase the extractability of phenolic compounds, which lead to an increase in the total phenolics readings. Phenolic compounds in potatoes are present in free forms and combined with cell-walls complexes. Chipping causes an increase in the surface area of potato tissues in contact with high temperature. This leads to disruption of the cell walls and breakdown of the phenolic compounds. Different frying (time and temperature) and chipping conditions (blanching or without blanching) produce different effects. Therefore, it is difficult to predict the effect of frying and chipping on potato bioactive compounds.

3.4.3.3 Baking

Baking potatoes with skin is considered a good cooking method because it retains most of the nutrients and limits migration of phenolic compounds from the peels into both the cortex and internal tissues of the tuber. Moreover, the peels also act as a barrier against the loss of phenolic acids (Xu et al., 2009). Baking at 170°C for 45 minutes resulted in a loss of chlorogenic acid in baked Atlantic and Yukon Gold potatoes (Madiwale et al., 2012), but total phenolic acid content was still significantly higher in baked samples compared to boiled and uncooked samples. Baking potatoes for 45 minutes at 212ºC resulted in a 100% loss in the chlorogenic acid content, which suggests the susceptibility of chlorogenic acid to heat (Dao and Friedman, 1992). Im et al. (2008) reported that chlorogenic acids content ranged from 3.3 mg/100 gfw to 637 mg/100gfw for Keneber and Purple Peruvian potatoes, respectively. Conflicting results between previous observations could be related to different processing conditions and methods of determination. Im et al. (2008) and Madiwale et al. (2012) wrapped their potatoes in aluminum foil, which contributes to retention of most of the chlorogenic acid content. Dao and Friedman (1992) assayed their samples using ultraviolet spectrometry whereas Im et al. (2008) employed advanced LC-MS/MS method. LC-MS/MS technique is more sensitive and can separate a very wide of organic compounds and used to confidently identify and quantify compounds if two compounds have similar UV spectra while ultraviolet spectrometry technique measures the absorption in the ultraviolet-visible region. Baking contributed to an increase in total phenolic content and antioxidant activity of eight potato genotypes (Blessington et al., 2010). Baking time is also critical for retention of potato bioactive compounds. Thirty minutes baking increased the total phenolic and chlorogenic acid content; however baking for 45 minutes reduced the total phenolic content (Navarre et al., 2010). The effect of baking on total phenolic content is also

genotype-dependent. Xu et al. (2009) reported that the total phenolic acid was significantly altered by baking for the Dakota Pearl cultivar, but not for the Nordonna cultivar. As the combined effect of temperature and time is genotype-dependent; therefore, it is a critical to study the effect of baking for each cultivar to reach the optimal baking time and temperature.

3.4.3.4. Boiling

Boiling results in either reduced, retained, or enhance the total phenolic content and antioxidant activity of potato genotypes when compared to uncooked samples. Dao and Friedman (1992) reported that boiling potatoes in water for 30 minutes resulted in a 60% loss of chlorogenic acid content. Im et al. (2008) observed that the loss of chlorogenic acid and its isomer were dependent on the salt concentration in the water. One percent salt contributed to 20% to 40% losses in chlorogenic acid content whereas 3% salt caused 40% and 70% loss in chlorogenic acid and its isomer, respectively. Chlorogenic acid loss is greatest with boiling in 3% salt, it it because the chlorogenic acid is leaching into the water. Boiling potatoes for a short time (20 minutes) did not reduce the phenolic acid content, but significantly decresed the anthocyanin content of color-fleshed potatoes (Mulinacci et al., 2008). Conflicting evidence was reported by Navarre et al. (2010), who found that boiling potatoes for 18 minutes resulted in an increase in the total phenolic content and chlorogenic acid content in white- and purple-fleshed potato genotypes. Chlorogenic acid contributes to the 95% of total phenolic acids in potato tubers. Kan et al. (2014) demonstrated that chlorogenic acid was isomerized to chlorogenic acid isomers (4-O- caffeoylquinic acid and 5-O- caffeoylquinic acid) after boiling. These isomers exhibited similar antioxidant activity measured by the DPPH (2,2-diphenyl-1-pikryl-hydrazyl) radical scavenging assay. It is important to note that different potato bioactive compounds have different behaviour during cooking. Therefore, the final effect of cooking on potato bioactive compounds

levels depends on the processing factors, the chemical nature of the compounds, and the structure of food matrix (Palermo et al., 2014).

3.4.3.5. Microwaving

Conflicting results similar to baking and frying effects were observed for microwaving. Blessington et al. (2010) observed an increase in the total phenolic content and antioxidant activity of eight potato genotypes post-microwaving. Potato samples had greater levels of chlorogenic acid, caffeic acid, para-coumaric acid, vanillic acid, and epicatechin. The same result was reported by Navarre et al. (2010), who found that microwaving increased the total phenolic content and chlorogenic acid content in white- and purple-fleshed potato genotypes. Dao and Friedman (1992) reported that microwaving resulted in a 45% loss in the chlorogenic acid content of potato. Decreases of 40% and 20% were observed in chlorogenic acid content and its isomer, respectively, in microwaved potatoes (Im et al., 2008).

CHAPTER THREE: EFFECT OF GENOTYPE AND STORAGE ON GLYCOALKALOID AND ACRYLAMIDE CONTENT AND SENSORY ATTRIBUTES OF POTATO CHIPS

Overview:

Potato chips are the most popular consumed snack food in Western countries. Potato chips contain beneficial bioactive compounds such as resistant starch, polyphenols, along with toxicants, naturally occurring glycoalkaloids (GA) and processing induced acrylamide (AL). Information on the effect of farm to fork operations on both GA and AL are limited. In this study the effect of cultivar and storage on both GA and AL content in potato chips were evaluated using four potato cultivars. In addition, reducing sugars and sensory attributes were measured in response to storage time and cultivar. Four potato cultivars: Atlantic, Yukon Gold, Purple Majesty, and CO97227-2P/PW were stored at 4°C for 90 days. Potato chips made from fresh and stored tubers were analyzed for total GA and AL using High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD) and Ultra Performance Liquid Chromatography (UPLC-DAD), respectively. Raw potatoes were analyzed for reducing sugars using a spectrophotometer. Sensory attributes of potato chips were assessed using 114 untrained panelists. The effect of storage on GA and AL content is cultivar dependent. Storage of potatoes at low temperature (4°C) resulted in a significant increase in GA and AL and reducing sugar content after 90 days. Positive correlations were observed for the overall acceptability, texture, taste, ranking, and GA and AL content, emphasizing the positive role of GA and AL on sensory qualities. These results indicate that an increase in GA and AL content with storage is dependent on cultivar thus, it is critical to measure GA and AL content not only in the fresh tubers but also

in the final potato products such as baked and chipped potatoes. Also within the acceptable range, GA and AL content positively correlated with sensory attributes of fresh and stored potato chips and increased consumer preference. Thus, it is critical to develop a food systems approach to lower GA and AL contents, while retaining sensory attributes in the final potato products.

1. Introduction

The potato (*Solanum tuberosum* L.) is one of the most widely consumed food crops around the world and is the world's 4th largest crop after rice, wheat, and maize (Burlingame et al., 2009) in production. Potato is the leading vegetable crop in the United States with per capita consumption of about 112 lbs annually including 35 lbs fresh market (Osteen, 2012) and 77 lbs processed potatoes (Lucier and Ali, 2006). Potato chips are the most popular consumed snack food in western countries and fried potato products and potato chips contribute to 38% of acrylamide (AL) in the American diet (Friedman and Levin, 2008). Potatoes are considered a good source of carbohydrates, vitamins, and minerals. Moreover, color-fleshed potatoes are also rich in beneficial bioactive compounds such as anthocyanins, polyphenols, and carotenoids (Liu, 2004; Madiwale et al., 2011). Consumption of colored potatoes increased by 17% while traditional potatoes decreased during the last 10 years (United States Potato Board, 2007). However, potatoes are also known to contain some undesirable compounds which are either naturally occurring such as GA (Friedman, 2006) or produced during processing of potatoes at high temperature like AL. Due to growing interest and awareness on health recent, efforts are focusing on reducing both GA and AL in potatoes.

Potato GAs have been reported to have anti-cancer and anti-inflammatory properties (Friedman, 2006) at low concentrations, but are toxic at higher concentrations. Potato GAs includes the more toxic α-chaconine and the less toxic α-solanine, which together form

approximately 95% of the total GA content. GA produce two toxic effects:1) disruption of phospholipid membranes and 2) the inhibition of both acetylcholinesterase and butyrylcholinesterase enzymes in the central and peripheral nerve system, which contribute to nerve impulses transmission (Krasowski et al., 1997). Potato GAs can be lethal when consumed in doses of 3-6 mg/kg of body weight (Morris and Petermann, 1985). According to Lachman et al. (2013), the content and the distribution of GA in the tuber were dependent on the potato cultivar. In Blaue St. Galler cultivar, 57% of original levels of GAs remain after peeling, whereas in the Agria cultivar only 5% of GAs remain (Lachman et al., 2013). Freshly harvested commercial potato cultivars contain GA below the acceptable limit of 200 mg/kgfw (Knuthsen et al., 2009), but potatoes can be stored at low temperature for up to one year before being processed and consumed in order to maintain product supply during the year. Harvested potatoes are stored at low temperatures to inhibit sprouting and maintain the quality of potato tubers. Storage conditions, sprouting, mechanical injury, fungal attack, and exposure to light can elevate the levels of GA above the acceptable safe limit (Friedman and McDonald, 1997). Exposure of potato tubers to fluorescent light resulted in greater increase in GA compared to indirect sunlight and storage in darkness under room and refrigeration temperatures (Machado et al., 2007). Storage at 3 ± 1°C resulted in more than two-fold increase in the content of GA compared to same cultivars stored at 10°C (Griffiths et al., 1998; Haase, 2010). Storage potato tubers at 34°C had no significant effect on GA levels (Petersson et al., 2013). GA content of the potatoes is also affected by storage period. Dao and Friedman (1992) reported that storing potatoes for three or sixteen days at room temperature resulted in an increase in potato GA by 62% and 300%, respectively. GA is an important component of potato flavor. Potatoes with GA levels greater than 140 mg/kgfw have a bitter taste while small quantities of GA below that level, improve

processed potato taste (Sinden et al., 1976). Processing such as baking, chipping, and frying can increase GA levels above the regulated safety limit (Iablokov et al., 2010).

Processing of potatoes such as frying and baking also generate AL which is formed from the reaction of reducing sugars (free glucose, fructose, hydrolyzed sucrose, and hydrolyzed starch during potato storage) with amino acid asparagine via the Maillard reaction, which occurs during processing temperatures above 120°C (Pelucchi et al., 2011). AL is considered a neurotoxicant and carcinogen in animal models (Friedman et al., 2008). EPA safety level for acrylamide is 0.002 mg/kg of body weight/day (United States Environmental Protection Agency, 1992). Potato products such as French fries and potato chips contain high levels of AL ranging from 424 to 1739 µg/kg. This is due to the presence of high amounts of amino acid asparagine 94 mg/100g (Martin et al., 2001) and reducing sugars 0.1 to 3 g/kg which may increase up to 20 g/kg potato in tubers stored at low temperatures (Amrein et al., 2004). The amino acid asparagine and reducing sugars are the precursors for AL formation, but the latter is considered as a critical component in the Maillard reaction due to reducing sugars is source of carbonyl group, which is required for AL formation via the Maillard reaction (Amrein et al., 2004).

Concentration of reducing sugars in raw potato is dependent on the cultivar and storage condition especially time and temperature (Al Viklund et al., 2008). Storage at low temperatures (4°C) enhances AL formation resulting from starch-sugar conversion in the tuber (Gamble et al., 1987). In addition to variation in asparagine and reducing sugars, AL content is also affected by processing conditions (e.g., frying time and temperature), cooking methods, moisture level, pH, the presence of additives, and storage conditions. Kita et al. (2004) and Pedreschi et al. (2005) reported that reducing frying temperatures from 190°C to 170°C and from 185°C to 160°C resulted in 60% and 75% reduction in AL formation in potato chips, respectively. Frying time

also affects AL formation, for example AL content was increased from 300 ppb to 1200 ppb when the bread baking time was increased from 18 to 32 minutes at 270°C (Surdyk et al., 2004). Thus, an opportunity exists to select cultivars and to optimize post-harvest storage and processing conditions to mitigate the GA and AL content. There is limited information available on how cultivar and storage affect both GA and AL particularly in color-fleshed potatoes. In this study we evaluated the GA and AL content in potato chips in purple- white- and yellow-fleshed potato cultivars/selections after storage in order to identify suitable cultivars and storage conditions that minimize these compounds while retaining sensory attributes.

2. Materials and methods

2.1. Chemicals

Methanol was supplied by EMD chemicals (Philadelphia, PA, USA). Monobasic ammonium phosphate was procured from Avantor Performance Materials (Phillipsburg, NJ, USA). Carrez I, II solutions, acetonitrile, acetone, and chloroform were purchased from Fisher Scientific (Fenton, MO, USA). α-solanine and α-chaconine standards were obtained from Indofine (Hillsborough, NJ, USA). Acrylamide, glucose reagent, phosphoglucoisomerase, glucose, and fructose standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol for reducing sugars extraction was purchased from the chemistry-stockroom at Colorado State University (Fort Collins, CO, USA).

2.2. Potatoes

Four commercial cultivars Atlantic (white-fleshed), Purple Majesty (purple-fleshed), Yukon Gold (yellow-fleshed), and the advanced selection CO97227-2P/PW were used for this study. The letters (P/PW) after the advanced selection indicates skin/flesh color: P, purple and PW, purple with white zones. Atlantic and Yukon Gold were used as standards for chipping and

baking potatoes, respectively. All four cultivars were grown at the San Luis Valley Research Center (latitude 37° 43'N and longitude 106° 9'W), Colorado State University, CO, USA. Potatoes were grown in Dunul cobbly sandy loam soil with 0.9% - 1.0 % organic matter and 7.5-8.0 pH for 100-110 days, starting from May until September. Total fertilizer applied during the growing season included 54 kg N, 27 kg P2O5, 18 kg K2O, and 1.1 Kg Zn/A. Gross application of 43 cm of irrigation was performed using a center pivot. Three weeks prior to harvesting, the potato plants were treated with sulfuric acid to kill vines. After harvest, the potatoes were initially cured at 16 ± 1°C for three weeks to allow sugar starch conversion and this is considered "0 days". Each potato cultivar was placed in numbered bags weighting 4.5 kg (10 lbs) each for different storage (4°C) intervals (0, 30, or 90 days of storage). Humidity was maintained at 85 - 90% during storage and Tuber weight was recorded at monthly intervals for moisture loss.

2.3. Chipping of the potatoes

Potatoes were chipped after curing at days 0 and after reconditioning at 30 or 90 days of storage. The raw potato tubers were cleaned under running tap water and then sliced into 1/16 sections. The raw potato slices were washed under running warm water for one minute to remove any water-soluble sugars present on the surface, were placed in strainer trays to remove excess water and fried in Bakers & Chefs™ Clear Frying Oil at 185°C until bubbling slowed (one minute 45 s to two minutes 15 s depending on tuber specific gravity). The fried chips were placed on paper towels to absorb excess oil and then allowed to cool for 10-15 minutes. The chips were labeled, bagged, and stored in the dark at either -20°C for further analysis or at 4°C for sensory evaluation.

2.4. Acrylamide extraction and quantification

The AL content of chipped potato samples was measured for four potato cultivars at 0, 30, or 90 days of storage using Ultra-performance Liquid Chromatography (UPLC). The acrylamide was extracted using the method established by Gokmen et al. (2005). A representative sample of ground chips (five gram) in three replicates for each cultivar was suspended in 10 mL methanol. The homogenized (IKA.T 25 digital ultra-turrax, IKA, Germany) samples were centrifuged at 10,000 rpm (11,180 xg) at 10°C for 10 minutes using an ultracentrifuge (Beckman J2-HS centrifuge, Beckman Coulter, CA, USA) and the supernatant was mixed with 100 µL of each Carrez-I and II solutions to precipitate the colloids. The suspension was centrifuged at 10,000 rpm (11,180 x g) at 10°C for five minutes and the supernatant was completely dried in glass test tubes by using an Analytical Nitrogen Evaporator (Berlin, MA, USA) set at 40°C. The residues were then re-dissolved in two mL of distilled water by vortexing for two minutes. The extract (one mL) was filtered through a 0.22 µm syringe filter and stored at -20°C for analysis. The final test solution (five µL) was injected into UPLC column for quantification.

AL quantification was performed by a Waters Acquity UPLC system equipped with a bio-Sample manager and bio-quaternary solvent manager with a diode array detector (DAD). The chromatographic separations were performed on BEH Shield RP18 1.7 µm 2.1 x 100 mm column (Waters, Ireland, USA). The injection volume was five µL. The mobile phase was filtered water adjusted to pH 9.5 by ammonium hydroxide (solvent A) and 100% acetonitrile (solvent B). The flow rate was adjusted to 0.5 mL per minute at room temperature. The initial solvent gradient was 98% of solvent A and 2% solvent B. Then, solvent B increased from 2% to 100% within 4.5 minutes. Finally, the solvent gradient was returned to 98% of solvent A and 2%

of solvent B for 4.5 minutes. AL was detected at 226 nm with continuous monitoring of the peak spectra between 190-350 nm and concentrations were calculated using standard acrylamide.

2.5. Glycoalkaloids extraction and quantification

GAs were extracted as previously described by Rodriguez-Saona et al. (1999). A five gram sample of potato chips was homogenized with 10 mL of acetone in three replicates. The homogenized sample was centrifuged at 1300 xg for 15 minutes using ultracentrifuge (Beckman J2-HS centrifuge, Beckman Coulter, CA, USA) and the clear supernatant was collected. The residue was re-extracted with 10 mL of 30% aqueous acetone and the supernatant was combined with the first extract. Two volumes of chloroform for each volume of acetone extract were added and stored overnight at 4°C after mixing. The upper aqueous portion was collected into glass vials and concentrated in Analytical Nitrogen Evaporate at 40°C until all residual acetone was evaporated. One mL of distilled water was added to the dried extract and filtered through a 0.45 µm syringe filter and stored at -20°C for analysis. Twenty µL of the final test solution were injected into HPLC column for quantification.

GAs were analyzed using HPLC following the method of Sotelo and Serrano (2000). The HPLC system (SPD-M10AVP, Shimadzu) equipped with an Atlantis dC18 (4.6 x 250 mm, 5 µm) column was used for glycoalkaloids separation. Acetonitrile with 0.05 M monobasic ammonium phosphate buffer (35:65 v/v) adjusted to pH 6.5 with ammonium hydroxide was used as mobile phase with one mL per minute flow rate. DAD detector was set at 200 nm. Calculations were based on the standard curves. GAs and AL was correlated to sensory data obtained by Madiwale et al. (2012).

2.6. Statistical analysis

The effects of genotype and storage on GA, AL, and reducing sugar content were determined by analysis of variance (ANOVA) using the SAS, Statistical Analysis System, v.9.3. (SAS Institute Inc., Cary, NC); general linear model (GLM) procedure. Fisher's protected t-test using the Least Square Means (LSD) test was used for comparing group differences with $p \leq 0.05$ being considered as statistically significant for GA, AL, sensory attributes, and reducing sugars. Pearson correlation coefficients were calculated using SAS. All results have been expressed as mean ± standard error.

3. Results and discussion

3.1. Effect of genotype and storage on acrylamide and glycoalkloid levels

Previous reports focusing on either acrylamide (AL) or glycoalkaloids (GA) suggest that several factors including, genotype, storage, and processing affect the AL and/or GA content in potato chips (Friedman and McDonald, 1997; Griffiths et al., 1998; Becalski et al., 2003; De Wilde et al., 2005; Matsuura-Endo et al., 2006; Mader et al., 2009). However, no information is available on how genotype and storage conditions alter both AL and GA levels in fried potato products. Potato chips are one of the primary consumed snack foods in the Western countries and contain both AL and GA. Results from this study reveal that the storage effect on AL and GA is genotype dependent, which is in agreement with previous reports on AL or GA levels by Bejarano et al. (2000) and Kumar et al. (2004). AL content of chips at 0 days of storage (tubers were preconditioned for three weeks before chipping) ranged from 135 ± 3 ppb for Yukon Gold to 463 ± 11 ppb for Atlantic. At 30 days of storage, the AL content increased from 135 ± 3 ppb to 399 ± 24 ppb for Yukon Gold. The AL content of chips from Atlantic, CO97227-2P/PW and Purple Majesty were not affected by 30 days of storage. However, after 90 days of storage, the

AL levels for Atlantic, CO97227-2P/PW and Purple Majesty increased significantly from 484 ± 0.8 ppb to 972 ± 68 ppb; 388 ± 38 ppb to 2,177 ± 89 ppb and 418 ± 27 ppb to 1,533 ± 101 ppb, respectively, showing a 2 - to 4-fold increase in AL content (Figure 3.1) compared to chips from fresh and 30 day stored potatoes. This is in accordance with previous studies that AL content of the Atlantic potato cultivar ranged from 193 ± 31 ppb to 1,123 ± 13 ppb fried at 150°C and 180°C, respectively (Granda et al., 2004). Storage for 90 days had minimal effect on AL content of Yukon Gold potato chips compared to 30 day storage. Yukon Gold had the lowest AL content and CO97227-2P/PW had the highest AL content among the four cultivars at 90 days of storage.

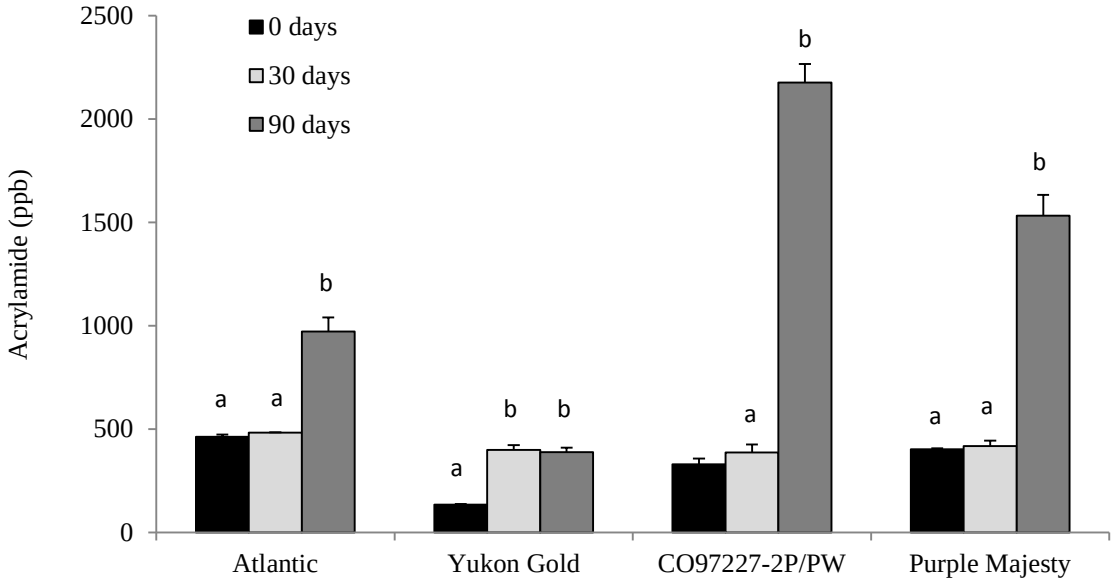

Figure 3.1. Effect of genotype and storage on acrylamide levels (ppb) in potato chips. 0, 30, or 90 indicate days of storage. Acrylamide content was determined using UPLC-DAD as described in the materials, and methods. Values with different letters (w, x, y and z) indicate differences (Least Square Means test; $p \leq 0.05$) among storage times for a particular cultivar/selection. Results are presented as the mean ± SE of three biological replicates and two technical replicates for each biological replicates. Each value is a mean of six determinations.

Storage potato tubers at low-temperature also causes conversion of starch to sugars and reducing sugars are reported to be the precursors of AL (Martin et al., 2001; Amrein et al., 2004;

Matsuura-Endo et al., 2006). The levels of reducing sugars (glucose and fructose) increased in all cultivars with storage, except in tubers from Yukon Gold that had lower glucose and fructose content (Table 3.1). Even though, Yukon Gold is not preferred for chipping these results point to the possibility of developing cultivars which are suitable for prolonged storage. Purple Majesty had the highest glucose (1587 ± 35 mg/kgfw) and fructose (706 ± 38 mg/kgfw) concentrations at day 0. Yukon Gold had the lowest glucose (421 ± 14 mg/kgfw) and fructose concentrations (504 ± 10 mg/kgfw) at the same period of storage (Table 3.1). For Atlantic cultivar, glucose and fructose concentrations increased from 858 ± 34 mg/kgfw and 524 ± 23 mg/kgfw to 1,292 ± 72 mg/kgfw and 842 ± 26 mg/kgfw after 90 days of storage, respectively. For CO97227-2P/PW, glucose and fructose concentrations increased from 589 ± 23 mg/kg and 569 ± 17 mg/kgfw ($p \leq 0.05$) to 2208 ± 50 mg/kgfw and 1,413 ± 42 mg/kgfw after 90 days of storage, respectively. Reducing sugars followed the similar trend as that of AL. Ohara-Takada et al. (2005) also investigated the effect of low-temperature storage on potato tubers and the potential for AL formation. They reported even short-term cold storage increased sugar accumulation in the tubers and the potential for AL formation. It is essential to select color-fleshed genotypes that are resistant to storage-induced accumulation of reducing sugars as red and purple-fleshed potato chips are becoming popular. Some of the processing steps can also alter AL content of chips. In this study rinsing potato slices before frying to mimic industry standard procedures might have caused reduction sugars. Thus, it is also critical to optimize storage and processing conditions to reduce sugar accumulation and potential AL formation. The safe limit for GA in freshly harvested commercial potato cultivars is 200 mg/kg (Knuthsen et al., 2009).

The predominant GA in potato is α-chaconine and α-solanine. GA (both α-solanine and α-chaconine) followed a similar trend as that of AL with respect to cultivar and storage (Figure

3.2. a - d). The lowest concentration of GA was observed in Yukon Gold potato chips at 0 days of storage (27 mg/kg) and the highest was recorded for Purple Majesty potato chips (78 mg/kg) among the four cultivars studied. This cultivar-dependent increase is in agreement with previous studies (Griffiths et al., 1998; Morris et al., 2010).

Table 3.1. Reducing sugar levels in four raw potato cultivars at 0 and 90 days of storage.

Cultivar/Selection	Glucose (mg / kg)		Fructose (mg / kg)	
	0 days	90 days	0 days	90 days
Atlantic	858 ± 34[b]	1,292 ± 72[b]	524 ± 23[a]	842 ± 26[b]
Yukon Gold	421 ± 14 [d]	N.D	504 ± 10[a]	N.D
CO97227-2P/PW	589 ± 23 [c]	2,208 ± 50[a]	569 ± 17[a]	1,413 ± 42[a]
Purple Majesty	1,587 ± 35[a]	2,333 ± 18[a]	706 ± 38[a]	1,148 ± 31[a]

N.D = Not detected

Atlantic, a common chipping cultivar accumulated two times more GA within 90 days of storage as GA content increased from 60 to 105 mg/kg chips (Figure 3.2.a). Previous studies reported that storage at 4°C without sprouting inhibitors increased the GA content of potato tubers during the first nine weeks of storage (Griffiths et al., 1998; Haase, 2010). GA content in chips from Yukon Gold potatoes was not affected by storage (Figure 3.2.b). Thus, it is possible to select cultivars that are resistant to increases in GA and AL due to storage.

Purple-fleshed cultivars, Purple Majesty and CO97227-2P/PW, followed a similar trend as that of Atlantic for GA. GA content increased ($p \leq 0.05$) in both the cultivars after 90 days of storage. GA content in Purple Majesty increased from 78 mg/kg to 101 mg/kg potato chips after 30 days of storage (Figure 3.2.d). GA content of CO97227-2P/PW potato chips showed a 77% increase after 90 days of storage. Storing for 30 days did not have any significant effect on GA content of CO97227-2P/PW (Figure 3.2.c), similar to that of AL. GAs in potatoes are affected by

the factors that enhance the sprouting such as light and temperature contributing to higher levels of GA (Haase, 2010).

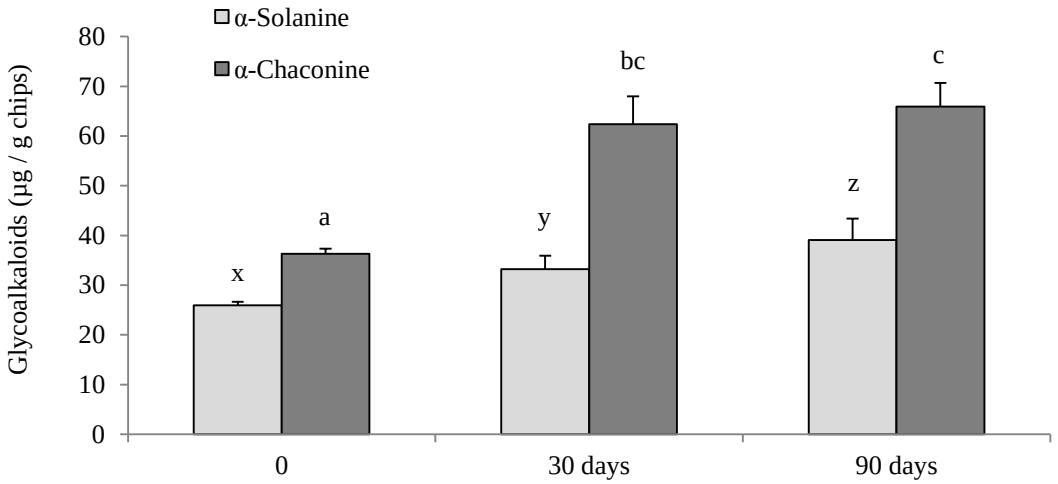

Figure 3.2.a. Effect of genotype and storage on total glycoalkaloid levels (α-solanine, and α- chaconine) (µg/g) in potato chips from Atlantic. 0, 30, or 90 indicate days of storage. Glycoalkaloid content was determined using HPLC-DAD as described in materials, and methods. Values with different letters indicate differences ($p \leq 0.05$) in α-chaconine (a, b, c) and α-solanine (x, y, z) content among different storage times. Results are presented as the mean ± SE of three biological replicates and two technical replicates for each biological replicate.

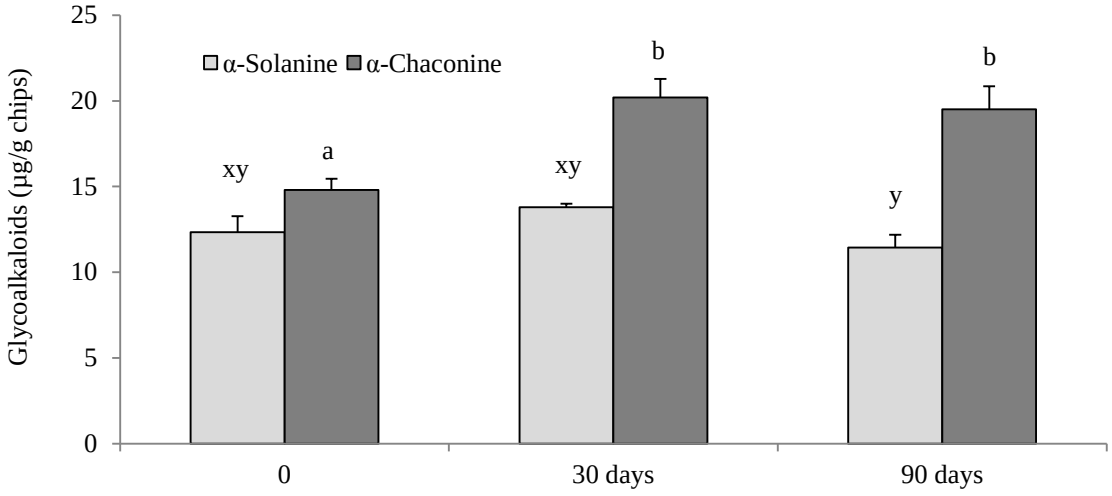

Figure 3.2.b. Effect of genotype and storage on total glycoalkaloid levels (α- solanine, and α- chaconine) µg/g) in potato chips from Yukon Gold. 0, 30, or 90 indicate days of storage. Glycoalkaloid content was determined using HPLC-DAD as described in materials, and methods. Values with different letters indicate differences ($p \leq 0.05$) in α-chaconine (a, b, c) and α-solanine (x, y, z) content among different storage times. Results are presented as the mean ± SE of three biological replicates and two technical replicates for each biological replicate.

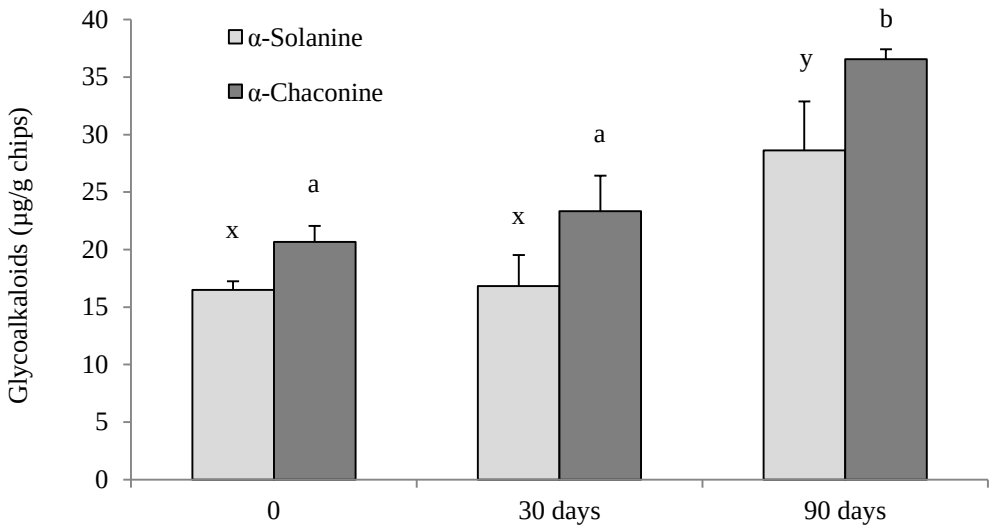

Figure 3.2.c. Effect of genotype and storage on total glycoalkaloid levels (α-solanine, and α-chaconine) (µg/g) in potato chips from CO97227-2P/PW. 0, 30, or 90 indicate days of storage. Glycoalkaloid content was determined using HPLC-DAD as described in materials, and methods. Values with different letters indicate differences ($p \leq 0.05$) in α-chaconine (a, b, c) and α-solanine (x, y, z) content among different storage times. Results are presented as the mean ± SE of three biological replicates and two technical replicates for each biological replicate.

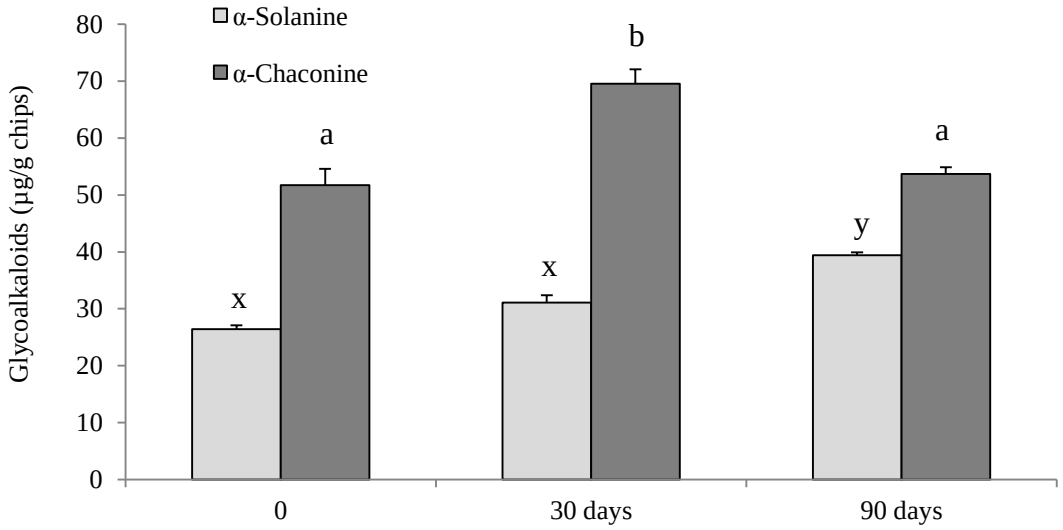

Figure 3.2.d. Effect of genotype and storage on total glycoalkaloid levels (α-solanine, and α-chaconine) (µg/g) in potato chips from Purple Majesty. 0, 30, or 90 indicate days of storage. Glycoalkaloid content was determined using HPLC-DAD as described in materials, and methods. Values with different letters indicate differences ($p \leq 0.05$) in α-chaconine (a, b, c) and α-solanine (x, y, z) content among different storage times. Results are presented as the mean ± SE of three biological replicates and two technical replicates for each biological replicate.

3.2. Sensory evaluation

Sensory analysis of tuber samples from Atlantic, Yukon Gold, Purple Majesty, and CO97227-2P/PW was carried out for chip samples after 30 and 90 days of storage by 114 untrained panelists (Madiwale, 2012). Five attributes namely appearance, taste, color, texture, and overall acceptability were assessed and scored on a 9-point hedonic scale (1 = disliked extremely; 9 = liked extremely). At the end, panelists were asked to rank the samples based on their preference (1 = liked most; 7 = liked least). Sensory evaluations were performed to understand the effect of cultivar and storage and to compare the acceptance of purple-fleshed potatoes over traditional white- and yellow-fleshed cultivars.

Previous researchers reported the effect of chemical and biological treatments used to reduce AL on sensory attributes (Jung et al., 2003; Gokmen and Senyuva, 2007b; Pedreschi et al., 2008; Capuano and Fogliano, 2011; Kalita and Jayanty, 2013), but limited information is available on the effect of cultivar and storage on the sensory attributes of potato chips. Atlantic, a popular chipping cultivar, was used as the standard for potato chips. According to the panelists all cultivars except CO97227-2P/PW received a mean score between 6 and 8 for all the sensory parameters. Atlantic chips received a mean score of 7.2 and 7.0 for overall acceptability after 30 and 90 days of storage, respectively. Atlantic chips received a rank mean score of 2.6 after 30 days of storage and 2.8 after 90 days of storage. Atlantic was ranked as being "liked most" among potato cultivars tested. Yukon Gold potato chips were ranked slightly lower than the Atlantic at day 30 (3.7), while Purple Majesty received a similar mean rank as that of Atlantic after 90 days of storage (2.9).

Sensory attributes were influenced by the cultivar ($p \leq 0.05$). Atlantic chips received highest mean scores for appearance, color, taste, and texture compared to the other three

cultivars. Yukon Gold potato chips received the highest mean score of 7.4 for texture after 30 days of storage. Atlantic potato chips received a mean texture score of 7.6 after 90 days of storage. After Atlantic, Purple Majesty chips were preferred for taste, texture, and overall acceptability after 90 days of storage. Thus, some purple-fleshed cultivars were comparable with traditional cultivars in terms of their sensory scores. Storage for 90 days improved the mean sensory scores for purple-fleshed cultivars and had minimal effect on the sensory parameters of Atlantic and Yukon Gold (Madiwale, 2012).

Eighty four percent (n = 94) of the panelists responded that they would prefer color-fleshed 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 for color-fleshed potato products, 55% of panelists said yes (n = 61) while 45% (n = 50) said no. On average panelists were willing to pay an extra $0.83 for a 10.5 - ounce bag of chips costing $3.20 if replaced with color-fleshed potatoes (Madiwale, 2012). These results indicate that it is critical to develop a food systems approach that minimizes GA and AL content not only in the fresh produce but also in the stored and processed potato products while retaining sensory attributes.

3.3. Correlations

3.3.1. Acrylamide, glycoalkaloids, and reducing sugars

Earlier researchers showed significant positive correlations between reducing sugars and AL (De Wilde, 2005; Zhu et al., 2010). Results from this study followed a similar trend where AL content in potato chips was correlated positively with glucose and fructose content in raw potato tubers ($r = 0.85$; $p \leq 0.05$ and $r = 0.94$; $p \leq 0.05$, respectively). A positive correlation was observed between α-solanine and glucose ($r = 0.59$; $p \leq 0.05$). Morris et al. (2010) also showed a positive correlation between α-solanine content and the sweetness of potatoes. Thus, higher

glucose not only acts as a precursor for greater AL in chips, but also positively correlates ($p \leq 0.05$) with α-solanine levels.

3.3.2. Acrylamide and sensory attributes

Sensory analysis revealed significant changes in sensory parameters depending on storage and cultivar. As GA and AL levels were influenced by storage and cultivar, we wanted to determine whether significant correlations exist between GA and AL content and sensory parameters. Pearson correlation coefficients were calculated for all sensory attributes (rank, appearance, color, taste, texture, and overall acceptability) and AL content after 30 and 90 days of storage (Table 3.2). At day 30, positive correlations were observed for the appearance, color, taste, texture, and overall acceptability and AL content (r = 0.58, 0.57, 0.64, 0.57 and 0.63, respectively). A negative correlation was observed between the rank and AL content in potato chips (r = - 0.66) as shown in Table 3.2. This suggests that AL content in acceptable limits improves sensory attributes and overall preference. All correlations were significant at $p \leq 0.05$. This may be because of formation of reducing sugars during reconditioning of potatoes after storage influences the color of the final product. After 90 days of storage, rank was positively correlated with AL level suggesting higher preference of potato chips (r = 0.82; $p \leq 0.05$). After 90 days of storage, positive correlations between the taste, texture, and overall acceptability and AL content were observed (r = 0.76, 0.69 and 0.71; at $p \leq 0.05$, respectively; Table 3.2).

3.3.3. Glycoalkaloids and sensory attributes

After 30 days of storage, the ranking was positively correlated with GA content (r = 0.65; $p = 0.022$) suggesting higher preference with higher GA content (1 = liked most; 7 = liked least). GA content contributes to the desirable taste in the potato chips (14-15 mg/100g; Osman, 1983). After 90 days of storage, positive correlations were observed between taste, texture, overall

acceptability, ranking and GA content (r = 0.95, 0.94, 0.93, and 0.82, respectively, at $p \leq 0.05$) as shown in Table 3.2. In this study Atlantic and Purple Majesty cultivars had the highest preferences with the ranking 5.08 and 5.03, respectively, and had the highest GA content at 90 days (105.05 µg/g, and 93.12 µg/g, respectively) of storage. These results suggest that focusing breeding efforts exclusively on sensory attributes might result in development of cultivars with higher content of toxic GA and AL. It is important to focus on reducing toxic compounds and improving health benefits along with consumer preference.

Table 3.2. Correlation coefficients between reducing sugars, and sensory attributes with acrylamide and total glycoalkaloids after 30 and 90 days of storage at 4°C.

Reducing Sugars /	30 days storage		90 days of storage	
Sensory Parameters	Acrylamide	Total Glycoalkaloids	Acrylamide	Total Glycoalkaloids
Glucose	----	----	0.85**	0.53*
Fructose	----	----	0.94**	0.37
Appearance	0.58**	-0.33	-0.19	0.24
Color	0.57*	-0.42	0.40	0.17
Taste	0.64**	0.12	0.76*	0.95**
Texture	0.57**	-0.53*	0.69**	0.94**
Overall Acceptability	0.63**	-0.04	0.71**	0.93**
Rank	0.66**	-0.65*	0.82**	0.82**

*Correlation is significant at $p \leq 0.05$
** Correlation is significant at $p \leq 0.01$

4. Conclusions

In this study, we determined the effect of storage and genotype on GA and AL formation in potato chips. Our results showed that storage had no effect on GA and AL in Yukon Gold potato chips even after 90 days of storage, but elevated the content of these toxic compounds in Purple Majesty, CO97227-2P/PW, and Atlantic potato chips. GA and AL content of potato chips correlated positively with the overall acceptability and negatively with the ranking of panelist' preferences at 90 days of storage (lower ranking suggested greater consumer preference). Reducing sugars of raw potato tubers of Atlantic, CO97227-2P/PW, and Purple Majesty

positively correlated with AL content of potato chips. These results indicate that within the acceptable range, GA and AL content positively correlate with consumer preference. Three months of storage elevated GA and AL content in most of the cultivars tested. Mostly, potatoes are stored up to 12 months before the products reach the consumers. Thus, it is critical to develop a food systems approach that establishes the farm to fork operation to minimize the GA and AL content and retain sensory attributes of potato chips.

CHAPTER FOUR: EFFECT OF GENOTYPE, STORAGE, AND PROCESSING ON ACRYLAMIDE AND REDUCING SUGARS CONTENT, AND SENSORY ATTRIBUTES OF POTATO CHIPS AND BAKED POTATOES

Overview:

Potatoes are a popular crop worldwide and a good source of micro- and macro-nutrients, and dietary fiber, along with health benefiting compounds such as polyphenols. Potato products are known to contain processing induced acrylamide. The information on the effect of cultivar, storage, and processing on acrylamide (AL) is limited. In this study, we investigated the effect of genotype, storage (either 4°C or 10°C for three, or six months) and processing (baking and chipping) on AL and reducing sugars content and correlated with sensory attributes of potato chips and baked potatoes using ten different potato genotypes. Five color-fleshed cultivars: Purple Majesty, All Red, All Blue, Mountain Rose, advanced selection CO97227-2P/PW, and five white-fleshed cultivars: Lenape, Rio Grande Russet, Russet Burbank, Atlantic, and AC99375-1RU were used for this study. Potato chips and baked potatoes made from fresh and stored tubers were analyzed for AL using Ultra Performance Liquid Chromatography-Diode Array Detector (UPLC-DAD). Raw potatoes were analyzed for reducing sugars using a spectrophotometer. Sensory attributes of baked potatoes and potato chips made from potato tubers stored for three months at 4°C or 10°C were assessed using 105 untrained consumer panelists and scored on a 9-point hedonic scale "1 = disliked extremely and 9 = liked extremely" and ranked on a scale of "1 = liked most to 7 = liked least". Concentration of reducing sugars in raw potato and acrylamide in potato chips and baked potatoes was dependent on the cultivar/ selection and storage condition. Storage of potato tubers at 4°C resulted in an increase of

reducing sugars in raw potato tubers, resulting in a higher level of AL in potato chips compared to un-stored potatoes. The interaction effect of storage temperature and storage time on AL content is cultivar-dependent. AC99375-1RU potato chips had the lowest AL content and Mountain Rose potato chips had the highest AL content among the ten cultivars after three months of storage. Baked potatoes of Atlantic cultivar had the lowest AL content and baked potatoes of CO97227-2P/PW had the highest AL content among the ten tested cultivars. The interaction effect of storage time and storage temperature on AL content in potato chips and baked potatoes resulted in either increased, decreased or no change after six months of storage. AL content in potato chips showed a strong correlation with reducing sugars in raw potato tubers, but reducing sugars weakly correlated with AL content in baked potatoes. Atlantic chips made from potato tubers stored at 4°C or 10°C received higher ($p \leq 0.05$) sensory attributes mean scores and were ranked "liked most" among the other six cultivars. All Red chips scored the lowest for all sensory attributes at 4°C or 10°C. Storage temperature had no effect on appearance of Russet Burbank and All Blue or on the color, taste, and overall acceptability of Atlantic and All Blue chips. Rio Grande and AC99375-1RU chips made from potato tubers stored at 4°C and 10°C, respectively, had a similar crispiness, taste, and overall acceptability mean scores compared to Atlantic chips. Baked Atlantic, Russet Burbank, and Rio Grande Russet from potato tubers stored at 4°C or 10°C had the highest mean scores for appearance, color, taste, texture, and overall acceptability. Storage had no effect on sensory attributes of all baked potato cultivars except baked All Red. Baked Atlantic, AC99375-1RU, and Russet Burbank from potato stored at 10°C ranked "liked most". Storage temperature had no effect on the density for potato cultivars at either 4°C or 10°C except Russet Burbank tubers stored at

10°C. However, storage temperature had a significant effect on texture for potato tubers stored at 4°C.

1. Introduction

The potato (*Solanum tuberosum* L.) is ranked the 4th largest food crop with per capita consumption of 112 lbs annually in the United States (Osteen, 2012). Among the snack foods, potato chips are considered the largest consumed snack in United States. Potato chips deliver beneficial bioactive compounds for humans such as phenolic acids, anthocyanins, carotenoids, and resistant starch (Prakash and Sharma, 2014). According to the 2010 U.S. Potato Board report (United States Potato Board, 2007), consumption of specialty/colored potatoes increased by 17%, while consumption of traditional potatoes declined. It was recently reported that purple-fleshed potatoes suppressed colonic/systemic oxidative stress in high fat-diet consuming pigs by suppressing inflammation markers such as TLR-4, NF-KB, and TNF-α (Radhakrishnan, 2014). Along with beneficial compounds, potato chips are also known to contain undesirable compounds, such as acrylamide (AL), a carcinogenic compound (Dearfield et al., 1995). Frying of potatoes generates AL formed by the reaction of reducing sugars (glucose, fructose, hydrolyzed sucrose, and hydrolyzed starch during potato storage) with asparagine, an amino acid, via the Maillard reaction, which occurs during processing temperatures above 120°C (Tareke et al., 2002). Irrespective of the natural variation, the limiting precursor in potatoes is reducing sugars, mainly glucose and fructose. Heated potato products such as French fries and potato chips contain a high concentration of AL ranging from 424 to 1739 µg/kg (Pedreschi, 2009). This is due to the presence of the high amount of reactants involved in the formation of AL; asparagine: 94 mg/100 (Martin et al., 2001) and reducing sugars: 0.1 to 3 g/kg (Amrein et al., 2004). Content of reducing sugars may reach 20 g/kg after storage at low temperatures.

Asparagine is most abundant amino acid in most cultivars and required for AL formation, but low temperature storage did not elevate asparagine levels. However, reducing sugars content increased during low temperature storage (Olsson et al., 2004). Variation in AL content was not only from the variability of the precursor levels present in selected cultivars, but also processing conditions e.g., frying, cooking time, and temperature (Williams, 2005). Consumption of processed potatoes in the United States is 77 lbs per capita (Osteen, 2012). There is considerable public concern about the relationship between cancer and consumption of AL-rich foods such as fried and baked potatoes (Michalak et al., 2011). This concern is increased when the health risk of dietary AL exposure is increased from one to four µg/kg body weight/day (Parzefall, 2008). Human dietary exposure to AL ranges from 272 to 590 µg/kg for fried potatoes; 229 to 890 µg/kg for coffee; 149 to 150 µg/kg for breakfast cereals and 75 to 1044 µg/kg for bakery products (European Food Safety Authority, 2012). When compared to other starchy foods, fried and baked potatoes account for the largest proportion of AL intake (Arvanitoyannis and Dionisopoulou, 2014). No AL is formed during boiling and microwaving whole potatoes with the skin (American Cancer Society, 2013). Baked potatoes are considered an alternative to reduce processing induced-AL in potato chips.

The effect of frying time and the frying method (atmospheric vs. vacuum frying) on the AL content of potato chips was studied by Granda et al. (2004), who found that the potato chips fried using vacuum frying (105°C for eight minutes) had 85-99% reduction of AL content compared with those chips fried with the traditional fryer, but the AL content increased with frying time. Palazoglu et al. (2010) clarified that AL content of fried potato chips increased significantly with frying temperature, whereas the level of AL was 19.6 ng/g, 39 ng/g, and 95 ng/g when potato chips were fried at 170°C, 180°C, and 190°C, respectively.

AL is also affected by the composition of the food, post-harvest handling differences in cooking methods, water content, pH, the presence of additives, and storage conditions. Low temperature storage (below 8°C) is not appropriate for potatoes designed for the processing market. Low temperatures led to an increase in the sugar levels through a process called cold-induced sweetening (Sowokinos, 2001). This phenomenon naturally occurs when the tuber has started to mobilize sugars from starch to protect itself from frost (Blenkinsop et al., 2002). Mobilization of sugars happens more rapidly at low storage temperatures or higher than 8°C, which correlates to the start of sprouting (Amrein et al., 2004). This is confirmed by Chuda et al. (2003), who found that the chips made from potato tubers stored at cold temperature (2°C) contained high AL content and positively correlated with reducing sugar levels. Additionally, reconditioning cold-storage potato tubers by warming the tubers at 15°C for three weeks reduced the sugar levels (Foot et al., 2007). Therefore, storage at 8°C or above will reduce AL formation in the potato product upon baking or frying. Thus, avoiding cold storage conditions can be done by short-term storage. This is a problem for the producers due to the necessity to maintain potato supplies throughout the year to the market. This can be achieved by chemical treatment of potatoes with sprout suppressing agents. This is also a problem as consumers due to health and safety concerns do not desire the chemicals. In order to lower AL levels, potatoes used for processing should contain reducing sugars levels below 3 g/kgfw at harvest (United States Department of Health and Human Services, 2007). Interestingly, selecting potato cultivars with low reducing sugars also results in the production of golden colored potato chips that are desirable to consumers. Information on the effect of storage, processing and genotype on AL and reducing sugars content in potato chips is limited. The objectives of this study were to investigate the effect of storage

(time and temperature), genotype, and processing (baking and frying) on AL content, reducing sugars, and sensory attributes of potato chips and baked potatoes.

2. Materials and methods

2.1. Chemicals

AL, glucose reagent, phosphoglucoisomerase, glucose, and fructose standards were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A). Methanol was supplied by EMD chemicals (Philadelphia, PA, U.S.A). Carrez I, II solutions, and acetonitrile were purchased from Fisher Scientific (Fenton, MO, U.S.A). Ethanol for reducing sugars extraction was purchased from the Chemistry stock room at Colorado State University (Fort Collins, CO, U.S.A).

2.2. Plant materials

Ten specialty potato cultivars were selected dependent on their content of reducing sugars as the precursors of AL formation. Commercial colored potato cultivars: All Red, All Blue, Purple Majesty, Mountain Rose, advanced selection CO97227-2P/PW, and five white-fleshed potatoes: Atlantic, Russet Burbank, AC99375-1RU, Lenape, and Rio Grande Russet were chosen for this study. All cultivars were grown at San Luis Valley Research Center, Center, CO. The growth was started in May 2012 and continued until late September 2012. Three weeks before harvest, potatoes were treated with sulfuric acid for vine killing. After harvesting, each potato cultivar was placed in six different numbered bags weighting 4.5 kg (10 lbs). Each cultivar was stored at two different temperatures (4ºC or 10°C) for three different storage intervals (initial, three, or six months). Their weight was recorded at monthly intervals before sampling for analysis. Raw potato tubers were diced and stored at -20ºC until analysis.

2.3. Chipping of the potatoes

Potatoes were chipped directly after completion of each storage interval (initial, three, or six months). The raw potato tubers were thoroughly cleaned under running tap water and then sliced into 1/16 inch sections. The raw potato slices were washed under running warm water for approximately one minute to remove any water-soluble sugars present on the surface, then were placed in strainer trays to remove excess water and fried in Bakers & Chefs™ Clear Frying oil at 185°C until bubbling slowed (one minute 45 s to two minutes 15 s depending on tuber specific gravity). The fried chips were placed on paper towels to absorb excess oil and were allowed to cool for 15 minutes. The chip samples were then labeled, bagged, and stored in the dark at either -20°C until AL analytical tests or at 4°C for sensory evaluation.

2.4. Baking of the potatoes

Fresh and stored potato tubers were baked using a conventional oven directly at initial, three, or six months of storage. Before baking, the potato tubers were washed and left to dry at room temperature and then pierced approximately 1.5 cm deep with a knife. Each tuber was wrapped in food grade aluminum foil and baked for one hour at a consistent temperature (204°C) in a preheated-oven. The baked potatoes were taken out of the oven and cooled for 30 minutes at room temperature. Finally, the baked potatoes were diced with the skin into small pieces and stored at -20ºC until analysis.

2.5. Acrylamide extraction and quantification

The concentration of AL was determined according to the method established by Gokmen et al. (2005). Briefly, 10 mL methanol was added to a representative sample of ground potato chips (two gram) in three replicates for each cultivar. Then the samples were homogenized using IKA.T 25 digital ultra-turrax (IKA, Germany) and centrifuged at 10,000 rpm

(11,180 xg) at 10°C for 10 minutes using an ultracentrifuge (Beckman J2-HS centrifuge, Beckman Coulter, CA, USA). One hundred µL of each Carrez-I and II solutions were mixed with the supernatant to precipitate the colloids. Finally, the suspension was centrifuged at 10,000 rpm (11,180 xg) at 10°C for five minutes and the supernatant was completely dried in glass test tubes by using an Analytical Nitrogen Evaporator (Berlin, MA, USA) set at 40°C. Immediately, two mL of distilled water were added to the completed drying samples to re-dissolve the residues by vortexing them for two minutes and stored at -20°C for analysis. For quantification of AL, one mL of the extract was filtered through a 0.22 µm syringe filter and five µL of the final solution was injected into a UPLC column.

The AL concentration of chipped potato samples was analyzed for ten potato cultivars at initial, three, or six months of storage using UPLC. AL levels were determined by a Waters Acquity UPLC system equipped with a bio-Sample manager and bio-quaternary solvent manager with a diode array detector. The chromatographic separations were done on BEH Shield RP18 1.7µm 2.1x100 mm column (Waters, Ireland, USA). The injection volume was five µL. The mobile phase was filtered water adjusted to pH 9.5 by NH_4OH (solvent A) and 100% acetonitrile (solvent B). The flow rate was adjusted to 0.5 mL per minute at room temperature. Initial solvent gradient was 98% of solvent A and 2% solvent B. Solvent B increased from 2% to 100% within 4.5 minutes. Finally, the solvent gradient was returned to 98% of solvent A and 2% of solvent B. AL was detected at 226 nm with continuous monitoring of the peak spectra between 190-350 nm and concentrations were calculated for standard curves.

2.6. Reducing sugars extraction and determination

Reducing sugars (glucose and fructose) were extracted using the protocol established by Viola and Davies. (1992). Two gram sample of raw potatoes from each cultivar was ground and

then suspended in 19 mL of 80% ethanol. The bottles were immersed in a shaking water bath at 80ºC for one hour during the extraction process. Then the bottles were centrifuged at 2200 xg for 15 minutes (centrifuge 5810 R 15 amp version, Eppendorf, Westbury, NY, USA). The supernatants were collected in 50 mL falcon tubes. The residues were re-extracted with 20 mL of 80% alcohol and the supernatants were added to the pervious supernatants. Two mL of the supernatants were concentrated down to 500 µL using an Analytical Nitrogen Evaporator (Berlin, MA, USA) set at room temperature. The final concentrated extract was used to determine the reducing sugars. First, 100 µL of potato extract and standard solutions were added to a micro-plate well and then 120 µL of distilled water were added. The absorbance of each well was recorded at 340 nm (A_1). Then, 30 µL of glucose reagent were added to each well and the absorbance at 340 nm was recorded again as A_2. The calculated difference between A_2 and A_1 was due to the conversion of glucose to 6-phosphogluconate and the corresponding reduction of NAD to NADH. Next, 10 µL phosphoglucoisomerase were added to all wells and the absorbance at 340 nm was recorded again as A_3. The calculated difference between A_3 and A_2 was due to the presence of fructose.

2.7. Sensory evaluation

Sensory evaluation of the chipped samples from seven potato cultivars: Rio Grande Russet, Russet Burbank, Atlantic, AC99375-1RU, All Blue, Mountain Rose, and All Red was conducted with 105 untrained consumer panelists. Evaluations were carried out using chip samples made from potato tubers stored for three months at 4°C or 10°C to study the effect of storage on sensory attributes. The chips were tested after three months of storage to imitate market conditions where the bagged chips may sit on shelves more than three months before sale and consumption. Atlantic is a leading cultivar for producing potato chips and was used as

control. The panelists were asked to score the chip samples based on the sensory attributes of appearance, taste, color, texture, and overall acceptability on a 9-point hedonic scale "1 = extremely disliked; 9 = extremely liked." The panelists then were asked to rank the chip samples based on their preference "1 = liked most; 7 = liked least." Each chip sample was assigned a 3-digit random code and randomly served in a 2-ounce plastic portion cup. Panelists were asked to rinse their mouths with distilled water and bite into an unsalted cracker between samples to clean their palates.

2.8. Texture analysis

Texture analysis performed on raw potato tubers after three months storage at 4°C or 10°C by using the texture analyzer (TA-XT2; Texture Technologies Corp, Scarsdale, NY, USA). The density for three biological replicates was measured by determining the weight (g) and the volume of tuber (mL) for each tuber. The value of density (g/mL) for each biological replicate was determined by dividing the weight value by the volume value. The maximum force (g) was determined by the texture analyzer and was set at distance of 20 mm, pretest speed 5.0 mm/s, test speed 4.5 mm/s, post test speed 8.3 mm/s, time 5 sec, temperature 0ºC and load cell 5 kg.

2.9. Statistical analysis

Data were grouped as raw, baked, and chipped for white-and pigmented-fleshed potatoes. Two-way analysis of variance (ANOVA) for the interactions of storage time, and storage temperature on AL, reducing sugars content and sensory attributes were determined by using the SAS, (Statistical Analysis System), v.9.3 (SAS Institute Inc., Cary, NC). General Linear Model (GLM) procedure and Least Squares Means (LSD) were applied on obtained results with a level of significant of 0.05 for multiple comparisons for means of the tested parameters. Pearson

correlation coefficients were calculated using SAS (Statistical Analysis System), v.9.3 (SAS Institute Inc., Cary, NC). All results have been expressed as mean ± standard error.

3. Results and discussion

3.1. Effect of genotype, storage, and processing on AL and reducing sugars levels in potato chips and baked potatoes

It is well known that the content of AL in potato products such as potato chips are influenced by several factors such as, genotype, post-harvest treatments, and processing. Recently, interest in the study of the precursor content involved in AL formation and their behavior during storage, has increased due to the fact that potato chips are one of the largely consumed snack foods in the Unites States. Cold storage of potato tubers is the best condition for long term storage since it reduces respiration and limits the use of sprout inhibitors. However, low-temperature storage leads to the accumulation of the reducing sugars and thereby increases the potential formation of cancer-causing AL in potato chips. In our previous work, we found that three months storage of potatoes at low temperature (3 ± 1°C) resulted in a significant increase in AL content in potato chips of Atlantic, Yukon Gold, Purple Majesty, and CO97227-2P/PW. In this study, we selected five white-fleshed potato cultivars, including Atlantic, as the standard for potato chips: Lenape, Rio Grande Russet, Russet Burbank, AC99375-1RU, and five color-fleshed potatoes involving, two red-fleshed potatoes, (All Red and Mountain Rose), and two purple-fleshed potatoes (All Blue and Purple Majesty), and purple-fleshed with specks (CO97227-2P/PW). Our findings in this study agreed with our previous results that the storage affects AL and it is cultivar-dependent. The storage temperature of the raw potato tubers affects the AL concentration in potato chips. Storage potato tubers at 4°C resulted in an increase in the content of reducing sugars in the raw potato tubers, which similarly led to a higher level of AL in

the potato chips (Matthaus et al., 2004). AL content of chips at initial time of storage ranged from 111 ± 18 ppb for Purple Majesty to 2450 ± 54 ppb for All Red (Figure 4.1.b). Potato genotype had a significant effect on AL, which can be determined by reducing sugars rather than the amino acid, asparagine (Williams, 2005). This was in accordance with published results of (Amrein et al., 2004), who found that the cultivar Nicola had the highest content of AL (2020 µg/kg), while the lowest AL content was reported for Panda (80 µg/kg). Our data was also in agreement with Granda's data who found that AL content of different potato cultivars fried using traditional method ranged from 358 ± 50 ppb form NDTX4930-5W advanced selection to 5021 ± 45 ppb for white Rose cultivar (Granda et al., 2004).

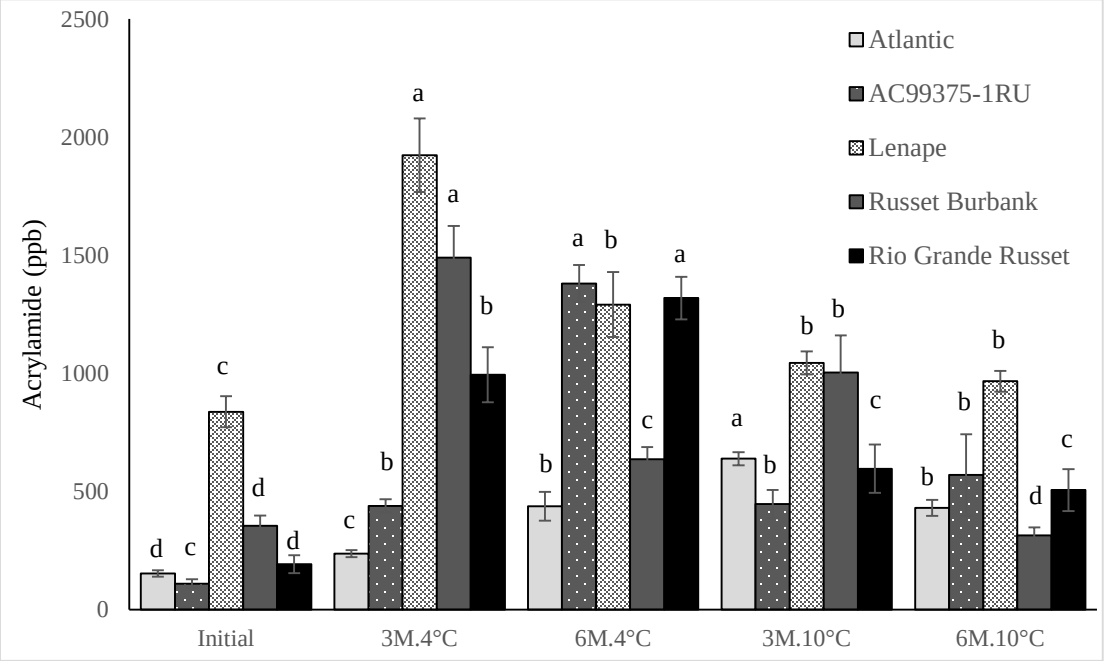

Figure 4.1.a. Effect of genotype and storage on acrylamide level (ppb) in potato chips of white-fleshed potatoes after 6 months of storage at 4°C or 10°C. Initial: no storage; M: month; 4°C or 10°C: storage temperatures. Acrylamide content was determined using UPLC-DAD as described in the materials and methods. Values with different letters indicate differences (Least Square Means test; $p \leq 0.05$) among different storage times. Results are presented at the mean ± SE of three biological replicates and two technical replicates for each biological replicate. Each value is a mean of six determinations.

Our data was also in agreement with Zhao's data who found that AL content in potato chips from Atlantic variety was 147.4 ± 0.077 ppb (Zhao et al., 2013) and AL content in our Atlantic chips samples was 153.75 ± 14 pbb at initial level of storage.

After three months of storage at 4°C, the AL content increased from 111 ± 18 ppb to 440 ± 28 ppb for ACC99-375-1R and from 2450 ± 54 ppb at initial of storage to 4837 ± 62 ppb for Mountain Rose (Figure 4.1.a; Figure 4.1.b). The AL content of chips from all tested cultivars significantly increased after three months of storage at 4°C (Figure 4.1.a and 4.1.b). This is in agreement with our previously published work (Amer et al., 2014) on AL in potato chips that showed two to four fold increase in AL content after three months of storage. In this study, after three months of storage, the AL concentration ranged from 440 ± 28 ppb for AC99375-1RU to 4837 ± 62 ppb for Mountain Rose, a 3.3-fold variation in AL concentration; however, the storage effect resulted in a 0.97 to 8.9 fold increase in AL content of all tested potato chips. AL content of Atlantic potato chips made from potato tubers stored for three months at 4°C was 237.14 ppb ± 15. However, Atlantic chips of Zhao's samples had higher AL content (484.7 ± 0.07 pbb) than our Atlantic chips samples (237.14 ± 15 pbb). The difference in AL content between Zhao's and our Atlantic chips samples because of the difference in AL determination. We used UPLC for AL determination and they used HPLC with cleaning up the extracts using solid-phase extraction (SPE) cartridge. Storage potato tubers for three months at 4ºC had minimal effect on AL content of Lenape, Russet Burbank, Rio Grande Russet, and All Blue potato chips. AC99375-1RU had the lowest AL content and All Red had the highest AL content among the ten cultivars, which agreed with our published data (Amer et al., 2014). Our data also agreed with Kalita and Jayanty (2013), who found that the advanced selections (CO00197-3W) had the highest level of AL among the chipping cultivars of potato tubers for two months at 7°C. However, in our study,

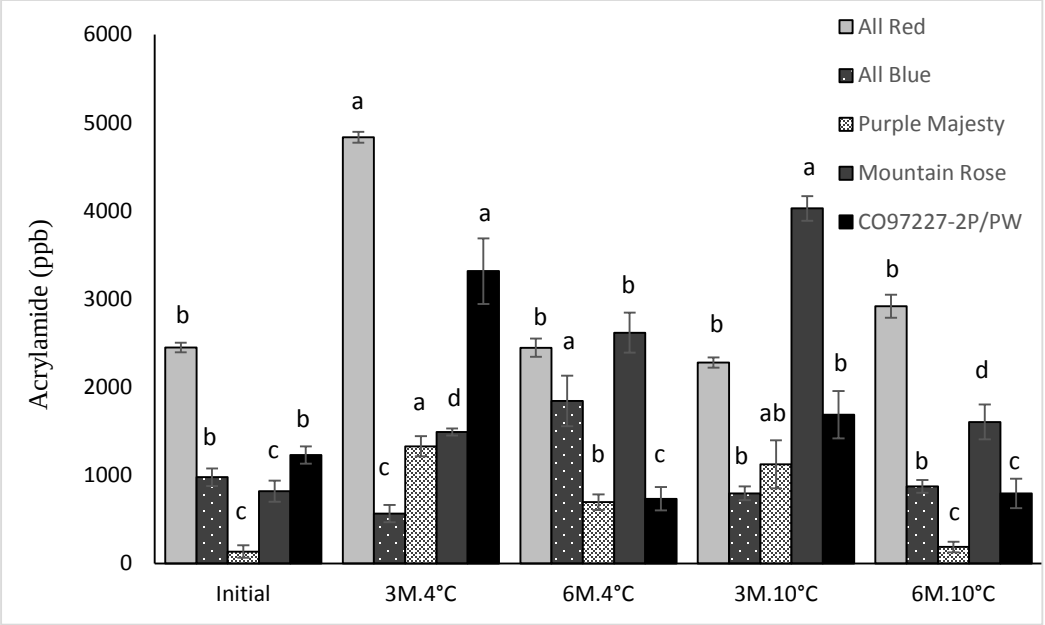

Figure 4.1.b. Effect of genotype and storage on acrylamide level (ppb) in potato chips of color--fleshed potatoes after six months of storage at 4°C or 10°C. Initial: no storage; M: month; 4°C or 10°C: storage temperatures. Acrylamide content was determined using UPLC-DAD as described in the materials and methods. Values with different letters indicate differences (Least Square Means test; $p \leq 0.05$) among different storage times. Results are presented at the mean ± SE of three biological replicates and two technical replicates for each biological replicate. Each value is a mean of six determinations

the AL levels in chips from All Red was 4837 ± 62 ppb after storage potato tubers for three months at 4°C and 2281 ± 563 ppb after storage potato tubers for three months at 10°C.

AL content of potato chips after six months of storage at 4°C ranged from 439 ± 61 ppb for Atlantic to 2618 ± 227 ppb for All Red. AL content of Atlantic chips made from potato tubers stored for six months at 4°C was 438.45 ± 61 ppb which agreed with Zhao who found that AL content in their Atlantic chips was 575.2 ± 0.07ppb (Zhao et al., 2013). AL content of chips from Rio Grande was 193 ± 7.9 ppb at initial level and then increased to 994 ± 147 ppb after three months of storage at 4ºC. However, AL content of chips from Rio Grande increased to 1319 ± 90 ppb after six months of storage at 4ºC. AL content of chips from both All Red and AC99375-1RU were 2450 ± 54 ppb and 111 ± 18 ppb, respectively, at initial. However, AL content of chips from All Red remained constant (2449 ± 102 ppb) and AL content of chips from

AC99375-1RU was 1381 ± 78 ppb after six months of storage. The AL content of chips from all tested cultivars significantly increased after six months of storage at 4°C except CO97227-2P/PW. Our findings agreed with those of Al Viklund et al. (2008), who found that the AL content in chips made from Hulda and Saturna cultivars were lowest at the initial time of storage and increased significantly after 12 weeks and then decreased after from 18 and 24 weeks, respectively. During cold storage, starch content gets converted to reducing sugars and used for respiration purposes. However, sugars start to accumulate when their production outweighs their use (Hertog et al., 1997). To avoid the increase in reducing sugars during storage, potato tubers should be preconditioned or reconditioned for three weeks at 15°C before or after storage (Pritchard and Adam, 1992; Edwards et al., 2002). In comparison to AL content of chips made from potato tubers stored for three months at 10°C, the AL content of chips from all tested cultivars except All Red, All Blue, and Mountain Rose, was increased by interaction effect of storage duration and temperature of storage compared to AL levels in chips made from un-stored potato tubers. However, storage potato tubers for six months at 10ºC resulted in increase in AL content of chips from all tested cultivars except Russet Burbank, All Blue, and CO97227-2P/PW. Storage at 4°C resulted in more accumulated reducing sugars compared to storage at 10°C at the same period of storage. Storing potato tubers at 8°C to 10°C is generally used to avoid an increase in the sugar content during cold storage (Blenkinsop et al., 2002). Potato chips made from tubers stored at 2°C contain 10 times higher of AL than that stored at 20°C (Chuda et al., 2003). This was also observed by Matsuura-Endo et al. (2006), who found that the content of reducing sugars in raw potato tubers increased markedly in all tested cultivars after storage at temperatures lower than 8°C, with similar increases in the AL levels of potato chips.

AL content of baked potatoes at initial time of storage ranged from 647 ± 42 for AC99375-1RU to 2211 ± 171 ppb for All Red (Figure 4.1.c; 4.1.d). Potato genotype had a significant effect on AL, which can be determined by reducing sugars rather than the amino acid, asparagine (Williams, 2005). After three months of storage at 4°C, the AL content in baked potatoes increased from 648 ± 42 ppb to 2107 ± 257 ppb for AC99-375-1RU and from 561 ± 12 ppb to 3719 ± 83 for Mountain Rose.

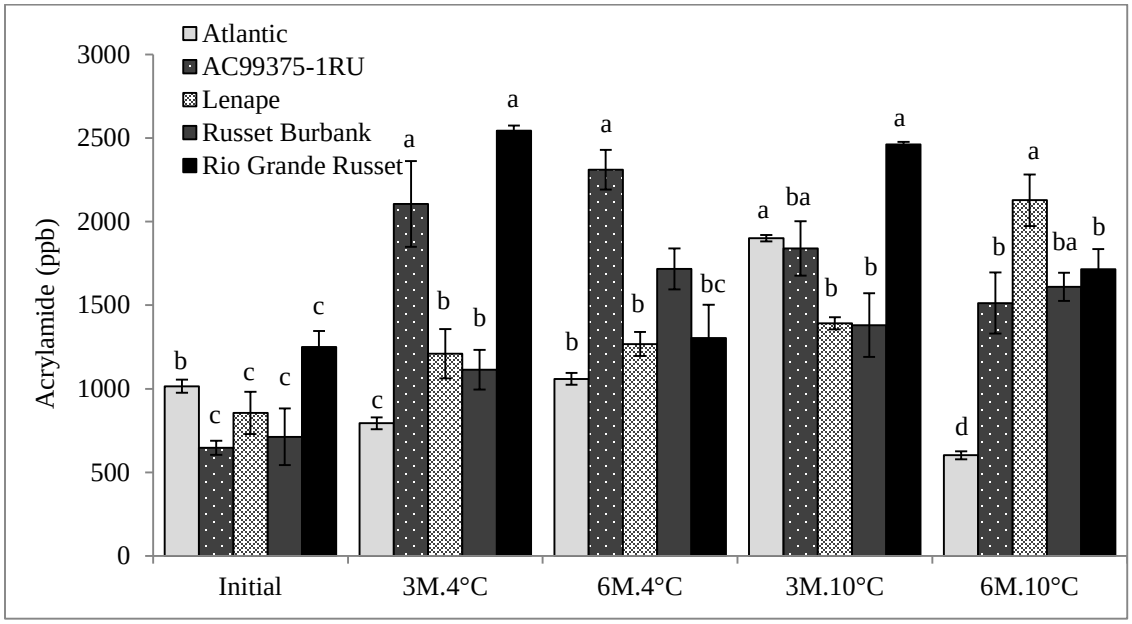

Figure 4.1. c. Effect of genotype and storage on acrylamide level (ppb) in baked potatoes of white-fleshed potatoes after six months of storage at 4°C or 10°C. Initial: no storage; M: month; 4°C or 10°C: storage temperatures. Acrylamide content was determined using UPLC-DAD as described in the materials and methods. Values with different letters indicate differences (Least Square Means test; $p \leq 0.05$) among different storage times. Results are presented at the mean ± SE of three biological replicates and two technical replicates for each biological replicate. Each value is a mean of six determinations.

Low storage temperature (4°C) for three months resulted in increased AL content in baked potatoes from all cultivars except baked potatoes from Atlantic and All Red cultivars. However, storage of potatoes for six months at 4°C resulted in an increase in AL content in baked potatoes from all tested cultivars except Mountain Rose cultivar. In comparison, storage at 10°C for three months resulted in an increase in AL content in baked potatoes from all tested cultivars. However, storage for six months at 10°C resulted in an increase in AL content in baked

potatoes from all tested cultivars compared to initial levels except Atlantic, All Blue, and Mountain Rose cultivars (Figure 4.1.c; Figure 4.1.d).

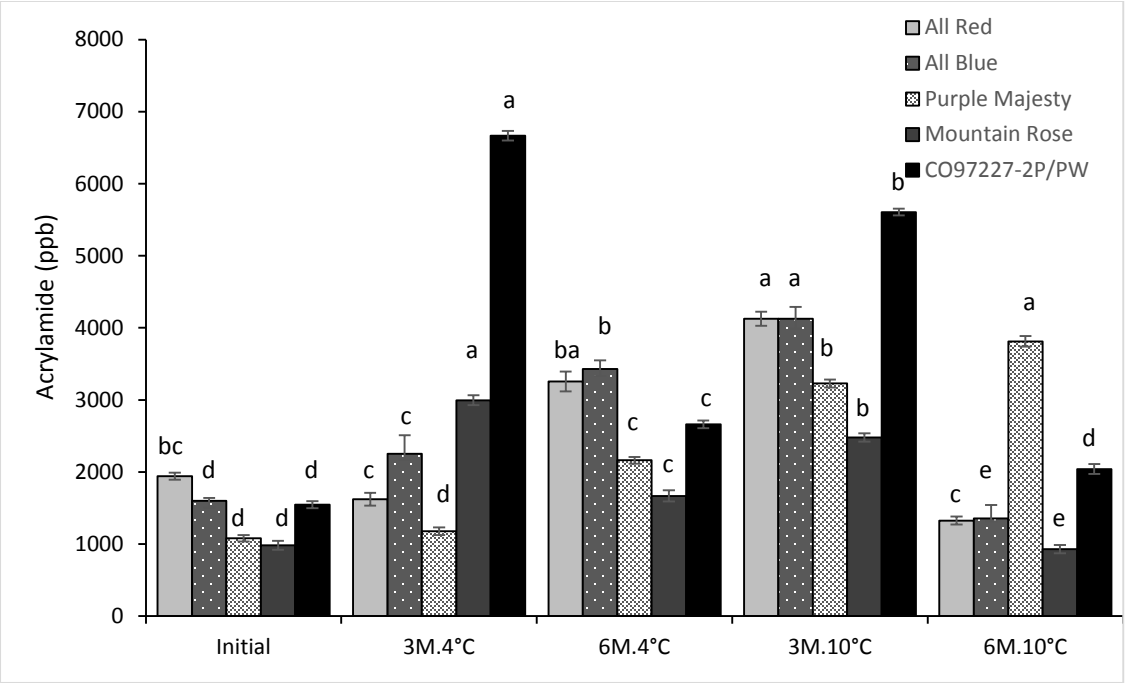

Figure 4.1. d. Effect of genotype and storage on acrylamide level (ppb) in baked potatoes of color-fleshed potatoes after 6 months of storage at 4°C or 10°C. Initial: no storage; M: month; 4°C or 10°C: storage temperatures. Acrylamide content was determined using UPLC-DAD as described in the materials and methods. Values with different letters indicate differences (Least Square Means test; $p \leq 0.05$) among different storage times. Results are presented at the mean ± SE of three biological replicates and two technical replicates for each biological replicate. Each value is a mean of six determinations.

The content of AL differed between cultivars, storage temperatures, and storage times (Al Viklund et al., 2008). This variation related to the difference in precursor concentrations. The results of this study show that the glucose concentration in raw potato tubers varied between 858 ± 15 mg/kg for Atlantic and 6,420 ± 59 mg/kg for All Red at initial storage. The glucose level followed similar trends as that of AL; increasing after three months of storage and then decreasing after six months of storage at 4°C (Table 4.1). Our findings agreed with published work by Olsson et al. (2004), who found that glucose and fructose levels markedly increased

from October until January (three months of storage) and then decreased at the end of the storage (June). Olsson et al. (2004) also demonstrated that small variations in reducing sugars occurred with storage time at 10°C which is in agreement with our results that the glucose and the fructose levels were higher when the potatoes had been stored at 4°C rather than 10°C. This likely occurs due to low-temperature sweetening (Zommick et al., 2014).

Fructose content ranged from 560 ± 22 mg/kg for Atlantic to 2037 ± 15 mg/kg for All Blue at initial storage (Table 4.2). At 4°C, the concentration of fructose significantly increased from 560 ± 22 to 833 ± 55 mg/kg for Atlantic and from 1698 ± 53 to 4355 ±77 mg/kg for All Red. After six months of storage at 4°C, the fructose content increased from 833 ± 55 to 1308 ± 9.6 mg/kg for Atlantic and from 1698 ± 53 to 4355 ±77 mg/kg for All Red. Our results showed that cultivar and storage conditions influence the levels of glucose and fructose in potatoes and correlated with the content of AL in potato chips. Glucose and fructose contributed to 2/3 of the total sugar content in potato tubers (Burton, 1948) and the ratio between the reducing sugars is normally 1:1, but storage at low temperature alters the ratio between the reducing sugars (Merlo et al., 1993). This may be because of the presence of a high concentration of fructokinase in potato extracts, which could cycle fructose back via the pool hexose-phosphates cycle (Merlo et al., 1993). Formation of AL in potato products is still a concern for researchers and food safety. Recently published work by Muttucumaru et al. (2014) observed that AL formation potentially was impacted by post-harvest storage, cultivar, and reducing sugar content in potato tubers. Fried potatoes are the greatest source of AL in US diet. AL formation can be decreased by choosing a suitable cultivar and by using appropriate cooking methods (Biedermann-Brem et al., 2003). Information on the effect of genotype, storage, and processing on AL content in baked potatoes is limited. The interaction effect of storage time (three months) and temperature (4°C) resulted in

an increase in the AL content in baked potatoes. The AL content increased from 454 ± 24 ppb to 1,255 ± 90 ppb for Russet Burbank and from 1,547 ± 50 ppb to 6,667 ± 68 ppb for advanced selection CO97227-2P/PW (Figure 4.3.c; Figure 4.4.d). After six months of storage at 4°C, the AL content in baked potatoes increased or decreased, depending on cultivar. The AL content in baked potatoes ranged from 794 ± 36 ppb to 1040 ± 53 ppb for Atlantic; 978 ± 23 ppb to 1360 ± 47 ppb for Lenape; 1255 ± 90 ppb to 1536 ± 48 ppb for Russet Burbank; 1622 ± 90 ppb to 3255 ± 137 ppb for All Red; 2271 ± 5 ppb to 3431 ± 98 ppb for All Blue; 1178 ± 22 ppb to 2162 ± 47 ppb for Purple Majesty. The AL content decreased in baked potatoes of AC99375-1RU from 2663 ± 18 ppb to 2405 ± 82 ppb, from 2544 ± 18 ppb to 1106 ± 31 ppb for Rio Grande Russet, from 2994 ± 29 ppb to 1667 ± 78 ppb for Mountain Rose and from 6667 ± 68 ppb to 2661 ± 24 ppb for CO97227-2P/PW. After three months of storage at 10°C, the AL content significantly increased for all tested cultivars compared to initial levels of AL. The AL content in baked potatoes ranged from 1,084 ± 44 ppb for Russet Burbank to 5,607 ± 48 ppb for CO97227-2P/PW. After six months of storage at 10°C, the interaction effect of storage time and temperature resulted in either increase or decrease in AL content of baked potatoes. The AL content ranged from 649 ± 44 ppb for Atlantic to 3,813 ± 73 ppb for Purple Majesty.

Table 4.1. Glucose levels (mg/kg) of raw white- red- and purple-fleshed potatoes from ten different cultivars at initial, three and six months of storage at 4°C or 10°C.

Cultivar/Selection	Raw white- red- and purple-fleshed potatoes				
	Initial	3M.4ºC	6M.4ºC	3M.10ºC	6M.10ºC
Atlantic	858 ± 15^{b}	1,292 ± 72^{a}	856 ± 23^{b}	802 ± 34^{b}	905 ± 86^{b}
AC99375-1RU	1,107 ± 12^{c}	4,287 ± 21^{a}	967 ± 62^{c}	3,360 ± 75^{b}	1,184 ± 13^{c}
Lenape	1,004 ± 61^{c}	2,222 ± 20^{a}	888 ± 56^{c}	1,516 ± 16^{b}	1,130 ± 58^{bc}
Russet Burbank	844 ± 11^{b}	2,611 ± 15^{a}	1,124 ± 113^{b}	845 ± 69^{b}	955 ± 50^{b}
Rio Grande Russet	2,677 ± 30^{ab}	2,885 ± 18^{a}	912 ± 12^{c}	2,361 ± 12^{b}	881 ± 71^{c}
All Red	6,420 ± 59^{a}	6,085 ± 52^{a}	1,618 ± 21^{c}	2,894 ± 16^{b}	2,189 ± 12^{bc}
All Blue	3,555 ± 12^{b}	4,654 ± 36^{a}	1,701 ± 31^{d}	2,710 ± 35^{cb}	1,944 ± 82^{cd}
Purple Majesty	3,403 ± 67^{ba}	4,864 ± 75^{a}	3,437 ± 15^{ba}	3,436 ± 42^{ba}	2,361 ± 12^{b}
Mountain Rose	1,514 ± 16^{c}	5,068 ± 92^{a}	1,525 ± 91^{c}	3,416 ± 37^{ab}	2,932 ± 30^{bc}
CO97227-2P/PW	1,407 ± 29^{c}	4,162 ± 27^{a}	2,384 ± 77^{b}	2,017 ± 86^{b}	2,309 ± 37^{b}

Means with different letters indicate significant differences in rows ($p \leq 0.05$) from the initial time point. Results are presented as mean ± SE of three biological replicates and two technical replicates for each biological replicate. Each value is a mean of six determinations.

Table 4.2. Fructose levels (mg/kg) of raw of white- red- and purple-fleshed potatoes from ten different cultivars at initial, three and six months of storage at 4°C or 10°C.

Cultivar/Selection	Raw white- red- and purple-fleshed potatoes				
	Initial	3M.4ºC	6M.4ºC	3M.10ºC	6M.10ºC
Atlantic	560 ± 22^{c}	834 ± 55^{b}	869 ± 24^{b}	1,308 ± 10^{a}	738 ± 36^{bc}
AC99375-1RU	919 ± 14^{b}	2,670 ± 12^{a}	2,533 ± 51^{a}	722 ± 36^{c}	682 ± 28^{c}
Lenape	799 ± 10^{c}	2,4070 ± 16^{a}	1,507 ± 65^{b}	822 ± 55^{c}	602 ± 30^{c}
Russet Burbank	864 ± 16^{c}	2,704 ± 49^{a}	1,256 ± 64^{b}	804 ± 80^{c}	1,025 ± 68^{bc}
Rio Grande Russet	1,797 ± 32^{b}	2,835 ±13^{a}	1,916 ± 13^{b}	1,847 ± 19^{b}	1,029 ± 50^{c}
All Red	1,698 ± 53^{d}	4,355 ± 77^{a}	2,920 ± 74^{b}	2,266 ± 14^{c}	1,593 ± 12^{d}
All Blue	2,037 ± 15^{b}	3,662 ± 81^{a}	1,675 ± 88^{c}	839 ± 58^{d}	604 ± 26^{d}
Purple Majesty	1,470 ± 13^{b}	1,149 ± 13^{b}	4,214 ± 16^{a}	443 ± 70^{c}	505 ± 70^{c}
Mountain Rose	809 ± 55^{c}	2,813 ± 32^{a}	1,971 ± 29^{b}	1,984 ± 49^{b}	1,144 ± 95^{c}
CO97227-2P/PW	1,263 ± 58^{c}	2,951 ± 10^{a}	1,557 ± 19^{bc}	1,647 ± 10^{b}	1,773 ± 91^{b}

Means with different letters indicate significant differences ($p \leq 0.05$) from the initial time point. Results are presented as mean ± SE of three biological replicates and two technical replicates for each biological replicate. Each value is a mean of six determinations.

3.2. Sensory evaluation

Chipped samples from seven cultivars: Rio Grande Russet, Russet Burbank, Atlantic, AC99375-1RU, All Blue, Mountain Rose, and All Red were used for sensory analysis by 105 untrained consumer panelists after three months of storage at 4°C or 10°C (Table 4.3). Five attributes (appearance, taste, color, texture, and overall acceptability) were assessed and scored on a 9-point hedonic scale "(1 = disliked extremely; 9 = liked extremely)". Panelists were then asked to rank the chip samples based on their preference "(1= liked most; 7 = liked least)". Information on the effect of cultivar and storage on the sensory attributes of potato chips is limited. That is why sensory evaluations were performed to understand the effect of cultivar and storage on sensory attributes of potato chips and to compare the acceptability of purple-fleshed potatoes and white-fleshed cultivars. Atlantic, a popular chipping cultivar was used as the standard cultivar for potato chips. Mean values for appearance for all chip cultivars ($p \leq 0.05$) ranged from 3.8 ("dislike moderately") to 7.6 ("like moderately" to" like very much") for chips made from potato tubers stored at 4°C and from 4.9 to 8.0 for chips from potato tubers stored at 10°C. Atlantic potatoes received higher mean scores of 7.6 and 8.0 for appearance when stored for three months at 4ºC or 10ºC, respectively ($p \leq 0.05$). All Red chips made from potato tubers stored at 4°C had a mean score of 3.8 for appearance ($p \leq 0.05$) which was lower than the Atlantic cultivar and all other cultivars except Mountain Rose. Panelists scored All Red chips lower for appearance than most cultivars, and a mean value of 3.8 corresponded to "disliked moderately" on the hedonic scale. Storage temperature had an effect on appearance of most cultivars except Russet Burbank and All Blue. However, appearance mean scores increased for potato chips made from potato tubers stored at 10°C compared to 4°C. For color, mean values for all chip cultivars ($p \leq 0.05$) ranged from 3.8 to 7.7 for chips made from potato tubers at 4°C,

and from 5.1 to 7.8 for chips made from potato tubers at 10°C. The standard chip cultivar, Atlantic, received the highest mean score for color of 7.7 and 7.8 stored for three months at 4ºC or 10ºC, respectively. Panelists scored All Red the lowest for color with mean values of 3.8 ("dislike moderately") and 5.1 ("neither like nor dislike") at 4°C or 10°C, respectively. Mean values for taste for all cultivars ($p \leq 0.05$) ranged from 3.1 to 7.0 for chips made from potato tubers stored at 4°C and from 5.0 to 6.8 for chips made from potato tubers stored at 10°C. According to the panelists, Atlantic chips made from potato tubers stored for three months at 4ºC or 10ºC received highest mean scores of 7.0 and 6.8 for taste. All Red chips received the lowest mean score of 3.1 and 5.0 for taste. No other potato cultivars tested were scored higher than the standard Atlantic for taste. Panelists scored All Red chips the lowest for taste with mean value of 3.1 at 4°C. Atlantic chips made from potato tubers stored for three months at 4°C or 10°C received the highest mean scores of 7.0 and 6.8 for taste. Among white-fleshed potatoes, AC99375-1RU potato chips made from potato tubers stored at 10°C received similar taste and overall acceptability mean scores compared to the standard. Mean scores of crispiness ($p \leq 0.05$) ranged from 2.0 ("dislike very much") to 7.3 ("like moderately") and from 5.5 ("neither like nor dislike") to 7.8 ("like very much") for chips made from potato tubers stored at 4°C or 10°C, respectively. Atlantic chips made from potato tubers stored at 4°C had the highest mean score of 7.3 for crispiness and All Red and Mountain Rose chips were lower than all at 4°C ($p \leq 0.05$). Panelists scored Rio Grande chips made from potato tubers stored at 10°C the lowest for crispiness, however, Rio Grande chips made from potato tuber stored at 4°C had similar crispiness score as that of Atlantic. Storage of potato tubers at 10°C resulted in improved crispiness of the chips except for Rio Grande cultivar. Mean values of overall acceptability for all cultivars ($p \leq 0.05$) ranged from 2.8 to 7.4 and from 5.4 to 7.1 "like moderately" for cultivars

made from potato tubers stored at 4°C or 10°C, respectively. Atlantic chips made from potato tubers stored at 4°C or 10°C had the higher mean score of 7.4 and 7.1, respectively, for overall acceptability. All Red chips made from potato tubers stored at 4°C had a lower mean score of 2.8 ("dislike moderately") for overall acceptability than other cultivars. Storage of potato tubers at 10°C had a significant effect on the overall acceptability of potato chips for all cultivars except All Blue and Atlantic. Ranking was conducted on a scale of 1 = "liked most" to 7 = "liked least" for potato chips. Potato reference for potato chips was for the Atlantic cultivar (1.7) with the 4°C stored samples ranked higher than samples stored at 10°C (2.6). Potato tubers stored at 4°C were ranked next in "liking" with All Blue, Burbank, Rio Grande and AC99375-1RU receiving similar values ($p \leq 0.05$). Mountain Rose was ranked next while All Red was "liked least" among the seven cultivars. It was interesting to note that the potato chips made from red-fleshed potatoes were liked less than other cultivars. At 10°C storage, potato chips from Atlantic and AC99375-1RU received highest ranking scores. These were followed by Burbank ($p \leq 0.05$), then Rio Grande and Mountain Rose ($p \leq 0.05$). Potato chips ranked liked least were All Blue and All Red ($p \leq 0.05$). Chips made from Atlantic potatoes stored at 4°C were liked most overall. Storage temperature had no effect on chips made from Rio Grande or Burbank potatoes. Chips were ranked higher for purple-fleshed potatoes stored at 4°C ($p \leq 0.05$). However, chips made from red-fleshed potato tubers stored at 10°C had higher ranking values than those stored at 4°C ($p \leq 0.05$). Atlantic chips received the highest mean score for appearance, color, taste, crispiness, and overall acceptability when compared to the other six cultivars ($p \leq 0.05$).

Sixty three percent of the 98 consumer panelists ranked potato chips from the Atlantic cultivar stored at 4°C as "liked most". Rio Grande (16%) and All Blue (12%) were ranked next for liking. All other cultivars received less than 5% ranking scores for "1 = liked most." Red

potatoes were liked least for chipping with only one panelist liking the All Red cultivar most among the chips at 4°C storage. At 10°C, 39% of the 97 consumer panelists ranked potato chips from the Atlantic cultivar as "liked most". AC99375-1RU (21%), Russet Burbank (14%) and Rio Grande Russet chips (11%) were ranked next for liking. Color-fleshed potatoes received less than 8% ranking scores for "1 = Liked most." All Red chips were liked least for chipping with only eight panelists liking the All Red cultivars most among color-fleshed potatoes. Our findings for potato chips agreed with the sensory data from (Madiwale, 2012), who found that Atlantic had highest mean scores for all sensory attributes among tested potato samples from Yukon Gold, Purple Majesty, and CO97227-2P/PW. For other cultivars evaluated in the current study, AC99375-1RU potato chips received highest mean score for appearance (7.2), color (7.2), taste (6.5), crispiness (7.3) and overall acceptability (6.8) from 105 consumer panelists after potatoes were stored for three months at 10ºC before chipping. Schwartz (1987) observed that French-fried products of sweet potatoes had similar color, flavor, and texture to each other even after 12 months of storage. This finding did not agree with our data due to difference in storage conditions. Their potato tubers were stored in frozen conditions (-18°C), while our potato samples were stored at 4°C or 10°C for six months. Potato cultivars significantly affected sensory attributes scores for sweet potato chips for color, flavor, crispiness, and overall acceptability (Abong, 2011), which agreed with our data from the current study. Therefore, chips processors should select the appropriate cultivars, storage temperatures, and processing parameters. Storage temperature had a significant effect on the color and flavor scores of white-fleshed French fries (Kirkpatrick, 1956). Color scores were significantly better for French fries ($p \leq 0.05$) made from potatoes stored at 13°C or 16°C than from those stored at 7°C or 10°C. While no significant differences were found between flavor scores of French fries made from

potatoes stored at 10°C, 13°C, and 16°C, the French fries improved in flavor over those made from potatoes stored at 7°C. Sensory data for French fries from (Kirkpatrick, 1956) agreed with our sensory data for potato chips since potato chips made from potato tubers stored at 10°C had better color and taste hedonic scores than those made from potatoes stored at 4°C.

Sensory analysis was conducted on baked potato samples from white-fleshed potatoes and color-fleshed potatoes stored for three months at 4ºC or 10ºC (Table 4.4). Potatoes were baked and evaluated by 94 untrained consumer panelists on a hedonic scale. Russet Burbank is the most common cultivar for baking. Mean values for appearance for all baked cultivars ranged from 3.8 ("dislike slightly") to 7.3 ("like moderately") for baked potatoes made from potato tubers at 4°C and from 3.3 to 6.9 for baked potatoes made from potato tubers stored at 10°C. Baked Atlantic, Russet Burbank, and Rio Grande Russet made from potato tubers stored at 4°C received highest mean scores of 7.3, 6.9 and 6.9, respectively, for appearance. Baked All Red and Mountain Rose made from potato tubers stored at 4°C or 10°C received lowest mean scores for appearance (4.0, 3.3; 3.8, 3.8, respectively). Baked Russet Burbank, Rio Grande Russet, Atlantic, and AC99375-1RU made from potato tubers stored at 10°C had highest similar mean scores for appearance of 6.9, 6.8, 6.9, and 6.5, respectively. Storage temperature had no effect on appearance of all baked potato varieties except for baked All Red ($p \leq 0.05$), which received lower liking scores at 10°C than potatoes stored at 4°C. For color sensory attributes, mean values for all baked cultivars ranged from 4.0 to 7.3 for baked potatoes made from potato tubers stored at 4°C and from 3.2 to 7.0 for baked potatoes made from potato tubers stored at 10°C. Rio Grande Russet, Atlantic, and Russet Burbank baked potatoes made from potato tubers stored at 4°C had mean scores for color of 7.1, 7.3, and 6.9, respectively.

Table 4.3. Sensory attribute mean scores for chips from white- red- and purple-fleshed potato cultivars after three months of storage at 4°C or 10°C.

Sensory Attributes	Chips from White- Red- and Purple-Fleshed Potatoes						
	Rio Grande	Burbank	Atlantic	AC99375-1RU	All Blue	Mountain Rose	All Red
	4°C	4°C	4°C	4°C	4°C	4°C	4°C
	10°C	10°C	10°C	10°C	10°C	10°C	10C
Appearance	5.7^{cA}	6.6^{bA}	7.6^{aB}	6.6^{bB}	4.7^{dA}	4.3^{edB}	3.8^{eB}
	6.9^{bB}	6.8^{bA}	8.0^{aA}	7.2^{bA}	4.9^{dA}	5.7^{cA}	4.9^{dA}
Color	5.6^{cB}	6.5^{bB}	7.7^{aA}	6.9^{bA}	4.9^{dA}	4.4^{dB}	3.8^{eB}
	6.9^{bA}	6.9^{bA}	7.8^{aA}	7.2^{bA}	5.1^{dA}	5.7^{cA}	5.1^{dA}
Taste	4.3^{cB}	5.0^{bB}	7.0^{aA}	5.0^{bB}	5.0^{bA}	3.9^{cB}	3.1^{dB}
	5.7^{bA}	5.9^{bA}	6.8^{aA}	6.5^{aA}	5.0^{cA}	5.5^{bcA}	5.0^{cA}
Crispiness	7.2^{aA}	4.3^{cB}	7.3^{aB}	3.7^{dB}	5.9^{bB}	2.4^{eB}	2.0^{eB}
	5.5^{dB}	7.2^{bA}	7.8^{aA}	7.3^{bA}	7.0^{bA}	7.1^{bA}	6.0^{cA}
Overall Acceptability	4.9^{bB}	4.9^{bB}	7.4^{aA}	5.0^{bB}	5.0^{bA}	3.8^{cB}	2.8^{dB}
	5.7^{bA}	6.2^{abA}	7.1^{aA}	6.8^{aA}	5.3^{bA}	6.2^{abA}	5.4^{bA}
Rank	3.8^{bA}[4]	3.7^{bA}[3]	1.7^{aA}[1]	3.8^{bB}[5]	3.5^{bA}[2]	5.5^{cB}[6]	6.0^{dB}[7]
	4.1^{cA}[4]	3.5^{bA}[3]	2.6^{aB}[1]	3.0^{abA}[2]	5.1^{dB}[7]	4.6^{cA}[5]	5.0^{dA}[6]

Mean scores based on hedonic scale 1-9 (1 = disliked extremely and 9 = liked extremely) from 105 untrained consumer panelists. Values in brackets indicate ranking order (1 = Liked most among the seven cultivars). Different letters indicate differences ($p \leq 0.05$) among the clone rankings. Lowercase letters (a-e) on the mean scores indicate differences in rows among seven cultivars at the same storage temperature, whereas uppercase letters (A, B) on the mean scores indicate the differences in columns between 4°C or 10°C for each cultivar of potato.

Baked All Red and Mountain Rose made from potato tubers stored at 4°C received lower mean scores for color (4.0, 4.0, respectively). At 10°C, baked All Red and Mountain Rose made from potato tubers stored at 4°C received lower mean scores for color (3.2, 3.6, respectively). Storage temperature had no effect on color of baked potatoes for all cultivars except for baked All Red ($p \leq 0.05$) which received lower liking scores at 10°C than potatoes stored at 4°C. Taste is an important attribute in sensory analysis. Taste mean scores ranged from 5.1 to 6.4 for baked potato cultivars made from potato tubers stored at 4°C and from 4.3 to 6.5 for baked potatoes made from stored potato tubers at 10°C. Baked Russet Burbank, Rio Grande Russet, Atlantic, and AC99375-1RU made from potato tubers stored at (4°C = 6.2, 6.2, 6.4, and 5.9) or (10°C = 6.5, 5.9, 6.5, and 6.4) had similar mean scores for taste. Baked All Red potato tubers stored at 4°C or 10°C and baked Mountain Rose potato tubers stored at (4°C = 5.1, 4.3 and 5.2) had the lowest taste mean scores. Storage temperature had no effect on the taste for all baked potato cultivars except for baked All Red ($p \leq 0.05$) which received lower liking scores at 10°C than potatoes stored at 4°C. Mean values for texture of all baked cultivars ($p \leq 0.05$) ranged from 5.4 to 6.5 for baked potatoes made from potato tubers stored at 4°C and from 4.8 to 6.6 for baked potatoes made from potato tubers stored at 10°C. Atlantic, Burbank, Rio Grande, and All Blue baked potatoes made from potato tubers stored at 4°C had the same mean texture scores of 6.3, 6.3, 6.5, 6.2, respectively ($p \leq 0.05$). At 10°C storage, baked Burbank, Atlantic, and AC99375-1RU scored the same texture mean scores of 6.6, 6.5, 6.1, respectively ($p \leq 0.05$). Baked Mountain Rose, All Blue and Rio Grande received similar texture mean scores of 5.6, 5.8, and 5.8, respectively ($p \leq 0.05$). Baked All Red received the lowest texture mean score of 4.8 ($p \leq 0.05$). Storage temperature had no effect on the texture for all baked potato cultivars except for All Red ($p \leq 0.05$) which received lower liking scores at 10°C than potatoes stored at 4°C. For

overall acceptability, mean scores for all baked potato cultivars made from potato tubers stored at 4°C ranged from 4.8 to 6.7 and from 3.9 to 6.5 for baked potato cultivars made from potato tubers stored at 10°C. Baked Atlantic, Burbank, and Rio Grande potatoes made from potato tubers stored at 4°C received the similar highest mean overall acceptability scores of 6.7, 6.5 and 6.3, respectively ($p \leq 0.05$). Baked All Blue and AC99375-1RU made from potato tubers stored at 4°C received the same mean overall acceptability scores of 5.7 and 6.2, respectively ($p \leq 0.05$). At 10°C storage temperature, baked Rio Grande, Burbank, Atlantic, and AC99375-1RU potatoes received the similar mean overall acceptability scores of 6.1, 6.4, 6.5, and 6.4, respectively ($p \leq 0.05$). Baked All Red and Mountain Rose made from potato tubers stored either 4°C or 10°C received the lowest mean overall acceptability score than the others. Storage temperature had no effect on the overall acceptability for any baked of the potato cultivars except All Red.

Ranking was conducted on a scale of 1 = "liked most" to 7 = "liked least". Russet Burbank is the most common cultivar for baking. Baked Atlantic potatoes made from potato tubers stored at 4°C ranked "liked most". Baked Burbank and Rio Grande ranked next, followed by All Blue and AC99375-1RU. Baked All Red and Mountain Rose made from tubers stored at 4°C were ranked "liked least" than the other cultivars. At 10°C, Baked Atlantic, Russet Burbank, and AC99375-1RU were ranked "liked most". Baked Rio Grande ranked next followed by All Blue, then Mountain Rose and All Red. Storage temperature had no effect on the ranking for any baked potato cultivars. For baked color-fleshed potatoes, All Blue (purple-fleshed cultivar) were preferred for all the sensory parameters followed by red-fleshed potatoes. Some purple-fleshed potatoes had comparable sensory scores with traditional cultivars. Sensory attributes were influenced by the cultivar ($p \leq 0.05$). Storage for three months at 10°C had a significant effect on

the sensory parameters for baked All Red potatoes compared to storage for three months at 4°C of the 89 consumer panelists, 27% ranked baked potatoes from the Atlantic cultivar stored at 4°C as "liked most". Rio Grande Russet (21%), Russet Burbank (21%), and All Blue (14%) were ranked next for liking. All other cultivars received less than 10% ranking scores for number 1 "liked most". Baked All Red potatoes were liked least for baking with only three panelists ranked them "liked least." All Blue potatoes were "liked most" among color-fleshed potatoes. At 10°C, 20% of the 84 consumer panelists ranked potatoes baked from the AC99375-1RU cultivar stored at 4°C as "liked most" Russet Burbank (22%) and Atlantic (22%) were ranked next for liking. All other cultivars received less than 12% ranking scores for "1=liked most" Our findings for baked potatoes agreed with the sensory data from (Madiwale, 2012), who used the Yukon Gold as the standard for baked potatoes. They found that only AC97521-IR/Y baked potatoes after three months of storage, received a higher score than the standard. In our study, after three months of storage, Atlantic and Rio Grande Russet potatoes baked from potato tubers stored at 4°C or 10°C had similar sensory attribute mean scores as baked Russet Burbank potatoes. Russet Burbank potatoes are a common cultivar for baking. For other cultivars evaluated in the current study, AC99375-1RU potatoes stored for three months at 10ºC received highest mean score for appearance (6.5), color (6.6), taste (6.4), texture (6.1), and overall acceptability (6.4) from 94 consumer panelists after baking. Kaspar et al., (2013) evaluated baked purple- white- and yellow-fleshed potatoes (204°C for 105 minutes) for the aroma and appearance. They found that the panelists ranked the aroma and appearance of white- and yellow-fleshed potatoes higher than purple-fleshed potatoes ($p \leq 0.05$). However, no significant differences were found in the overall acceptability among the potato cultivars. This finding agreed with our sensory data for baked white-fleshed potatoes (204°C for 60 minutes) in that white-fleshed potatoes were ranked higher

than purple- or red-fleshed potatoes. Purple-fleshed potatoes were comparable to white-fleshed potatoes. This suggested that consumers tend to like white- and color-fleshed potatoes (All Blue) better than other cultivars.

3.3. Texture analysis for raw potatoes

Texture analysis was performed on raw potato tubers after three months of storage at 4°C or 10°C (Table 4.5) by using the texture analyzer (TA-XT2; Texture Technologies Corp, Scarsdale, NY, USA). The density for three biological replicates was measured by dividing the weight (g) for each tuber, with the volume of tuber (mL). Storage temperature had no effect on the density for all potato tuber cultivars stored either at 4°C or 10°C, except Russet Burbank tubers stored at 10°C. The density Russet Burbank tubers decreased when stored at 10°C compared to those stored at 4°C. However, storage temperature had a significant effect on maximum force for all potato cultivars tested (Table 4.6). Storage of potato tubers at 4°C for long time caused softening of the tubers (Pardede, 2005). This is related to biochemical and physical changes in potato tubers. The biochemical change is usually related to starch degradation due to the activation of enzymes α-, and β-amylase enzymes (Cochrane et al., 1991), while the physical changes caused by the degradation of pectic polysaccharides by cell wall-degrading enzymes (Willats et al., 2001). No correlations were observed between the density and maximum force ($r = - 0.17$).

Table 4.4. Sensory attribute mean scores for baked potatoes from white- red- and purple-fleshed potato cultivars after three months of storage at 4°C or 10°C.

Sensory Attributes	Baked White- Red- and Purple-Fleshed Potatoes						
	Rio Grande	Burbank	Atlantic	AC99375-1RU	All Blue	Mountain Rose	All Red
	4°C	4°C	4°C	4°C	4°C	4°C	4°C
	10°C	10°C	10°C	10°C	10°C	10°C	10°C
Appearance	7.0^{abA}	6.9^{abA}	7.3^{aA}	6.5^{bA}	4.9^{cA}	3.8^{dA}	4.0^{dA}
	6.8^{aA}	6.9^{aA}	6.9^{aA}	6.5^{aA}	4.5^{bA}	3.8^{cA}	3.3^{cB}
Color	7.1^{aA}	6.9^{abA}	7.3^{aA}	6.5^{bA}	5.0^{cA}	4.0^{dA}	4.0^{dA}
	6.9^{aA}	7.0^{aA}	7.0^{aA}	6.6^{aA}	4.7^{bA}	3.6^{cA}	3.2^{cB}
Taste	6.2^{abA}	6.2^{abA}	6.4^{aA}	5.9^{abA}	5.8^{bA}	5.2^{cA}	5.1^{cA}
	5.9^{abA}	6.5^{aA}	6.5^{aA}	6.4^{aA}	5.6^{bA}	4.9^{cA}	4.3^{dB}
Texture	6.3^{abA}	6.3^{abA}	65^{aA}	5.9^{bcA}	6.2^{abA}	5.6^{cA}	5.4^{cA}
	5.8^{bA}	6.6^{aA}	6.5^{aA}	6.1^{abA}	5.8^{bA}	5.6^{bA}	4.8^{cB}
Overall Acceptability	6.3^{abA}	6.5^{abA}	6.7^{aA}	6.2^{bcA}	5.7^{cA}	4.9^{dA}	4.8^{dA}
	6.1^{aA}	6.4^{aA}	6.5^{aA}	6.4^{aA}	5.3^{bA}	4.5^{cA}	3.9^{cB}
Rank	3.3^{bA}[3]	3.3^{bA} [2]	3.1^{aA} [1]	3.9^{cA}[4]	4.2^{cA}[5]	4.2^{dA}[6]	5.4^{dA}[7]
	3.8^{bA}[4]	2.9^{aA}[1]	2.9^{aA}[2]	3.0^{aA}[3]	4.4^{cA}[5]	5.2^{dA}[6]	5.7^{eA}[7]

Mean scores based on hedonic scale 1-9 (1= disliked extremely and 9 = liked extremely) from 94 untrained consumer panelists. Values in brackets indicate ranking order (1 = Liked most among the four cultivars). Different letters indicate differences ($p \leq 0.05$). Lowercase letters (a-e) on mean scores indicate differences in rows among seven cultivars at the same storage temperature; whereas, uppercase letters (A, B) on mean scores indicate differences in columns between 4°C or 10°C for each cultivar/selection.

Table 4.5. Texture analysis for raw from white- red- and purple-fleshed potatoes from seven different cultivars after three months of storage at 4°C or 10°C.

Cultivar/Selection	Raw White- Red- and Purple-Fleshed Potatoes		
	Storage temperature (ºC)	Density(g/mL)	Maximum Force (g)
Atlantic	4	1.09 ± 0.01[a]	14,468 ± 694[b]
	10	1.06 ± 0.03[a]	24,397 ± 989[a]
AC99375-1RU	4	1.05 ± 0.02[a]	15,041 ± 176[b]
	10	1.03 ± 0.02[a]	24,563 ± 848[a]
Russet Burbank	4	1.03 ± 0.00[a]	19,131 ± 274[b]
	10	0.99 ± 0.02[b]	30,004 ± 200[a]
Rio Grande Russet	4	1.00 ± 0.03[a]	14,890 ±163[b]
	10	1.04 ± 0.02[a]	26,444 ± 217[a]
All Red	4	1.06 ± 0.00[a]	14,382 ± 502[b]
	10	1.05 ± 0.05[a]	27,090 ± 357[a]
All Blue	4	1.02 ± 0.03[a]	18,691 ± 542[b]
	10	0.98 ± 0.02[a]	33,881 ± 169[a]
Mountain Rose	4	0.97 ± 0.04	14,235 ± 896[b]
	10	1.00 ± 0.00[a]	25,787 ± 149[a]

Means with different letters indicate significant differences in columns ($p \leq 0.05$). Results are presented as mean ± SE of three biological replicates.

3.4. Correlations

3.4.1. Acrylamide and reducing sugars

Positive correlations between reducing sugars and AL were observed by Kalita and Jayanty (2013) and Halford et al. (2012). Our results followed a similar trend where AL content in potato chips was correlated positively with glucose and fructose content in raw potato tubers stored for six months either at 4°C or 10°C (r = 0.25, $p \leq 0.05$, r = 0.83, $p \leq 0.05$, at 4°C and r = 0.70, $p \leq 0.05$, and 0.82, $p \leq 0.05$ at 10°C, respectively). In baked potatoes, weak correlations were observed between AL content and reducing sugars in raw potato tubers (r = 0.40, $p \leq 0.05$ and r = 0.24, $p \leq 0.05$, at 4°C and r = 0.10, $p \leq 0.05$ and r = - 0.17, $p \leq 0.05$ at 10°C, respectively).

3.4.2. Acrylamide and sensory attributes

It is well known that the AL content in potato tubers and potato products are affected by multiple factors such as storage and cultivar (Friedman and Levin, 2008; Noti, 2003). Therefore, we attempted to clarify the correlation between sensory attributes and AL formation in potato chips and baked potatoes. Pearson correlation coefficients were calculated for all sensory attributes (appearance, color, taste, crispiness, overall acceptability, and rank) after three months of storage and AL content. At three months of storage at 4°C, negative correlations were observed between all sensory attributes and AL content (r = - 0.54, - 0.53, - 0.43, - 0.55 and - 0.46, respectively). All correlations were not significant (p = 0.21, 0.22, 0.33, 0.20 and 0.30, respectively). Positive correlation was observed between the rank and AL content in potato chips also after three months of storage (r = 0.59). Our results agreed with our previous work that the appearance of potato chips negatively correlated with AL content. However, conflicting results of the correlation between sensory attributes (color, taste, crispiness, overall acceptability, and

rank) and AL content of potato chips were seen in this study when compared with our previous study. Similarly, at three months of storage at 10ºC, negative correlations were observed between sensory attributes (appearance, color, taste, crispiness, and overall acceptability) and AL content (r = - 0.36, - 0.40, - 0.30, 0.072 and - 0.027, respectively). This suggests that high levels of AL affect the sensory attributes and overall preference of potato chips. All correlations were not significant at p = 0.21, 0.22, 0.34, 0.19, 0.30 for chips made from potato tubers stored for three months at 4°C while p = 0.43, 0.37, 0.51, 0.88, 0.95 for potato chips made from potato tubers stored for three months at 10°C. After three months of storage, rank was positively correlated with AL levels, suggesting higher preference of potatoes (r = 0.59, 0.35; 0.44 at 4°C or 10ºC ($p \leq$ 0.16), respectively). Baked potatoes are considered as a healthy food due to the fact that potato chips have processing induced-AL and oil-containing chips. Recent reports suggested that a strong correlation between AL formation and the crust color of the bread was observed when the bread was baked at temperature higher than 200°C (Ahrne et al., 2007). Therefore, we tried to clarify the correlation between sensory attribute and AL formation in baked potatoes. Pearson correlation coefficients were calculated between attributes (appearance, color, taste, texture, overall acceptability, and rank) and AL content of baked potatoes made from potato tubers stored for three months at 4ºC or 10ºC and AL content (Table 4.6). After three months of storage at 4°C, negative correlations were observed between the appearance, color, taste, texture, and overall acceptability of baked potatoes and AL content (r = - 0.42, - 0.39, - 0.45, - 0.47 and - 0.45, respectively). A positive correlation was observed between the rank and AL content of baked potatoes as well (r = 0.41). Also after three months of storage at 10ºC, negative correlations were observed for the appearance, color, taste, texture, and overall acceptability of baked potatoes and AL content (r = - 0.71, - 0.67, - 0.64, - 0.65, and - 0.63, respectively). This

suggests that high levels of AL affect the sensory attributes and overall preference of potato chips. After three months of storage, rank was positively correlated with AL level suggesting higher preference of potatoes (r = 0.41, 0.70; $p \leq 0.36$, 0.08 at 4°C and 10ºC of storage temperature, respectively). Correlation coefficients for this study were lower than those previously published (Amer et al., 2014). Differences could be due to different cultivars used in the current study. Also, in the first study four potato cultivars were used, while seven cultivars were evaluated in the current study. Panelists could have been fatigued by the larger number of samples used in the current study.

Table 4.6. Correlation coefficients between reducing sugars and sensory attributes with acrylamide in potato chips and baked potatoes.

Reducing Sugars / Sensory Parameters	Chips		Baked	
	4°C	10ºC	4°C	10ºC
Glucose	0.25	0.70*	0.40	0.10
Fructose	0.83**	0.82**	0.24	-0.17
Appearance	-0.54	-0.36	-0.42	-0.71
Color	-0.53	-0.40	-0.39	-0.67
Taste	-0.43	-0.30	-0.45	-0.63
Crispiness	-0.55	-0.072	-0.47	-0.65
Overall Acceptability	-0.46	-0.028	-0.45	-0.63
Rank	0.59	0.35	0.40	0.70**

Note that sensory attributes were correlated with acrylamide in potato chips and baked potatoes stored for three months either at 4°C or10ºC. The content of reducing sugars of raw potatoes stored for six months either at 4°C or 10ºC were correlated with acrylamide in potato chips and baked potatoes.
*Correlation is significant at $p = 0.08$
**Correlation is significant at $p = 0.01$

3.4.3. Acrylamide, reducing sugars (glucose and fructose), total phenolic, anthocyanins, total glycoalkaloids and maximum force

Significant positive correlations previously were observed between glucose and fructose content in raw potato tubers and AL content in potato chips (r = 0.85; $p \leq 0.05$ and r = 0.94; $p \leq 0.05$, respectively; Amer et al., 2014). Low storage temperature (4°C) caused softening of the

potato tubers (Pardede, 2005). In the current study, we observed that storage potato tubers at 4°C had lower maximum force when compared to potato tubers stored at 10°C. This can be explained by starch degradation (Cochrane et al., 1991), which led to increased reducing sugar content in raw potato tubers which contributed to AL formation in potato products. Pearson correlation coefficients were calculated between AL, reducing sugars (glucose and fructose) and maximum force. Weak negative correlations were observed between AL in potato chips made from potato tubers stored either at 4°C or 10°C and maximum force, although not significant (r = - 0.32, $p \leq 0.1$; -0.18 , $p \leq 0.4$, respectively). Similar results was observed between glucose in raw potato tubers stored either at 4°C or 10°C and maximum force (r = 0.30; $p \leq 0.2$ and r = -0.35; $p \leq 0.1$, respectively), and fructose in raw potato tubers stored either at 4°C or 10°C and maximum force (r = 0.21; $p \leq 0.3$ and r = -0.18; $p \leq 0.4$, respectively). More research needs to be done on the relationships between AL content in potato chips, and reducing sugars in raw potato tubers, and maximum force. This finding can be applicable for using the measurement of maximum force as an indicator for reducing sugars content and thereby, to predict AL content in final products. In the current study, weak negative correlations were observed between AL, reducing sugars, and maximum force for potato tubers subjected to maximum force measurement. Measurements were completed on potatoes used for baking rather than chipping. Chipped potatoes were subjected to reconditioning for three weeks at 15°C after storage at low temperatures for sugar-starch conversion purposes.

Table 4.7. Correlation coefficients between reducing sugars, total phenolic, anthocyanins, total glycoalkaloids and acrylamide of potato chips with maximum force after three months of storage at 4°C or 10°C.

Compound	Maximum Force	
	4°C	10°C
Glucose	- 0.30	-0.35
Fructose	0.21	-0.18
Acrylamide	- 0.32	-0.18
Total Phenolic	0.36	-0.53
Anthocyanin	-0.69	-0.99*
Total Glycoalkaloids	0.22	0.57

*Correlation is significant at $p = 0.05$

Total phenolics, anthocyanins, and total glycoalkaloids (Table 4.2) also were correlated with maximum force of potato tubers stored at either 4°C or 10°C. There was a strong undirected relationship between anthocyanin in potatoes at 10°C ($r = - 0.99$, $p \leq 0.05$) and maximum force. Potatoes at 4°C were softer due to low storage temperature which yielded more degradation of pectic substances in the cell walls by cell wall-degrading enzymes (Willats et al., 2001). Anthocyanins also are higher in the skins of potatoes. No other correlation coefficients were significant ($p \leq 0.05$).

4. Conclusions

Storage of potato tubers at 4°C resulted in an increase of the reducing sugars in the raw potato tubers, which led to a higher level of AL in potato chips. The interaction effect of storage temperature and time is cultivar-dependent. AC99375-1RU had the lowest AL content and Mountain Rose had the highest AL content among the 10 cultivars after three months of storage. The interaction effect of storage time and temperature on AL in potato chips resulted in either increased, constant or decreased AL content after six months of storage. AL content in potato chips positively strongly correlated with reducing sugars in raw potato tubers, but weakly correlated with AL in baked potatoes. Atlantic chips made from potato tubers stored at 4°C or

10°C ranked "liked most" among the other six cultivars. Storage temperature had no effect on appearance of Russet Burbank, All Blue, or on the color, taste, or overall acceptability of Atlantic and All Blue chips. Moreover, storage had no effect on sensory attributes of all baked potato cultivars except All Red. Storage temperature had no effect on the density for potato cultivars at either 4°C or 10°C except Russet Burbank tubers stored at 10°C. However, storage temperature had a significant effect on maximum force for those cultivars stored at 4°C.

CHAPTER FIVE: EFFECT OF GENOTYPE, STORAGE, AND PROCESSING ON TOTAL PHENOLICS, ANTHOCYANIN CONTENT, ANTIOXIDANT ACTIVITY, AND VITAMIN C CONTENT OF WHITE-FLESHED VERSUS COLOR-FLESHED POTATOES

Overview:

Potatoes are a rich source of health-benefiting bioactive compounds. The effect of cultivar, storage (time and temperature), and processing (baking and chipping) on bioactive compound content and antioxidant activity was evaluated using 10 potato cultivars. Five color-fleshed cultivars (Purple Majesty, All Red, All Blue, Mountain Rose, advanced selection CO97227-2P/PW), and five white-fleshed cultivars (Lenape, Rio Grande Russet, Russet Burbank, Atlantic, and advanced selection AC99375-1RU) were stored at 4°C or 10°C for three or six months. Raw, baked, and chipped samples from initial and stored tubers were analyzed for total phenolic content (FCR), anthocyanin content (pH differential), antioxidant activity (DPPH and ABTS), and vitamin C content (Ultra-Performance Liquid Chromatography-Diode Array Detector; UPLC-DAD). Purple-fleshed potatoes had significantly higher total phenolic content, anthocyanin content, and antioxidant activity followed by red-fleshed and white-fleshed potatoes. The effect of storage time and temperature on total phenolic content, anthocyanin content, and antioxidant activity was cultivar-dependent. However, processing had a pronounced effect – for example, baking led to a significant ($p \leq 0.05$) increase in total phenolic content, anthocyanin content, and antioxidant activity. Where as chipping and frying led to significant losses in total phenolic content, anthocyanin content, and antioxidant activity. Purple- and red-fleshed potatoes could serve as potential sources of anti-oxidant and anti-inflammatory

anthocyanin compounds in the human diet even after storage and processing. Storage of potato tubers at either 4°C or 10°C for three months resulted in a rapid decline in vitamin C content in all potato genotypes compared to non-stored potatoes. After three months of storage, vitamin C content remained constant until the end of storage. Vitamin C content was significantly reduced in chipped potatoes compared to baked and unprocessed potatoes in all cultivars. Thus, it is critical to evaluate the effect of farm to fork operation on health-benefiting compounds before releasing the cultivars for food crops and develop novel processing technologies such as vacuum frying methods to retain the health-benefiting compounds.

1. Introduction

Potatoes (*Solanum tuberosum* L.) follow maize, wheat, and rice in food crop production across the world (Ezekiel et al., 2013). In addition to carbohydrates, protein, and resistant starch, potatoes are also a good dietary source of vitamins and minerals (Öhrvik, 2010). Potatoes have significant amounts of health-benefiting phytochemicals and antioxidants (Wu et al., 2004). Compared to fruits such as apples (contain from 1642 to 4728 mg gallic acid equivalent; (GAE) per kg gram fresh weight (gfw) in the peel and from 160 to 1056 mg GAE/kgfw in the flesh (Wang, 2014) and from 406 µg/gfw to 1694 µg/gfw in citrus fruits (Ramful et al., 2011), potatoes have less phenolic compounds (39 -106 mg GAE /100 gfw; Clark, 2011). However, potatoes ranked 3rd in their contribution of polyphenol content in the American diet due to their high level of consumption (51 kg / person/year; Osteen, 2012).

There are more than 5,000 potato cultivars around the world (Lutaladio and Castaidi, 2009) with different shape, color, size, textures, and cooking characteristics (Food and Agriculture Organization, 2008). Navarre et al. (2009) reported a 15-fold variation in phenolic

content between 100 tested potato cultivars. This also was confirmed by Andre et al. (2007), who observed a 11-fold difference in phenolic content between 74 Andean potato cultivars.

Potatoes are a seasonal crop and the necessity of providing potatoes into the market; potatoes can be stored up to one year before being processed and consumed. Moreover, the majority of potato consumption in most cultures involves baked, fried, boiled, and microwaved, which represents the use of 68% of potatoes sold (Lucier and Ali, 2006). Factors including storage and processing can cause damage to plant cells rapidly transforming potato phenolics into different reaction products (Spooner et al., 2005; Cheynier, 2005), thus reducing total phenolic content and antioxidant activity (Nicoli et al., 1999; Dewanto et al., 2002).

The concentration and composition of phenolic compounds in potatoes are dependent on cultivar, environmental stresses, post-harvest storage, processing parameters, and the extraction process. The phenolic compounds are normally present between the cortex and peel of the tuber, but reduced towards the center of the tuber (Friedman, 1997). The most abundant of these compounds are phenolic acids, carotenoids, and anthocyanins such as chlorogenic acid, violaxanthin, and petunidin, respectively (Payyavula et al., 2013). Potato polyphenols range from 530 to 1770 µg/g (Alsaikhan et al., 1995). Purple-fleshed potato cultivars were reported to contain a higher amount of phenolic content ranging from 5 to 6 mg/g dry weight (dw) more than white-fleshed potato cultivars (Navarre et al., 2009). A wider variation of chlorogenic acid content among cultivars (13.2 to 68.3 mg/100 gfw) was reported by Reddivari et al. (2007b). The chlorogenic content is 10-fold higher in pigmented cultivars (e.g., Mountain Rose and Purple Majesty) compared to non-pigmented cultivars (e.g., Yukon Gold). This is also confirmed by Hamouz et al. (2013), who found a significant effect of genotype on chlorogenic acid content, which ranged from 0.074 mg/gfw in Agria cultivar to 0.825 mg/gfw in Vitelotte cultivar. Total

anthocyanins in color-fleshed potatoes are also cultivars-dependent. Total anthocyanins ranged from 0.2485 mg/gdw to 2.258 mg/gdw (Lachman et al., 2012). Color-fleshed potatoes contain glycosylated anthocyanins, which range from 17 to 20 mg/100 gfw in red-fleshed potatoes and from 20 to 38 mg/100 gfw in purple-fleshed potatoes (Brown et al., 2005). Lachman et al. (2009) studied the total anthocyanins in 15 red- and purple-fleshed potato cultivars produced in five different locations in the Czech Republic and Blaue St. Galler plus an additional purple-fleshed potato cultivar from Switzerland. They found total anthocyanins ranged from 0.7 to 74.4 mg C-3- G equivalents /100 gfw. Phenolic content of potatoes is not only influenced by cultivar type but due to genotype/cultivar interaction with environmental stress, agronomic, and processing parameters. Potato tubers contain L-ascorbic acid (Keijbets and Ebbenhorstseller, 1990) and in American diet, it is estimated that one medium potato (5.3 oz) with the skin contains 45% of the recommended daily value for vitamin C (Percent daily values are based on a 2,000 calorie diet; United States Potato Board, 2007). Vitamin C content in potato tubers is approximately 10 to 40 mg /100 gfw (Dale et al., 2003; Love et al., 2008). Vitamin C content in Colorado-grown potato cultivars ranged from 14.5 mg /100 gfw in CO97226-2R/R to 32.1 mg /100 gfw in Yukon Gold (Kulen et al., 2013). In addition to genetic differences, vitamin C content is known to be degraded by storage, cooking, and processing of potatoes (Burgos et al., 2009). Storage conditions including storage time, temperature, and light may also have an influence on the retention of potato bioactive compounds. Storage time and temperature are important factors that may decrease potato bioactive compounds in final products. The phenolic content was elevated up to 100% in some cultivars, whereas in other cultivars it remained constant after storage (Stushnoff et al., 2008). Blessington et al. (2010) reported that only the reconditioned potatoes at 20°C after storage at 4°C had a significant increase in their phenolic content in comparison with

just storage at 4°C or 20°C. We have previously shown that total phenolic content increased with storage only in purple-fleshed cultivars, CO97227-2P/PW and Purple Majesty (Madiwale et al., 2011). This is confirmed by Kulen et al. (2013), who found that the total phenolic content was significantly higher in pigmented potato cultivars compared to yellow- and white-fleshed cultivars after storage. Additionally, the total phenolic content was higher at harvest and fluctuated after two and four months of storage and finally was increased after seven months of storage, but not significantly in all tested cultivars except the advance potato selection CO97227-2P/PW and CO97222-IR (Kulen et al., 2013). Storage of potato tubers at 4°C influenced total anthocyanins, but was dependent on cultivars. Total anthocyanin content increased by 18.5% and 12.1% in the Violette and Highland Burgundy Red cultivars, respectively, whereas, a 33.9% decrease of total anthocyanin was found in the Valfi cultivar (Lachman et al., 2012). Lewis et al. (1998) observed that the concentration of anthocyanins in four pigmented cultivars increased by storage at 4°C for six months. However, stored potato tubers at 10°C or 18°C did not show a significant increase in anthocyanin content. Lachman et al. (2012) studied the effect of cultivar, storage, and baking on the content of anthocyanins in color-fleshed potatoes. Storage at 4°C on anthocyanins in the Violette and Highland Burgundy Red cultivars resulted in an increases by 18.5% and 12.1%, respectively. However, the anthocyanin content of the Valfi cultivar decreased by 33.9%. Storage conditions can up-regulate gene coding for dihydroflavonol reductase (DFR) and anthocyanidin synthase (ANS) by conversion of the starch to sugar (Isherwood, 1976). These enzymes are utilized in anthocyanin biosynthesis and thereby, cause an increase in the anthocyanin concentration (Vitrac et al., 2000; Gollop et al., 2002; Solfanelli et al., 2006). Moreover, low storage temperatures can induce the activity of phenylalanine

ammonia-lyase (PAL), a key regulatory enzyme in the biosynthesis of polyphenols, which causes an elevation in the phenolic content (Jiang and Joyce, 2003).

Vitamin C is one of the most important micronutrients and has many biological activities in the human body. The vitamin C content in potato tubers after storage for four months at 4°C of 33 *Solanum tuberosum* genotypes was studied by Dale et al. (2003). They found a significant difference in vitamin C content in all tested cultivars after storage. Vitamin C degradation in potato tubers after storage for two months at 4°C was dependent on potato cultivar. However, after four months of storage at 4°C, the vitamin C levels in all potato cultivars decreased rapidly (Kulen et al., 2013). The effect of three cooking methods (boiling, baking, and microwaving) and storage time on vitamin C content in potato tubers was studied by Burgos et al. (2009). They found that boiled potato tubers had lower vitamin C than baked or microwaved potato tubers and the content of vitamin C decreased as the storage time increased.

Baked, fried, and microwaved potato samples resulted in higher total phenolic content than boiled and uncooked samples (Blessington et al., 2010). Frying of potatoes resulted in greater levels of phenolic acids compared to uncooked and boiled samples. The boiled, baked, and uncooked samples had lower phenolic acids content than the fried or microwaved samples (Blessington et al., 2010). Our lab recently showed the phenolic content of the baked and chipped potato samples ranged from 11.3 to 307.8 mg GAE/100 gfw and from 1.9 to 18.7 mg GAE/100 gfw, respectively, over the entire storage period (Madiwale, 2012). The effect of chipping resulted in retention from only 4% to 7% of total phenolics when compared to unprocessed samples (Madiwale, 2012). Chipping resulted in 97% losses of total phenolics as compared with unprocessed samples over the entire storage time. The increase of total phenolic

and antioxidant content in baked and fried samples is explained by improved extractability of the phenolic compounds from the cellular matrix of cooked samples.

Potato bioactive compounds could be retained during food processing operations by controlling processing factors. Processing of potatoes may have positive or negative influence on the content of phenolic compounds. The effect of various cooking methods was studied by Perla et al. (2012), who found that boiling, microwaving, and baking reduced the total phenolics in all tested cultivars, but boiling retained more compared to another two cooking methods. The time of baking affects the total phenolic acid and chlorogenic acid content. Baking at 204°C for 45 minutes resulted in a loss of chlorogenic acid in Atlantic and Yukon Gold potatoes.

In comparison to 30 minutes baking, the total phenolic and chlorogenic acid content were increased; however, it was still higher than that of unprocessed potatoes (Navarre et al., 2010). The effect of baking on total phenolic content resulted in both decreased and increased phenolic content, depending on the cultivars (Madiwale, 2012). Anthocyanins are very sensitive, unstable compounds affected by several factors such as pH, light, oxygen, enzyme activity, concentration, vitamin C, sugars, genotype, storage conditions, processing, and cooking methods (Patras et al., 2010; Cavalcanti et al., 2011; Lachman et al., 2013). Thus, frying of potatoes resulted in 38 to 70% degradation of anthocyanin compounds (Kita et al., 2004). The effect of thermal treatments resulted in a significant decrease of total anthocyanin content in all cultivars in comparison with unprocessed tubers. Thermal processing was reported to cause degradation of anthocyanins and enzymes in the presence of polyphenol oxidase. The anthocyanin content of purple-fleshed potatoes ranged from 13.4 to 81.3 mg C-3-G equivalents/100 gfw in baked samples, and from 0.8 to 3.2 mg C-3-G equivalents/100 gfw in chipped samples (Madiwale, 2012). Anthocyanins have been shown to have the highest retainability amongst tested phytochemicals after cooking

methods (boiling > microwave > baked; (Lachman et al., 2013). The variation in anthocyanin content between baked and chipped potatoes was due to the processing effects (Dick Vreugdenhil, 2011). The chipping process of potatoes led to significant losses in phenolic acid content.

Potatoes are considered as antioxidant-rich crop (Evers and DeuBer, 2012). Antioxidant activity is also determined by the potato cultivar and is slightly influenced by cooking conditions (Nicoli et al., 1999; Reddivari et al., 2007; Blessington et al., 2010). Antioxidant activity of baked, baked, and uncooked potatoes was reported to be lower compared to fried and microwaved potatoes (Blessington et al, 2010). The highest values of antioxidant activity were observed for steamed and baked potatoes (Lachman et al., 2012). Color-fleshed potatoes had a higher antioxidant activity due to high anthocyanin and phenolic acid content (Madiwale et al., 2012). Vitamin C is not stable after postharvest and cooking methods are responsible for its degradation in potato products. Thermal treatment of potato tubers contributed to 30 % loss of vitamin C and 10 % loss of vitamin C after keeping cooked potatoes hot for one hour (Hagg et al., 1998). Lachman et al. (2013) observed that vitamin C content was reduced in all cooking treatments with the highest decrease in baked potatoes and the lowest reduction in boiled potatoes. After baking, the vitamin C content decreased from 23% (Highland Burgundy Red) to 56% (Agria) of the initial levels of raw non-peeled tubers. Only a few publications are available on the effect of genotype and combined effect of both storage time and temperature, and processing on potato bioactive compounds and vitamin C. Thus, it is very critical to study the effect of genotype, storage conditions, and processing on the content of bioactive compounds and vitamin C of different potato cultivars, as well as their corresponding antioxidant activities.

2. Materials and methods

2.1 Chemicals

Methanol for phenolic extractions was purchased from EMD chemicals (Philadelphia, PA, USA). Gallic acid was acquired from Fisher Scientific (Pittsburgh, PA, USA). Potassium persulfate, sodium phosphate (monobasic and dibasic), sodium chloride, potassium chloride, sodium acetate, sodium carbonate, Folin-Ciocalteu reagents, ABTS, and DPPH were purchased from Sigma (St. Louis, MO, USA). Metaphosphoric acid and 1, 4-Dithio-DL-threitol were obtained from Alfa Aesar (Ward Hill, MA, USA).Vitamin C standard was purchased from BDH Chemicals (Radnor, PA, USA).

2.2. Potato materials

Ten potato cultivars: Atlantic, Purple Majesty, All Red, All Blue, Lenape, Rio Grande Russet, Russet Burbank, Mountain Rose, and two advanced selections (CO97227-2P/PW; AC99375-1RU) were grown at San Luis Valley Research Center, CO. The growth period for all cultivars in Dunul cobbly sandy loam soil was for 100 to 110 days, beginning from the middle of May until September. Potato plants were treated with sulfuric acid for vine killing purpose approximately three weeks before harvesting time. Each potato cultivar was randomly divided to three groups and placed in plastic bags (initial, three, or six months of storage). Potato tubers were stored at 4ºC or 10ºC. At the initial and at the end of each storage period, the numbered bags of potatoes were weighed before sampling for analysis to obtain weight loss during storage.

2.3. Potato baking and chipping

For unprocessed potatoes as control, potato tubers were diced and stored at -20°C. For baking, potato tubers from two red-fleshed cultivars, three purple-fleshed cultivars, and five white-fleshed potatoes cultivars were baked at initial, three, or after six months of storage in a

preheated conventional oven at 204°C for one hour. Each potato tuber before baking was washed, dried, wrapped in food-grade aluminum foil, and pierced approximately 1.5 cm deep with a knife at approximately three cm intervals. Baked potatoes were cooled for 15 to 20 minutes after cooking, then diced with the skin into small pieces weighing from seven to ten gram and stored at -20°C. For chipping, potato tubers were cleaned and chipped by an industrial chipper (Ditto Dean Food Prep, model TRS 23 with C-2 blade; Wasserstrom Company, Ohio, USA). The final thickness of raw potato chips was 2 mm. Raw chips were washed under warm running water for one minute. The chipped slices were then placed in strainer trays to remove excess water and fried in Bakers & Chefs Clear Frying oil at 185°C until the bubbling stopped (one minute 45 s to two minutes 15 s depending on tuber specific gravity). The fried potato chips were placed on paper towels to absorb any extra oil, and were cooled for 15 to 20 minutes. Potato chips were then labeled, bagged, and stored at -20°C until analysis.

2.4. Preparation of potato extracts

Ten grams of raw or baked potato samples were homogenized with 25 mL of 80% methanol acidified with formic acid (0.1% v/v) for at least one minute. For potato chips samples, 20 mL of 80% acidified methanol were added and five mL of distilled water were added for each tube. Homogenized samples were then poured into chloroform resistant tubes and were kept on ice. All tubes of potato extracts were then vortexed every 15 minutes for one hour. After that 15 mL of chloroform were added to the tubes and then the tubes were vortexed every ten minutes for 30 minutes. Next, the tubes were centrifuged at 2200 x g for 10 minutes. Finally, all tubes were stored overnight at 4ºC to allow layer separation. The next day, the 15 to 25 mL of supernatants were collected and stored at -20 ºC for further analysis.

2.5. Quantification of total phenolics

Folin-Ciocalteu colorimetric method was used to determine total phenolic content in potato extracts (Singleton et al., 1999). Thirty five µL of diluted potato extracts were reacted with 150 µL of 0.2 M Folin-Ciocalteu reagent in triplicate microplate wells. After five minutes at room temperature, 115 µL of sodium carbonate solution (7.5% w/v) were added for all the plate. The plate was incubated at 45ºC and then cooled for 30 minutes at room temperature. The absorbance was read at 765 nm using a microplate reader (Synergy-2, Biotech Instruments Inc., Winooski, VT). Total phenolic content was calculated based on a gallic acid standard curve and expressed as milligrams of gallic acid equivalents per 100 g of fresh potato.

2.6. Quantification of total monomeric anthocyanin content

Total monomeric anthocyanin content was estimated using the pH differential method (Wrolstad et al., 1989). Ten µL of diluted potato extracts were added to 290 µL of buffers (pH 1.0 and pH 4.5) in triplicate microplate wells. The absorbance (A) and the total monomeric anthocyanin (MAC) were calculated by using the formulas mentioned below. MAC was expressed as cyaniding-3-glucoside using an extinction coefficient (ε) of 62900 L/cm/mol, a molecular weight (MW) of 449.2 g/mol, a standard path length of 1cm, and a dilution factor (DF) of 10.

$$A = (A_{525} - A_{700})_{pH1.0} - (A_{525} - A_{700})_{pH\ 4.5}$$

$$MAC\ (mg/L) = (A^* MW\ ^*DF^*\ 1000) / (\varepsilon\ ^*\ 1)$$

2.7. ***Determination of antioxidant activity***

The antioxidant activity was measured using DPPH (2, 2-diphenyl-1-picryhydrazyl radical), a colorimetric method. Modified 2, 2-azino-bis- 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was also used to determine the antioxidant activity (Blois, 1955). The absorbance of the

potato extract was measured with a microplate reader (Biotech Instruments Inc., Winooski, VT, USA) at 517 nm for DPPH and at 734 nm for ABTS assay. The antioxidant activity was calculated based on trolox standard curve and expressed as milligrams of trolox equivalents per 100 gram of fresh potato.

2.8. Vitamin C extraction and quantification

Vitamin C was extracted using the method established by Dale et al. (2003). Raw, baked and chipped samples (five gram) were extracted two times for 15 minutes at 4°C in the dark with six mL of a 5 % (w/v) aqueous solution of methaphosphoric acid containing 1 % w/v of 1, 4-Dithio-DL-threitol. The samples were homogenized at high-speed (IKA.T 25 digital Ultra-turrax, IKA, Germany) and were centrifuged for 15 minutes at 1771 xg at 4°C using an ultracentrifuge (Beckman J2-HS centrifuge, Beckman Coulter, CA, USA). The 1^{st} and 2^{nd} supernatants were combined and filtered through a 0.45 µm nylon syringe filter before injection into UPLC.

The vitamin C level was detected using the method established by Waters Corporation, Milford, MA, USA. The vitamin C content of raw, baked, and chipped potato samples was measured for ten potato cultivars at initial, three, or six months storage at 4°C or 10°C using UPLC. Vitamin C quantification was performed by a Waters Acquity UPLC system equipped with a bio-sample manager, bio-quaternary solvent manager and temperature controlled column oven with a diode array detector (DAD). The chromatographic separations were performed on HSS T3 1.8 µm, 2.1x150 mm, column (Waters, Ireland, USA). The column temperature was 30°C, and the injection volume was two µL. The mobile phase was water; LC/MC grade containing 0.1% formic acid (solvent A) and 100 % methanol containing 0.1% formic acid (solvent B). The flow rate was adjusted to 0.25 mL per minute at room temperature. The solvent gradient was 99% of solvent A, and 1% of solvent B at initial time. After three minutes 45% of

solvent A and 55% of solvent B was applied. A 99% of solvent B was gradiened after five minutes. Finally, the solvent gradient was returned to 99 % of solvent A and 1 % of solvent B. Vitamin C content was detected at 254 nm with continuous monitoring of the peak spectra between 200 to 400 nm. Standard curves of vitamin C were made by analyzing standard concentrations of 0, 2.5, 5, 10, 20, 40, 80, 160, and 320 µg/mL.

2.9. Statistical analysis

Data were grouped as raw, baked, and chipped for white-and pigmented-fleshed potatoes. Two-way analysis of variance (ANOVA) for the interactions of storage time, and storage temperature on total phenolic, anthocyanins, antioxidants activity and vitamin C were determined by using the SAS, (Statistical Analysis System), v.9.3 (SAS Institute Inc., Cary, NC). Genotype and processing effect was determined in this study as well. General Linear Model (GLM) procedure and Least Squares Means (LSD) were applied on obtained results with a level of significant of 0.05 for multiple comparisons for means of the tested parameters. All results have been expressed as mean ± standard error of three biological replicates and two technical replicates for each replicates and each value is a mean of six determinations.

3. Results and discussion

3.1. Total phenolic content (TPC).

Genotype and storage play an important role in the content of potato bioactive compounds (Dale et al., 2003). Storage at either 4°C or 20°C contributes to an increase in the total phenolic content compared to non-stored potatoes (Mulinacci et al., 2008; Blessington et al., 2010). Blessington et al. (2010) observed that TPC was either unaltered or increased when compared to uncooked samples. Raw, baked, and chipped samples of ten Colorado-grown potato cultivars were measured for TPC at three time points: initial, three , or six months storage at two

different storage temperatures (4°C or 10°C) by using Folin-Ciocalteu assay established by Wrolstad et al. (1989) and Spanos and Wrolstad (1990). TPC in potato tubers is cultivar-dependent (Hamouz et al., 2013). Color-fleshed potatoes have high amount of TPC compared to white-fleshed compound. This is due a high level of anthocyanin in potato tubers. In this study, TPC ranged from 8.8 ± 3.6 to 92.7 ± 14.3 mg/100 gfw (Figure 5.1.a). Similar results were found by Hamouz et al (2013), who observed that the content of phenolic was significantly different in tested cultivars.

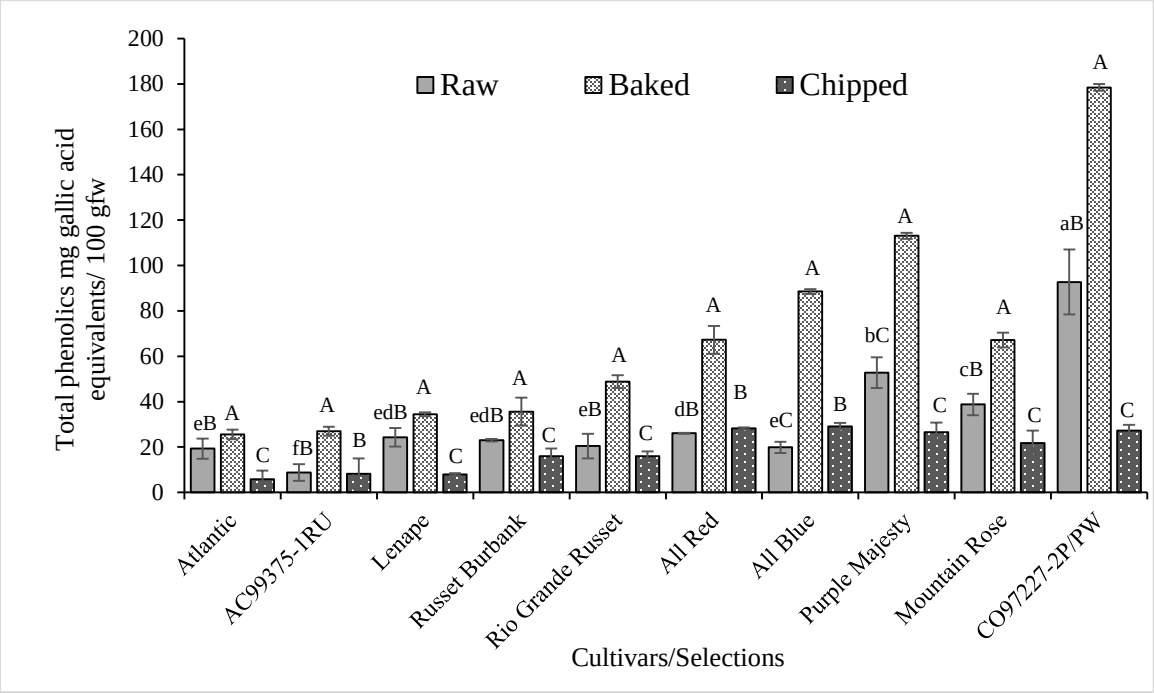

Figure 5.1.a. The effect of genotype and processing on total phenolic content in raw versus baked and chips of white-fleshed potatoes and color-fleshed potatoes. Total phenolic content was expressed as mg gallic acid equivalents/100 gfw. Different letters on the bars represent significant differences ($p \leq 0.05$) among the cultivars/selections. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations. Lowercase letters (a-e) on the bars indicate (genotypic effect) differences in raw potatoes among the cultivars, whereas uppercase letters (A-C) on the bars of raw and processed potatoes (baked and chipped) indicate (processing effect) differences compared to total phenolic content of raw potatoes.

TPC in potato tubers is enhanced by post-harvest storage and processing parameters (Blessington et al., 2010). For ten cultivars over the entire period of storage, TPC of the raw potato samples ranged from 8.8 ± 3.6 to 160.4 ± 3.8 mg GAE/100 gfw (Figure 5.1. a-g).

Stushnoff et al. (2010) also reported that TPC significantly increased after seven months of storage at 5 ± 1ºC in pigmented potato cultivars. They reported that advance selection CO97227-2P/PW had a sharp increase in TPC compared to other pigmented cultivars. Our data showed a high level of TPC specifically in CO97227-2P/PW, Purple Majesty, and Mountain Rose, which agreed with the Stushnoff finding in which the pigmented cultivars Purple Majesty and Mountain Rose contained higher levels of chlorogenic isomers than the non-pigmented cultivars. The interaction effects of storage period and storage temperature on TPC either decreased or increased, depending on the cultivars. In raw white-fleshed potatoes, the interaction effects of storage period and storage temperature (4°C) on TPC in AC99375-1RU and Lenape cultivars resulted in a gradually significant increase ($p \leq 0.05$) in TPC even after six months of storage 4°C. For other cultivars such as Atlantic and Rio Grande, the interaction effects of storage period and storage temperature resulted in an increase in TPC after three months of storage and a decline after six months of storage at 4°C. For Russet Burbank, TPC was high at the initial phase and declined after three months of storage, then increased to almost harvest level after six months of storage 4°C. Storage at 10°C for three months resulted in an increase in TPC of white-fleshed potatoes compared to harvest level even after six months of storage, but TPC was not significant compared to storage at 4°C. For raw pigmented-fleshed potatoes (All Red, All Blue, Purple Majesty, and CO97227-2P/PW), the interaction effects of storage period and storage temperature at 4°C resulted in a gradually significant increase in TPC even after six months of storage. After six months of storage, there was no significant difference in TPC for CO97227-2P/PW and All Red cultivars compared to initial levels at 4°C. TPC of the raw, baked, and chipped potato samples ranged from 8.8 ± 3.6 to 160.4 ± 3.8 mg GAE/100 gfw, 5.3 ± 2.0 to 193.9 ± 5.5 mg GAE/100 gfw and 5.6 ± 0.7 to 62.5 ± 3.1 mg GAE/100 gfw, respectively (Figure

5.1. a-b). Our data also agreed with both data of Madiwale et al. (2011) and Kulen et al. (2013), who found that CO97227-2P/PW contain the highest level of TPC among tested potato cultivars. Another study conducted by Blessington et al. (2010) on stored Russet Burbank for four months at 4ºC, TPC decreased after storage. These results points to a critical need for developing appropriate farm-to-fork operations for each cultivar.

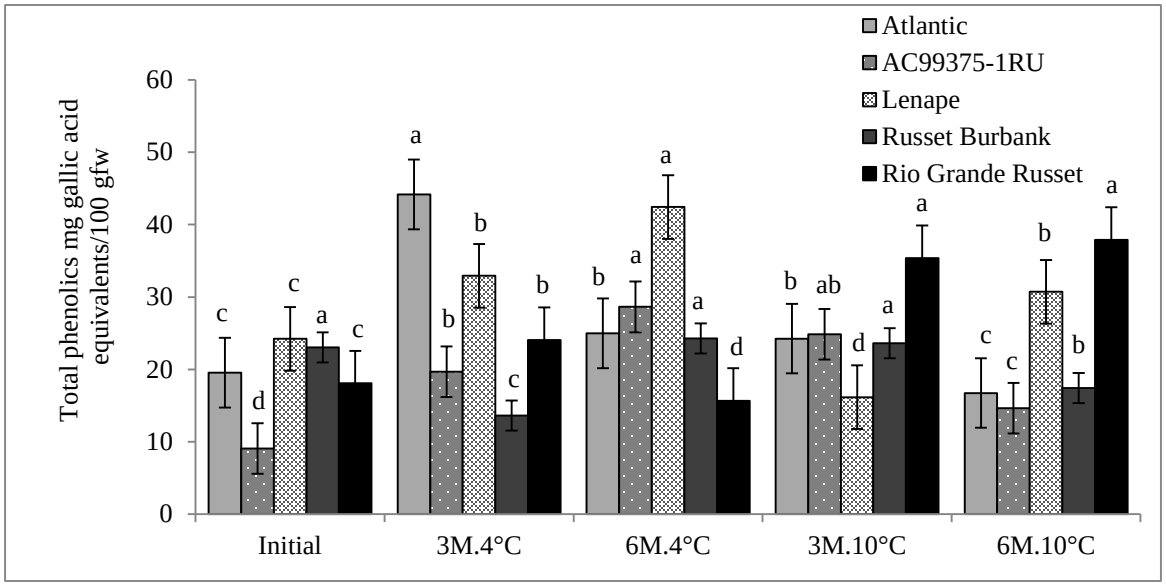

Figure 5.1.b. Total phenolic content in raw white-fleshed potatoes as assessed by Folin-Ciocalteu reagent assay described in the materials and methods. Total phenolic content was expressed as mg gallic acid equivalents/100 gfw. Different letters on the bars represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

TPC in potato tubers is also affected by processing parameters (Lachman et al., 2013). TPC of baked color- and white-fleshed potatoes increased with storage ($p \leq 0.05$) after three months of storage at 4°C and declined after six months of storage. TPC of the baked potato samples ranged from 5.3 ± 2 to 193.9 ± 5.5 mg GAE/100 gfw (Figure 5.1. c-f). When compared with unprocessed samples at the same time point of storage (initial, three, or six months of storage at 4°C), baking decreased and increased the TPC; depending on the genotype. In All Red and Atlantic cultivars, baking resulted in a decline of TPC after three or six months of storage at 10°C compared to unprocessed samples at the same time points of storage.

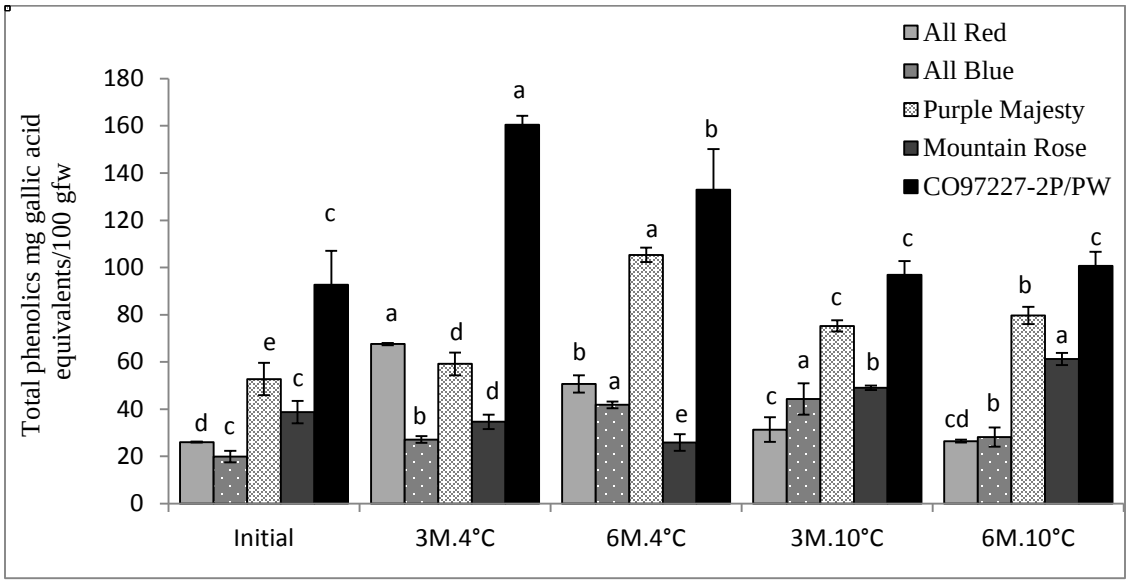

Figure 5.1.c. Total phenolic content in raw color-fleshed potatoes as assessed by Folin-Ciocalteu reagent assay described in the materials and methods. Total phenolic content was expressed as mg gallic acid equivalents/100 gfw. The letters P/PW after the advanced selection indicates skin / flesh color: P, Purple; PW, Purple with white patches. Different letters on the bars represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

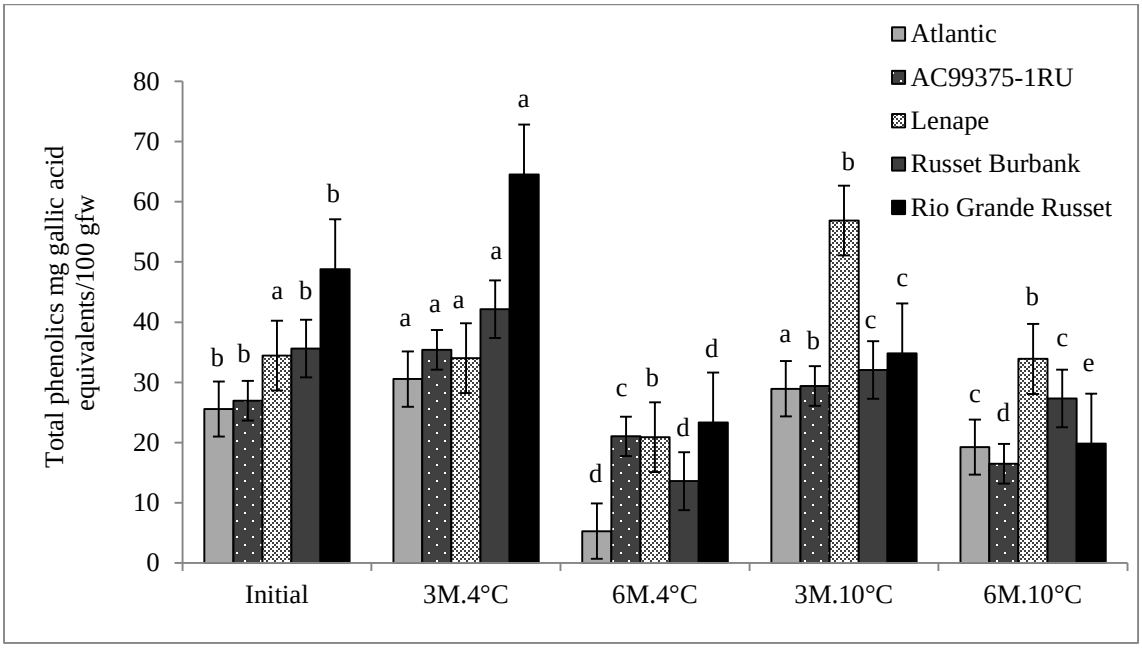

Figure 5.1.d. Total phenolic content in baked white-fleshed potatoes as assessed by Folin-Ciocalteu reagent assay described in the materials and methods. Total phenolic content was expressed as mg gallic acid equivalents/100 gfw. Different letters on the bars represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

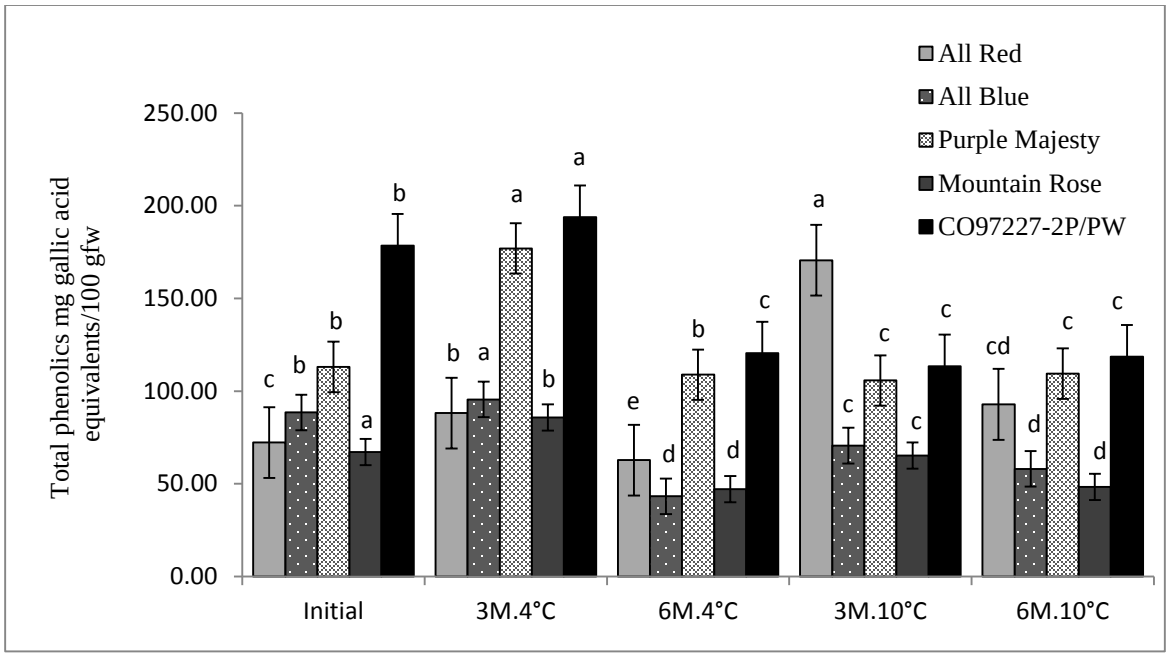

Figure 5.1.e. Total phenolic content in baked color-fleshed potatoes as assessed by Folin-Ciocalteu reagent assay described in the materials and methods. Total phenolic content was expressed as mg gallic acid equivalents/100 gfw. The letters P/PW after the advanced selection indicates skin / flesh color: P, Purple; PW, Purple with white patches. Different letters on the bars represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

Baked purple-fleshed potatoes had higher phenolic content ($p \leq 0.05$) when compared with baked white-fleshed potatoes over the entire storage period. Total phenolic results were also consistent with Madiwale et al. (2011), who used three of the same potato cultivars (Atlantic, Purple Majesty, and CO97227-2P/PW) also grown at San Luis Valley Research Center, Center, CO and stored for three months at 3 ± 1°C. In their study, TPC increased with storage. Both their results, and ours may be explained because at lower temperatures, the activity of polyphenol oxidase enzyme is low. However, there is greater polyphenol content at 4°C compared to 10°C (Mondy et al., 1966). This is due to the enzyme activity being indirectly related to monomeric polyphenol content: the less activity, the lower the transformation of monomeric polyphenols to polymeric polyphenols. At a higher storage temperature (10°C), there is greater discoloration due

to the transformation of polyphenols to polymerics, which is related to the higher polyphenol oxidase activity.

TPC of baked samples follow the same trend as raw samples. When compared with unprocessed samples at the same time point of storage, such as fresh baked with fresh raw for all cultivars, baking increased TPC for all cultivars after three months of storage at 4°C or 10°C. At the same time, baking also decreased the phenolic content for certain cultivars such as Atlantic, Lenape, Burbank, and All Red after six months of storage at 4°C or 10°C. Therefore, it is difficult to predict the effect of processing and storage temperature on TPC, which is dependent on potato genotype (Xu et al., 2009; Blessington et al., 2010; Navarre et al., 2010). This further confirms that it is essential to develop farm-to-fork operations that retain bioactive compounds (and reduce toxicants) for each cultivar. The effect of chipping and frying resulted in a greater loss in TPC when compared to unprocessed samples for all potato cultivars. TPC of the raw, baked, and chipped potato samples ranged from 8.8 ± 3.6 to 92.7 ± 14.3 mg GAE/100 gfw from 5.3 ± 2.0 to 193.9 ± 5.5 mg GAE/100 gfw and 5.6 ± 0.7 to 62.5 ± 3.1 mg GAE/100 gfw, respectively (Figure 5.1.e-f). This finding agreed with published data from Tudela et al. (2002) and Im et al. (2008), who found that chipping and frying resulted in the highest loss in phenolic content when compared to raw potato samples. Our results also agreed with previously established data by Madiwale (2012), in our lab, who found that frying and chipping resulted in significant losses in phenolic content compared to raw samples at the same period of storage. The greater loss of potato bioactive compounds could be related to chipping processes such as chipping and washing under running warm water to remove any water-soluble sugars present on the surface.

3.2. Antioxidant activity

Antioxidant activity of ten methanol-potato extracts from potato tubers at initial, after three, or six months of storage at 4°C or 10ºC measured by ABTS and DPPH assays showed an increase with storage (Figure 5.2. a-f). The antioxidant activity measured by ABTS assay for raw white-fleshed potatoes at initial ranged from 73.1 ± 6.1 mg TE/100 gfw for Atlantic cultivar to 294.8 ± 23.3 ± mg TE/100 gfw for Russet Burbank.

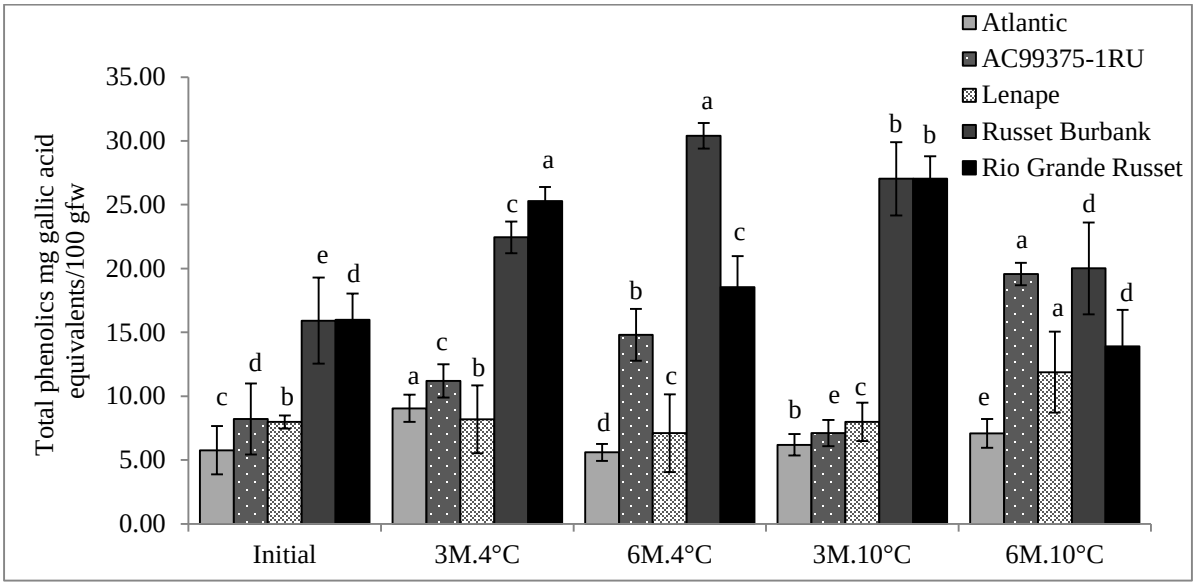

Figure 5.1.f. Total phenolic content in chipped white-fleshed as assessed by Folin-Ciocalteu reagent assay described in the materials and methods. Total phenolic content was expressed as mg gallic acid equivalents/100 gfw. Different letters on the bars represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

After three months of storage at 4ºC, the antioxidant activity for Atlantic decreased from 73.1 ± 6.1 mg TE/100 gfw to 65.7 ± 9.2 mg TE/100 gfw. However, storage of Atlantic potato tubers for six months at 4ºC resulted in an increase in the antioxidant activity (179.2 ±10.9 mg TE/100 gfw). At 10ºC, the antioxidant of stored of raw Atlantic potatoes for three months increased from 73.1 ± 6.1 to 86.7 ± 6.8 mg TE/100 gfw. The antioxidant activity (ABTS) of raw Russet Burbank decreased from 294.8 ± 23.3 mg TE/100 gfw at initial storage to 232.9 ± 2.7 mg TE/100 gfw after six months of storage at 4ºC. However, storage raw Russet Burbank for either

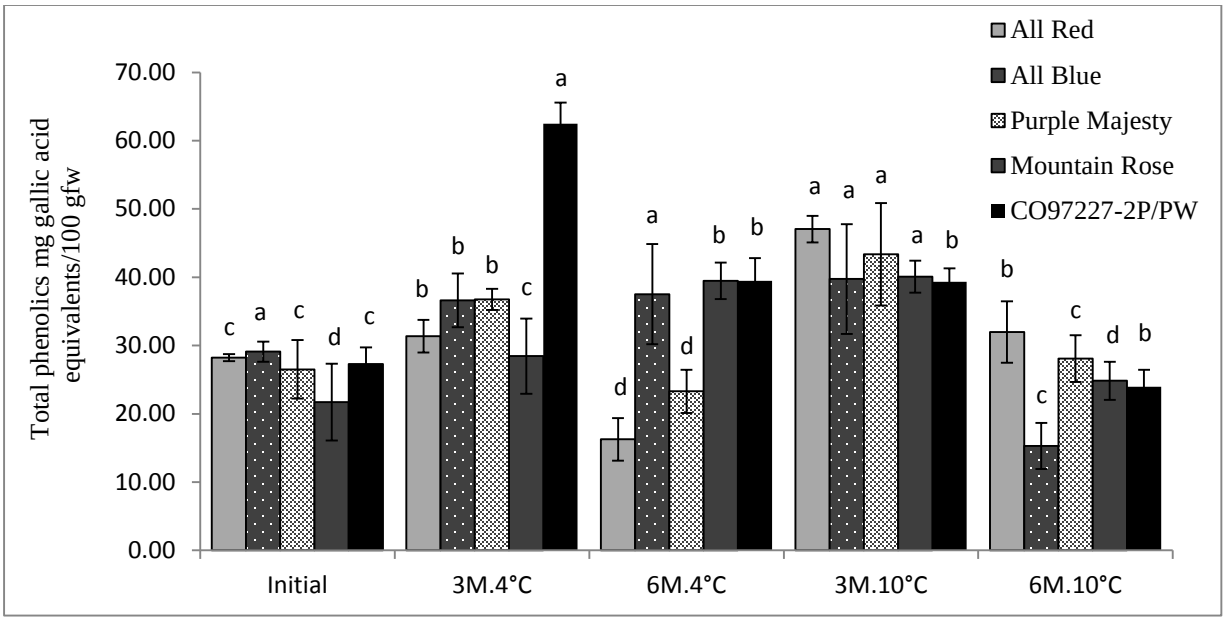

Figure 5.1.g. Total phenolic content in chipped color-fleshed potatoes as assessed by Folin-Ciocalteu reagent assay described in the materials and methods. Total phenolic content was expressed as mg gallic acid equivalents/100 gfw. The letters P/PW after the advanced selection indicates skin/flesh color: P, Purple; PW, Purple with white patches. Different letters on the bars represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

three or six months at 10ºC resulted in decrease in the antioxidant activity (ABTS) compared to initial levels. For colored-fleshed potatoes, the antioxidant activity (ABTS) at the time of initial storage ranged from 392.3 ± 7.9 mg TE/100 gfw for Purple Majesty to 525.3 ± 10.1 mg TE/100 gfw for CO97227-2P/PW. Storage of potato tubers of the Purple Majesty cultivar for three months at 4ºC resulted in a decrease in the antioxidant activity (293.9 ± 12.6 mg TE/100 gfw) compared to initial levels. However, storage for six months at 4ºC resulted in increase in the antioxidant activity of raw Purple Majesty potatoes (445.2 ± 48.9 mg TE/100 gfw). In comparison to initial levels, storage at 10ºC either for three or six months increased the antioxidant activity of raw Purple Majesty potatoes increased to 527.7 ± 13.0 and 618 ± 93.9 mg TE/100 gfw, respectively. The antioxidant activity of raw CO97277-2P/PW increased from 525 ± 10 mg TE/100 gfw at the time of initial storage to 572 ± 12.9 mg TE/100 gfw after six months of storage at 4ºC and to 557 ± 22.5 mg TE/100 gfw after six months of storage at 10ºC.

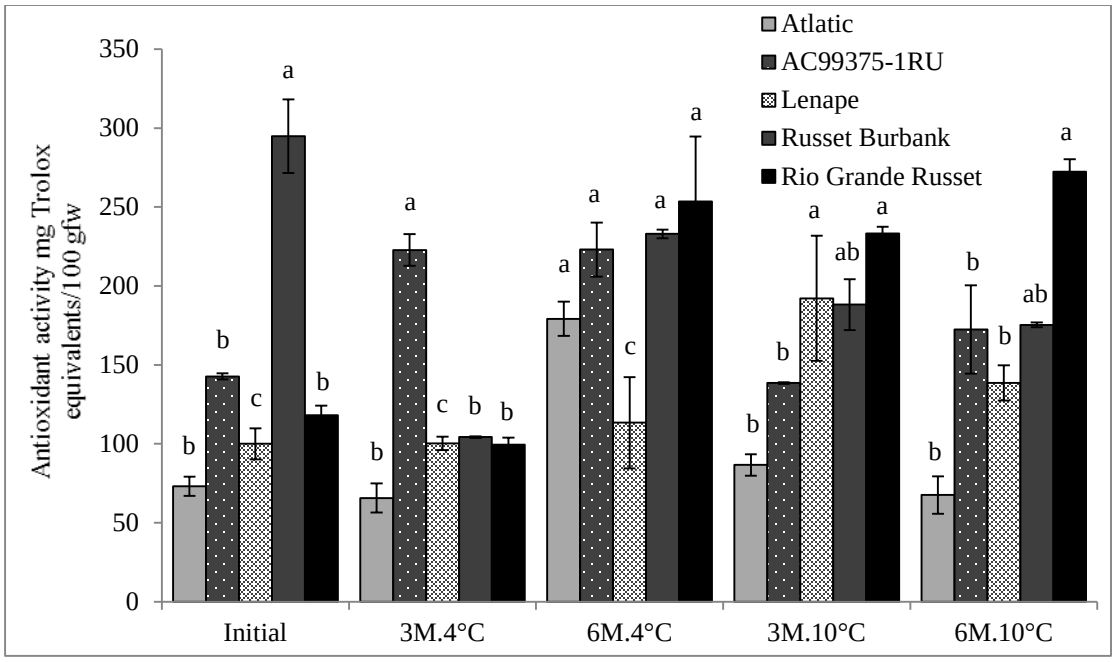

Figure 5.2.a. Antioxidant activity of raw white-fleshed potatoes as assessed by ABTS described in the materials and methods. Antioxidant activity was expressed as mg Trolox equivalents/100 gfw. Different letters on the bars for antioxidant activity represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

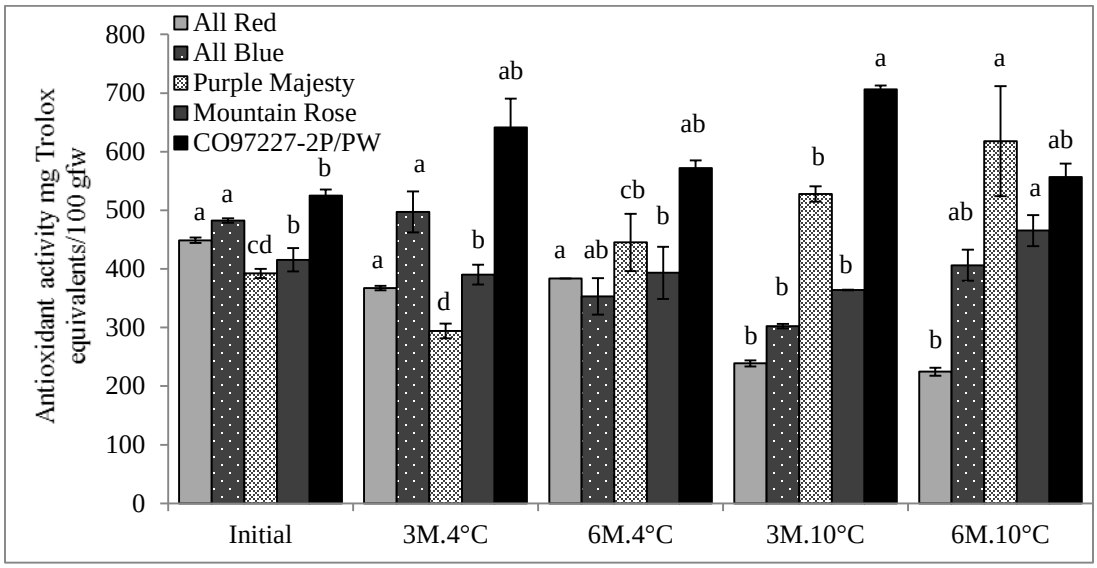

Figure 5.2.b. Antioxidant activity of raw color-fleshed potatoes as assessed by ABTS described in the materials and methods. Antioxidant activity was expressed as mg Trolox equivalents/100 gfw. The letters P/PW after the advanced selection indicates skin / flesh color: P, Purple; PW, Purple with white patches. Different letters on the bars for antioxidant activity represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

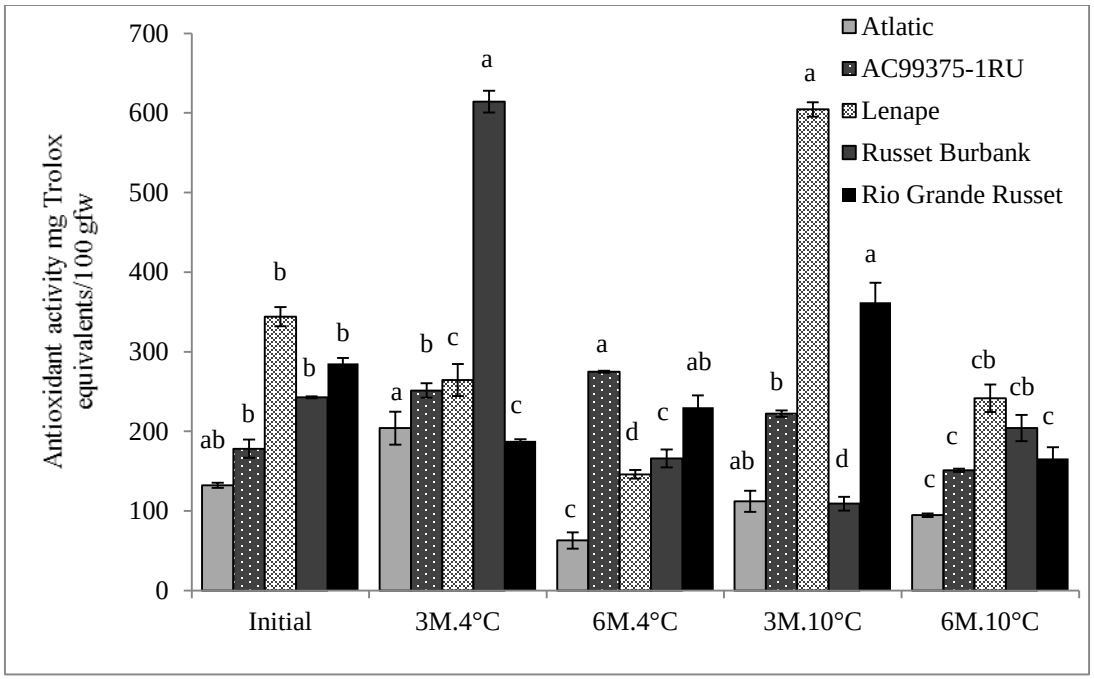

Figure 5.2.c. Antioxidant activity of baked white-fleshed potatoes as assessed by ABTS described in the materials and methods. Antioxidant activity was expressed as mg Trolox equivalents/100 gfw. Different letters on the bars for antioxidant activity represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

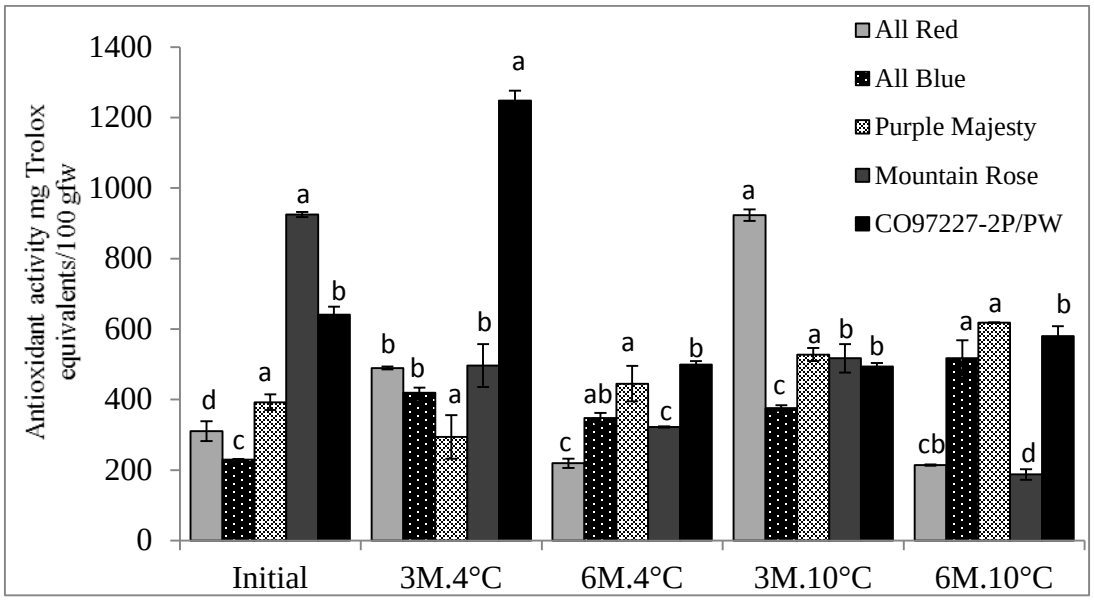

Figure 5.2.d. Antioxidant activity of baked color-fleshed potatoes as assessed by ABTS described in the materials and methods. Antioxidant activity was expressed as mg Trolox equivalents/100 gfw. The letters P/PW after the advanced selection indicates skin/flesh color: P, Purple; PW, Purple with white patches. Different letters on the bars for antioxidant activity represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

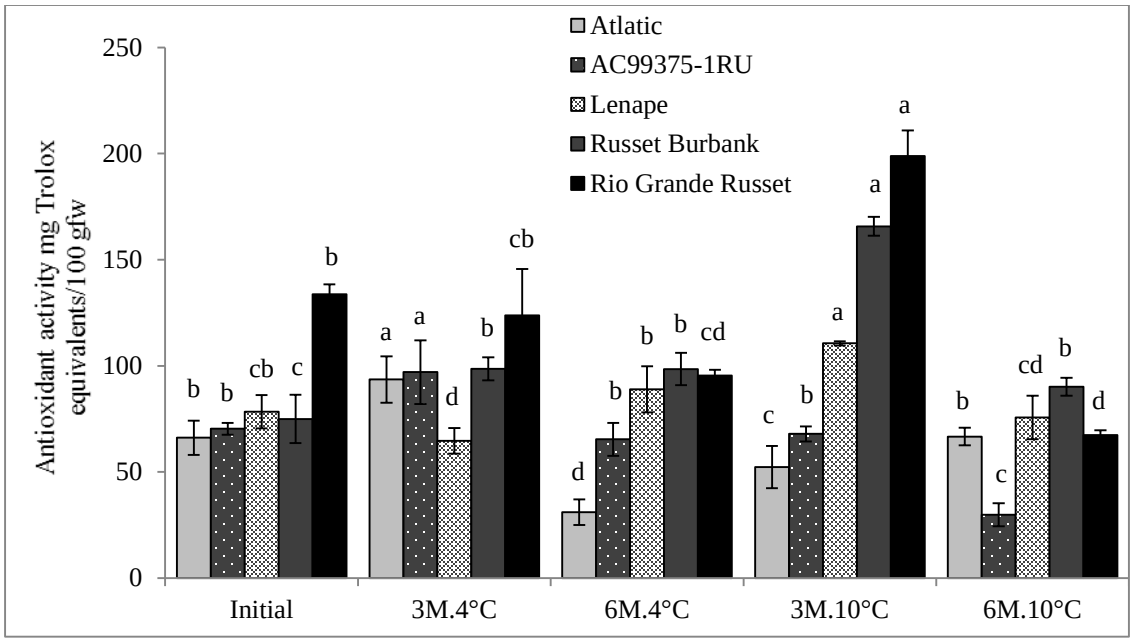

Figure 5.2.e. Antioxidant activity of chipped white-fleshed potatoes as assessed by ABTS described in the materials and methods. Antioxidant activity was expressed as mg Trolox equivalents/100 gfw. Different letters on the bars for antioxidant activity represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

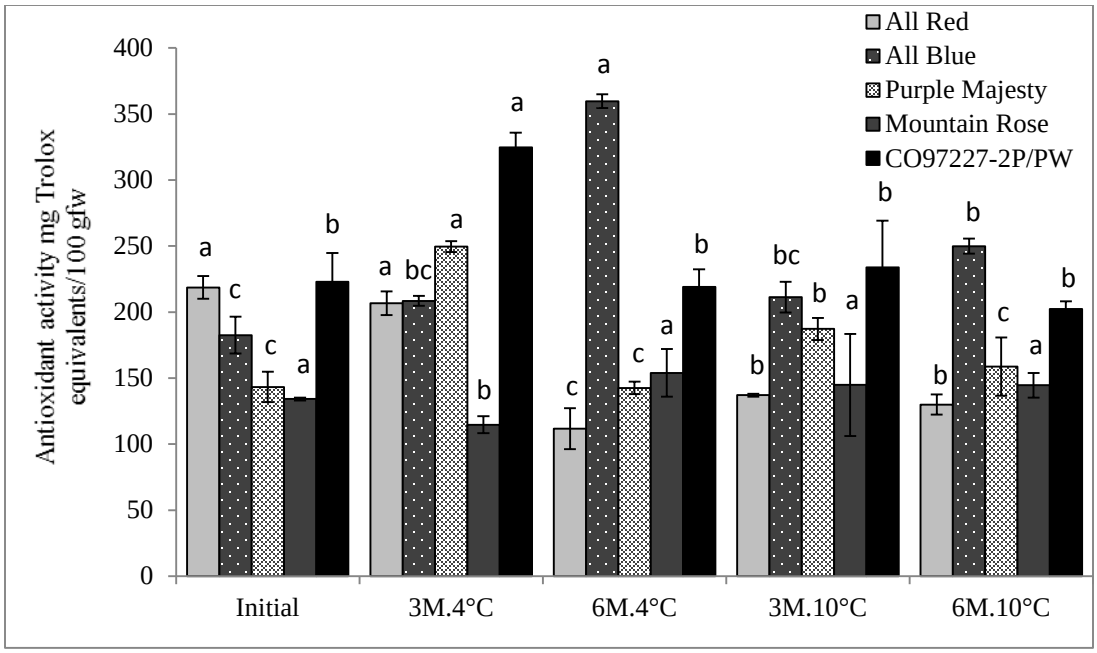

Figure 5.2.f. Antioxidant activity of chipped color-fleshed potatoes as assessed by ABTS described in the materials and methods. Antioxidant activity was expressed as mg Trolox equivalents/100 gfw. The letters P/PW after the advanced selection indicates skin/flesh color: P, Purple; PW, Purple with white patches. Different letters on the bars for antioxidant activity represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

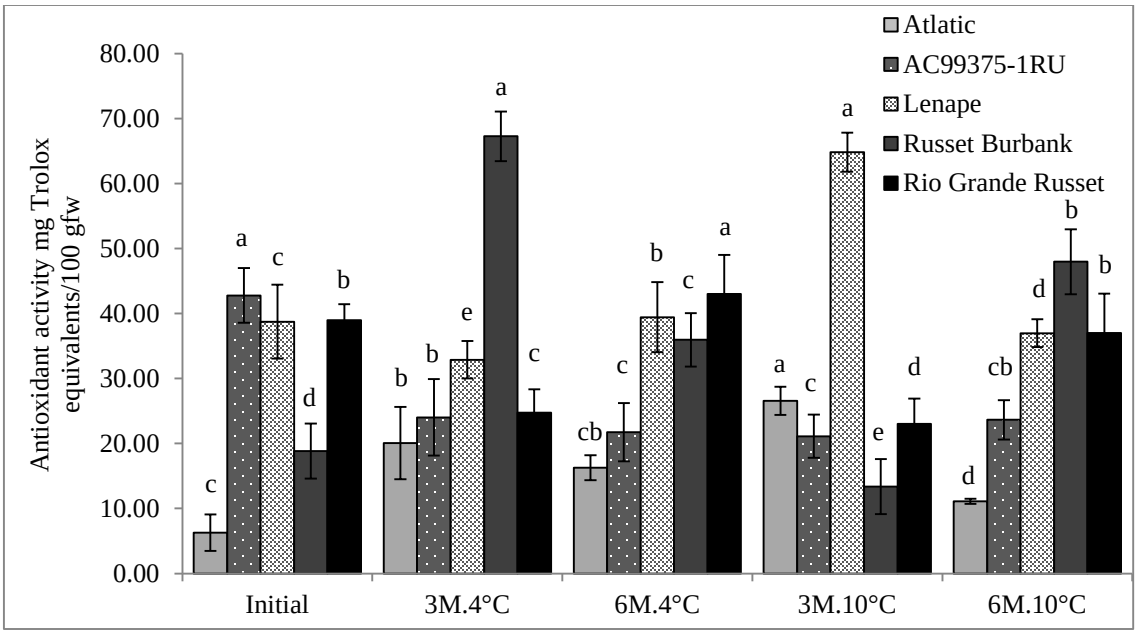

Figure 5.3.a. Antioxidant activity of raw white-fleshed potatoes as assessed by DPPH described in the materials and methods. Antioxidant activity was expressed as mg Trolox equivalents/100 gfw. Different letters on the bars for antioxidant activity represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

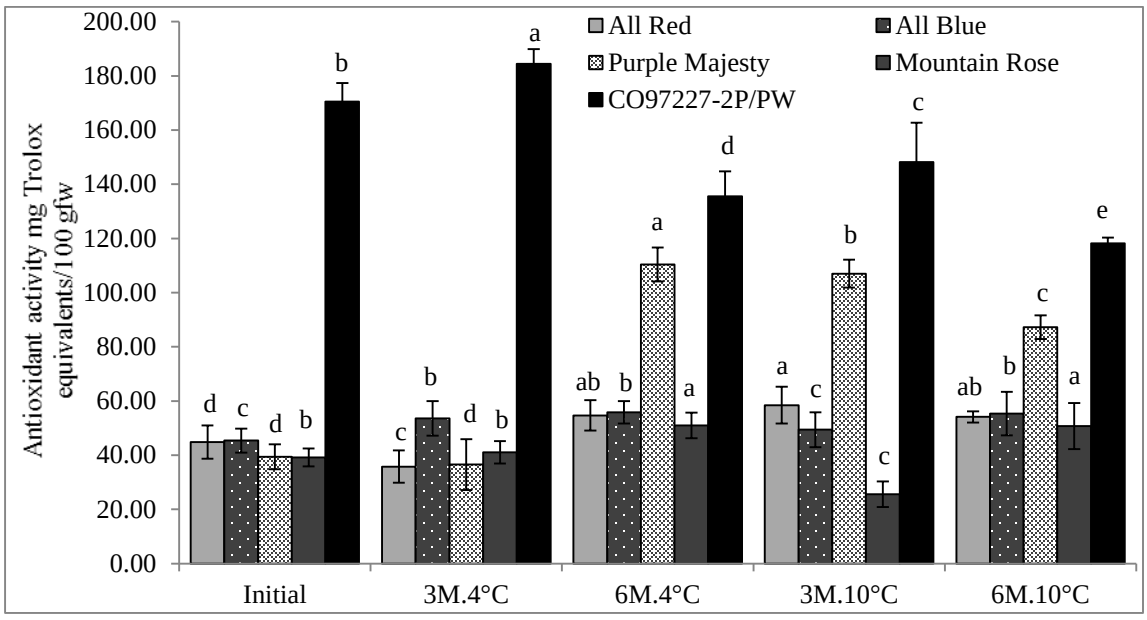

Figure 5.3.b. Antioxidant activity of raw color-fleshed potatoes as assessed by DPPH described in the materials and methods. Antioxidant activity was expressed as mg Trolox equivalents/100 gfw. The letters P/PW after the advanced selection indicates skin/flesh color: P, Purple; PW, Purple with white patches. Different letters on the bars for antioxidant activity represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

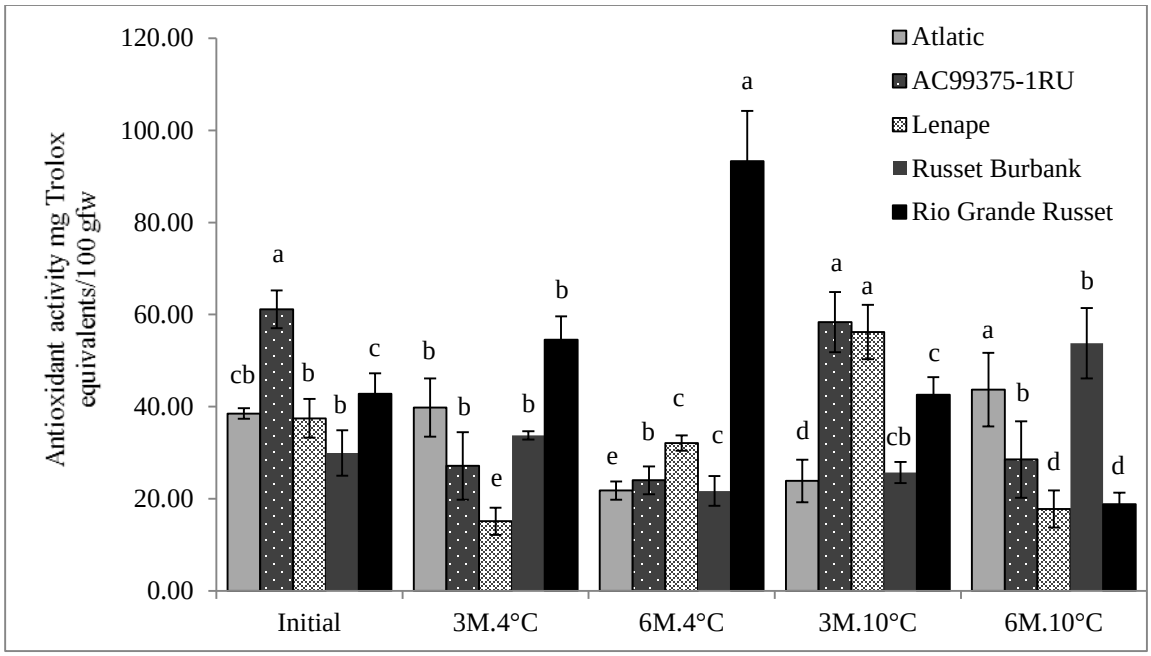

Figure 5.b.c. Antioxidant activity of baked white-fleshed potatoes as assessed by DPPH described in the materials and methods. Antioxidant activity was expressed as mg Trolox equivalents/100 gfw. Different letters on the bars for antioxidant activity represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

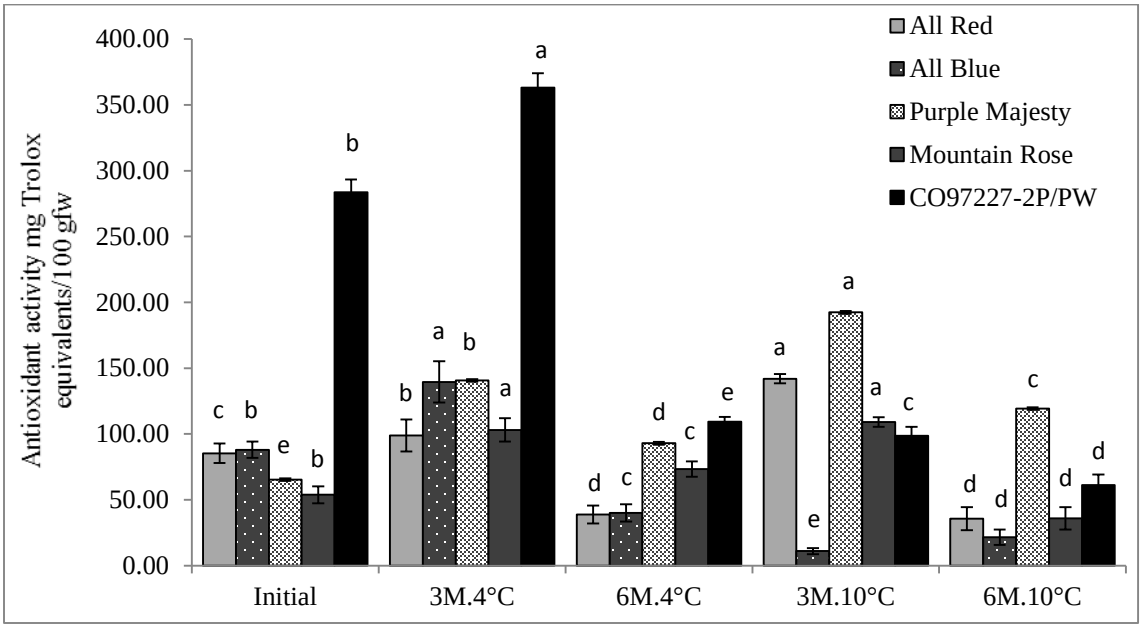

Figure 5.3.d. Antioxidant activity of baked color-fleshed potatoes as assessed by DPPH described in the materials and methods. Antioxidant activity was expressed as mg Trolox equivalents/100 gfw. The letters P/PW after the advanced selection indicates skin/flesh color: P, Purple; PW, Purple with white patches. Different letters on the bars for antioxidant activity represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

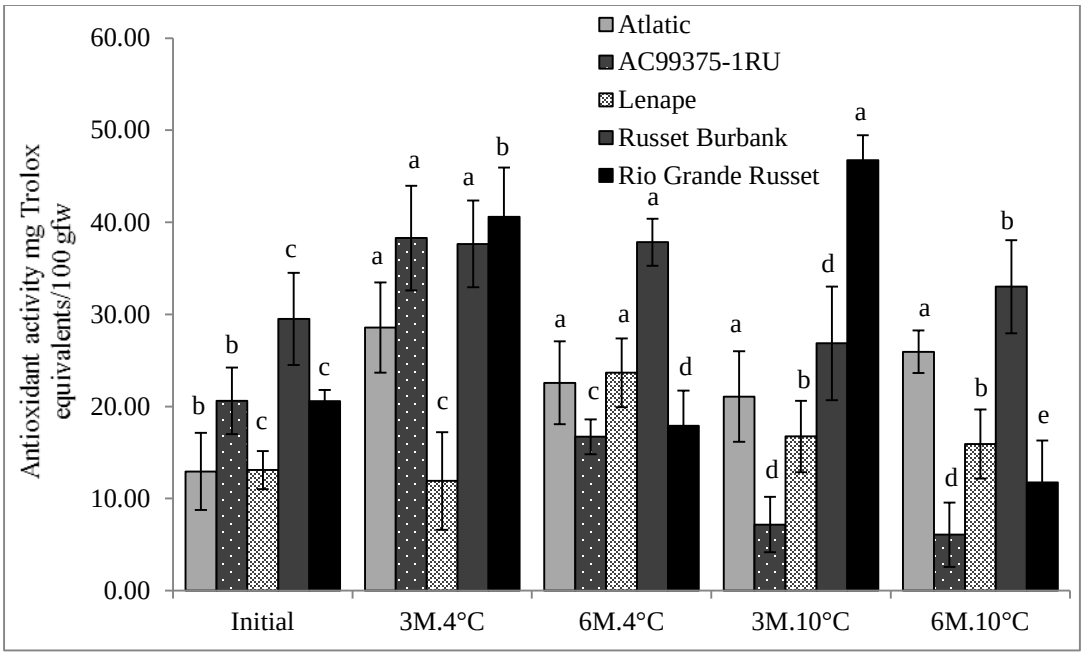

Figure 5.3.e. Antioxidant activity of chipped of white-fleshed potatoes as assessed by DPPH described in the materials and methods. Antioxidant activity was expressed as mg Trolox equivalents /100 gfw. Different letters on the bars for antioxidant activity represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

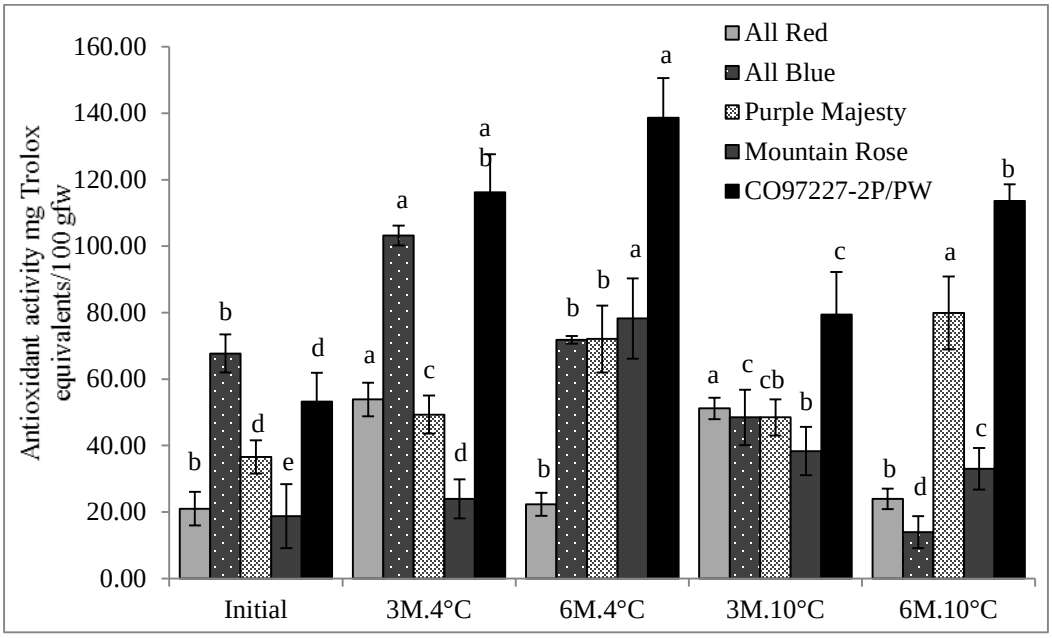

Figure 5.3.f. Antioxidant activity of chipped of color-fleshed potatoes as assessed by DPPH described in the materials and methods. Antioxidant activity was expressed as mg Trolox equivalents/100 gfw. The letters P/PW after the advanced selection indicates skin/flesh color: P, Purple; PW, Purple with white patches. Different letters on the bars for antioxidant activity represent significant differences ($p \leq 0.05$) among cultivars/selections compared with the initial time point. Results are presented as the mean ± SE of three biological replicates and three technical replicates for each biological replicate. Each value is a mean of nine determinations.

For baked potatoes, antioxidant activity measured by ABTS for white-fleshed potatoes ranged from 62.9 ± 10.3 mg TE/100 gfw after six months of storage at 4ºC to 614.6 ± 13.8 mg TE/100 gfw for Russet Burbank after storage for three months at 4ºC. The antioxidant activity of pigmented-fleshed potatoes ranged from 219.2 ± 13.0 mg TE/100 gfw for All Blue to 1248.3 ±27.9 mg TE/100 gfw for CO97277-2P/PW over the entire of storage period. The pigmented cultivars have from two to eight-fold higher antioxidant activity compared with non-pigmented cultivars due to the presence of anthocyanins, carotenoids, and phenolic acids (Kulen et al., 2013). The antioxidant activity values after six months of storage at 4°C or 10ºC were significantly ($p \leq 0.05$) higher compared with initial levels for all cultivars, irrespective of the tuber flesh color. Our findings agreed with Stushnoff et al. (2008), who found that the phenolic content was elevated by up to 100% in some cultivars, whereas, in the other cultivars it remained constant. The interaction effects of storage time and storage temperature at 4ºC resulted in a significant ($p \leq 0.05$) increase in TPC and antioxidant activity than storage at 10ºC. This can be explained by the fact that the low storage temperature can induce the activity of phenylalanine ammonia-lyase (PAL), a key regulatory enzyme in the biosynthesis of polyphenols including anthocyanins, which can cause elevation in the phenolic content (Jiang and Joyce, 2003). At the same time, a storage temperature inhibits the activity of polyphenol oxidase enzyme with greater polyphenol content at 4°C compared to 10°C (Mondy et al., 1966), which explains the higher antioxidant activity at 4°C compared to 10°C. The antioxidant activity for chipped potato samples ranged from 66.1 ± 8.0 to 223.0 ± 21.8 mg TE/100 gfw for Atlantic and CO97227-2P/PW, respectively, measured by ABTS assay at the time of initial storage. The latter showed the highest antioxidant activity among the 10 cultivars tested (Figure 5.2. a-b and Figure 5.3. a-b). For the ABTS assay, the range was from 66.1 ± 8.0 at initial storage to 93.6 ± 10.9 mg

TE/100 gfw after three months of storage at 4ºC and then decreased to 30.9 ± 6.0 mg TE/100 gfw after six months for Atlantic chips. At 10°C storage, the Atlantic chips antioxidant activity measured by ABTS showed a decrease after three months of storage (52.4 ± 9.9 mg TE/100 gfw) and then an increase to initial levels 66.7 ± 4.2 mg TE/100 gfw after six months of storage. The antioxidant activity measured by DPPH for Atlantic was 12.9 ± 4.2 mg TE/100 gfw at initial storage and then increased to 28.6 ± 4.9 mg TE/100 gfw after three months of storage, followed by a decrease to 22.6 ± 4.5 after six months of storage at 4ºC. At 10ºC storage, the antioxidant activity was 12.95 ± 4.18 mg TE/100 gfw at initial storage and increased to 21.1 ± 4.9 mg TE/100 gfw after three months of storage, then increased to 25.9 ± 2.3 mg TE/100 gfw after six months of storage. The antioxidant activity measured by ABTS assay for chips of the CO97227-2P/PW ranged from 252 ± 3 mg TE/100 gfw at initial storage and increased to 326.3 ± 11.3 mg TE/100 gfw after three months of storage at 4ºC then decreased to 222.1 ± 13.3 mg TE/100 gfw after six months of storage at 4ºC. At 10ºC, the antioxidant activity for CO97227-2P/PW chips was 252.3 ± 21.8 mg TE/100 gfw at initial storage and increased to 264 ± 35.5 mg TE/100 gfw after three months of storage, then decreased to 203.9 ± 6.0 mg TE/100 gfw after six months.

3.3. Total monomeric anthocyanin content.

In color-fleshed potatoes, the total anthocyanin content decreased from 16 to 29% after cooking and microwaving of unpeeled potatoes (Mulinacci et al., 2008). The anthocyanin content of raw purple-fleshed cultivars ranged from 9.0 ± 5.6 to 49.2 ± 2.4 mg cyanidin-3-glucoside equivalents (C-3-G-equiv)/100 gfw at initial storage (Table 5.1). The anthocyanin in purple-fleshed cultivars ranged from 11 to 174 mg C-3-G equiv/100 gfw (Reyes et al., 2004). Our data agreed with work published by Lachman et al. (2009), who found that the total anthocyanin in 15 red- and purple-fleshed potatoes cultivars ranged from 0.7 mg C-3-G

equiv/100 gfw to 74 mg C-3-G equiv/100 gfw. CO972272P/PW had the highest anthocyanin content among all five cultivars irrespective of the storage time. The initial anthocyanin content of raw CO97227-2P/PW tubers was 49.2 ± 2.4 mg C-3-G equiv/100 gfw, which then increased to 91.3 ± 1.7 mg C-3-G equiv/100 gfw after three months of storage at 4ºC and gradually decreased to 64.8 ± 0.1 mg C-3-G equiv/100 gfw after six months of storage at 4ºC. At 10ºC storage, the anthocyanin content of raw CO97227-2P/PW was 89.9 ± 6.5 mg C-3-G equiv/100 gfw after three months of storage, then gradually decreased to 58.7 ± 1.7 mg C-3-G equiv/100 gfw after six months of storage. All Blue, which showed the lowest anthocyanin content among the three purple-fleshed cultivars tested, had 1.2 ± 0.0 mg C-3-G equiv/100 gfw at initial storage and numerically increased to 1.9 ± 0.7 mg C-3-G equiv/100 gfw after three months of storage at 10ºC. All Red had the lowest anthocyanin content among the red-fleshed cultivars tested. The initial level of anthocyanin content in raw All Red cultivar was 0.9 ± 0.0 mg C-3-G equiv/100 gfw and increased to 11.9 ± 6.9 mg C-3-G equiv/100 gfw after three months of storage, then slightly decreased to 10.1 ± 5.8 mg C-3-G equiv/100 gfw after six months of storage at 4ºC. At 10ºC, the anthocyanin content of raw All Red cultivars increased to 2.4 ± 1.4 mg C-3-G equiv/100 gfw after three months of storage and then slightly increased to 2.7 ± 0.1 mg C-3-G equiv/100 gfw after six months (Table 5.1). For the purple-fleshed baked samples, the anthocyanin content ranged from 25.7 ± 0.6 to 134.6 ± 4.0 mg C-3-G equiv/100 gfw at initial storage. The anthocyanin content for red-fleshed cultivars ranged from 17.6 ± 0.2 for All Red to 23.9 ± 2.0 mg C-3-G equiv/100 gfw for Mountain Rose at initial storage (Table 5.1). Most phytochemicals are enhanced by one or both postharvest processing parameters such as storage and cooking (Blessington et al., 2010). The anthocyanin content of chipped Purple-fleshed cultivars ranged from 6.3 ± 3.6 mg C-3-G equiv/100 gfw for All Blue and to 13.5 ± 8.0 mg C-3-

G equiv/100 gfw for the CO97227-2P/PW (Table 5.1). Baking either completely retained or increased of the anthocyanin content in tested purple-fleshed potatoes. Our anthocyanin data of baked samples were consistent with published work by Madiwale (2012), who used two of the same purple-fleshed potato cultivars, Purple Majesty and CO97227-2P/PW, also grown at San Luis Valley Research Center, Center, CO, USA and also stored for three months at 3 ±1°C. In comparison, chipping resulted in 78% losses in the anthocyanin content as compared to unprocessed samples at the same interval of storage. CO97227-2P/PW samples consistently had the highest anthocyanin content (Madiwale, 2012). In the current study, the anthocyanin content of baked potato cultivars was slightly decreased or increased after three months of storage at 4°C of storage temperature, then decreased after six months of storage at 4°C when compared to initial levels. The anthocyanin content contributes to the polyphenols portion in Purple-fleshed potatoes. It has been reported that the starch gets converted to sugar by storage temperature conditions, which can up-regulate genes coding for enzymes such as dihydroflavonol reductase and anthocyanidin synthase, which play a role in the anthocyanin pathway and thereby results in increasing the level of anthocyanin. Thus, there is a potential to adjust the farm-fork operations need to maximize content of health-benefiting compounds in color-fleshed potatoes.

3.4. Vitamin C content

Potato tubers are considered a good source for vitamin C among different fruits and vegetables. All cultivars as raw, baked, and chipped potatoes at initial storage and after three or six months of storage at 4°C or 10°C were evaluated for vitamin C content (Table.5.2). Vitamin C in potato tubers at initial ranged from 21.7 ± 0.3 mg/100 gfw for Atlantic to 37.5 ± 0.9 mg/100 gfw for Rio Grande Russet. The vitamin C content in potato tubers ranged between 10 to 40 mg/100 gfw (Dale et al., 2003; Burgos et al., 2009).

Vitamin C data were consistent with Kulen et al. (2013), who studied the effect of storage (4°C) on vitamin C level in 12 Colorado-grown specialty potato cultivars. They used five of the same potato cultivars (CO97227-2P/PW, Purple Majesty, Mountain Rose, All Blue, and Russet Burbank) and were also grown at San Luis Valley Research Center, Center, CO, USA. Their data agreed with our data in which vitamin C content in potato tubers was cultivar-dependent and declined with storage time and temperatures.

Storage temperature and time are the most influential to maintain vitamin C of potato tubers. Ascorbate oxidase has been proposed to be the major enzyme responsible for enzymatic degradation of vitamin C, which was increased under stress (Lee and Kader, 2000). In the current study, potato tubers showed a gradual decline in vitamin C content as the storage temperature or time increases. Our findings support the fact that vitamin C content in potato tubers can be influenced by many factors such as storage time and temperature and processing parameters (Lee and Kader, 2000). Storage potato tubers at either 4°C or 10°C for three or six months resulted in a decline in vitamin C content in all potato genotypes compared to non-stored potatoes (Table 5.2). This finding agreed with previous studies by Pal et al. (2008), decreases in vitamin C content were rapid up to three months of storage at 4°C. After that, vitamin C content remained constant until the end of storage. Storage potato tubers at 4°C for three months resulted in 82% to 90% loss of vitamin C content in white-fleshed potatoes, while 85% to 94% loss of vitamin C in color-fleshed potatoes. After six months storage at 4°C, vitamin C content remained constant except in CO97227-2P/PW, Atlantic, AC99375-1RU and All Red, which increased slightly.

Table.5.1. Total monomeric anthocyanin content of potato cultivars after storage and processing.

Cultivar / Selection			Three months storage		Six months storage	
	Processing	Initial	4ºC	10ºC	4ºC	10ºC
All Red	Raw	0.9 ± 0.0^{dC}	11.9 ± 6.9^{a}	2.4 ± 1.4^{c}	10.1 ± 5.8^{b}	2.7 ± 0.1^{c}
	Baked	17.6 ± 0.2^{a}	14.7 ± 8.5^{b}	11.4 ± 0.1^{c}	8.5 ± 0.0^{d}	5.2 ± 0.0^{e}
	Chipped	5.9 ± 3.4^{a}	2.4 ± 1.4^{c}	3.7 ± 2.2^{b}	1.3 ± 0.7^{e}	1.9 ± 1.0^{d}
Mountain Rose	Raw	0.12 ± 0.8^{cB}	5.1 ± 0.0^{d}	5.9 ± 0.2^{d}	16.4 ± 0.5^{a}	13.9 ± 0.9^{b}
	Baked	23.9 ± 2.0^{b}	20.2 ± 12^{c}	11.9 ± 0.1^{d}	26.3 ± 15^{a}	9.9 ± 0.8^{e}
	Chipped	3.3 ± 2.0^{b}	5.5 ± 0.1^{a}	1.9 ± 1.0^{d}	2.8 ± 1.6^{c}	2.6 ± 1.5^{c}
All Blue	Raw	1.2 ± 0.0^{cC}	2.4 ± 0.0^{b}	1.9 ± 0.7^{c}	2.5 ± 0.0^{b}	$2.9 \pm 0.0a$
	Baked	25.7 ± 0.6^{a}	22.2 ± 13.0^{b}	15.3 ± 0.0^{d}	12.5 ± 0.2^{e}	17.1 ± 0.5^{c}
	Chipped	6.3 ± 3.6^{b}	4.6 ± 2.6^{c}	7.4 ± 0.2^{a}	5.6 ± 0.0^{d}	1.1 ± 0.1^{e}
Purple Majesty	Raw	9.0 ± 5.6^{cB}	3.9 ± 2.3^{d}	25 ± 14.5^{b}	26.9 ± 1.3^{b}	29.4 ± 0.2^{a}
	Baked	34 ± 1.3^{c}	45.9 ± 2.9^{b}	34.9 ± 0.1^{c}	35.3 ± 0.1^{c}	110.7 ± 1.2^{a}
	Chipped	9.2 ± 0.3^{b}	10.2 ± 0.4^{a}	6.1 ± 0.2^{e}	8.4 ± 0.0^{c}	6.9 ± 0.1^{d}
CO97227 -2P/PW	Raw	49.2 ± 2.4^{cA}	91.3 ± 1.7^{a}	89.9 ± 6.5^{a}	64.8 ± 0.1^{b}	58.7 ± 1.7^{bc}
	Baked	134.6 ± 4^{b}	169.4 ± 4^{a}	109.3 ± 13^{c}	42.0 ± 0.9^{e}	84.2 ± 3.1^{d}
	Chipped	13.5 ± 8.0^{c}	20.2 ± 3.9^{b}	28.7 ± 0.5^{a}	28.7 ± 0.3^{a}	21.9 ± 0.9^{b}

Results are presented as mean ± SE of three biological replicates and three technical replicates for each biological replicates. Each value is a mean of nine determinations for each time point and expressed as mg of cyaniding-3-glucoside equivalents/100 gfw. Means with different letters indicate significant differences in rows ($p \leq 0.05$) from the initial time point. Right uppercase letters (A-C) on the mean of total monomeric anthocyanin content of raw at initial storage indicate (genotype effect) differences in column between cultivars/selections.

Table.5.2. Vitamin C content (mg/100 gfw) of potato cultivars after storage and processing.

Cultivar / Selection	Processing	Initial	Three months storage		Six months storage	
			4ºC	10ºC	4ºC	10ºC
Atlantic	Raw	$^{A}21.7 \pm 0.3^{a}$	3.5 ± 0.30^{c}	2.0 ± 0.03^{d}	4.7 ± 0.26^{b}	2.0 ± 0.4^{d}
	Baked	$^{B}10.0 \pm 1.2^{a}$	0.5 ± 0.06^{b}	0.5 ± 0.11^{b}	0.2 ± 0.03^{b}	0.2 ± 0.07^{b}
	Chipped	$^{C}2.4 \pm 0.02^{a}$	0.7 ± 0.0^{cb}	0.8 ± 0.11^{b}	0.7 ± 0.04^{c}	0.4 ± 0.01^{b}
Lenape	Raw	$^{A}22.9 \pm 0.5^{a}$	2.4 ± 0.2^{cb}	3.0 ± 0.02^{b}	2.8 ± 0.13^{b}	1.7 ± 0.08^{c}
	Baked	$^{B}11.9 \pm 0.5^{a}$	0.7 ± 0.1^{cb}	0.3 ± 0.1^{cb}	1.1 ± 0.5^{b}	0.2 ± 0.03^{c}
	Chipped	$^{C}3.1 \pm 0.05^{a}$	0.3 ± 0.06^{d}	0.6 ± 0.02^{b}	0.5 ± 0.02^{c}	0.4 ± 0.09^{c}
Rio Grande Russet	Raw	$^{A}37.5 \pm 0.9^{a}$	4.9 ± 0.2^{b}	5.4 ± 0.34^{b}	5.3 ± 0.15^{b}	2.3 ± 0.24^{c}
	Baked	$^{B}20.8 \pm 0.3^{a}$	3.5 ± 0.35^{c}	7.0 ± 0.2^{b}	0.3 ± 0.04^{d}	0.9 ± 0.05^{d}
	Chipped	$^{C}1.7 \pm 0.09^{a}$	0.7 ± 0.06^{d}	1.5 ± 0.02^{b}	0.9 ± 0.02^{c}	0.6 ± 0.01^{e}
Russet Burbank	Raw	$^{A}23.0 \pm 0.9^{a}$	2.0 ± 0.13^{c}	7.8 ± 0.23^{b}	3.0 ± 0.23^{c}	2.5 ± 0.20^{c}
	Baked	$^{B}13.2 \pm 0.7^{a}$	2.5 ± 0.21^{b}	3.1 ± 0.29^{b}	0.1 ± 0.08^{c}	1.0 ± 0.07^{c}
	Chipped	$^{C}2.5 \pm 0.21^{a}$	0.5 ± 0.17^{d}	0.7 ± 0.08^{b}	0.8 ± 0.10^{b}	0.6 ± 0.05^{c}
AC99375-1RU	Raw	$^{A}24.6 \pm 0.6^{a}$	2.8 ± 0.24^{c}	6.6 ± 0.12^{b}	4.8 ± 0.33^{b}	2.8 ± 0.18^{c}
	Baked	$^{B}15.6 \pm 1.0^{a}$	2.0 ± 0.1^{b}	2.2 ± 0.07^{b}	0.1 ± 0.03^{c}	2.1 ± 0.07^{b}
	Chipped	$^{C}2.1 \pm 0.2^{a}$	0.7 ± 0.03^{b}	0.9 ± 0.04^{b}	0.2 ± 0.02^{c}	0.7 ± 0.68^{b}
All Red	Raw	$^{A}20.1 \pm 2.8^{a}$	1.1 ± 0.11^{b}	3.0 ± 0.11^{b}	2.1 ± 0.12^{b}	2.0 ± 0.13^{b}
	Baked	$^{B}13.5 \pm 0.5^{a}$	1.7 ± 0.22^{b}	1.4 ± 0.11^{b}	0.3 ± 0.07^{c}	0.7 ± 0.08^{c}
	Chipped	$^{C}3.4 \pm 0.20^{a}$	0.1 ± 0.0^{c}	0.5 ± 0.02^{b}	0.2 ± 0.02^{c}	0.3 ± 0.02^{b}
All Blue	Raw	$^{A}25.9 \pm 0.9^{a}$	2.9 ± 0.29^{c}	5.1 ± 0.22^{b}	3.9 ± 0.8^{cb}	4.3 ± 0.11^{b}
	Baked	$^{B}14.2 \pm 0.7^{a}$	2.5 ± 0.34^{b}	2.8 ± 0.14^{b}	0.1 ± 0.01^{d}	1.4 ± 0.12^{c}
	Chipped	$^{C}1.8 \pm 0.12^{a}$	0.8 ± 0.1^{cb}	0.9 ± 0.02^{b}	0.4 ± 0.04^{d}	0.7 ± 0.01^{c}
Purple Majesty	Raw	$^{A}26.5 \pm 3.0^{a}$	3.1 ± 0.36^{b}	5.2 ± 0.41^{b}	3.1 ± 0.08^{b}	5.0 ± 0.32^{b}
	Baked	$^{B}11.8 \pm 0.2^{a}$	2.9 ± 0.13^{b}	1.7 ± 0.08^{c}	0.3 ± 0.04^{e}	1.4 ± 0.05^{d}
	Chipped	$^{C}3.9 \pm 0.07^{a}$	0.7 ± 0.05^{c}	0.9 ± 0.06^{b}	0.5 ± 0.03^{d}	0.5 ± 0.02^{d}
Mountain Rose	Raw	$^{A}31.5 \pm 1.8^{a}$	2.5 ± 0.28^{b}	2.8 ± 0.06^{b}	2.6 ± 0.12^{b}	0.9 ± 0.04^{c}
	Baked	$^{B}16.0 \pm 1.9^{a}$	1.9 ± 0.16^{b}	0.9 ± 0.07^{b}	0.3 ± 0.02^{b}	0.5 ± 0.0^{b}
	Chipped	$^{C}1.8 \pm 0.06^{a}$	0.3 ± 0.02^{d}	0.7 ± 0.01^{b}	0.2 ± 0.02^{d}	0.5 ± 0.01^{c}
CO97227-2P/PW	Raw	$^{A}24.1 \pm 0.7^{a}$	1.6 ± 0.19^{c}	4.2 ± 0.22^{b}	3.6 ± 0.13^{b}	2.2 ± 2.21^{c}
	Baked	$^{B}13.9 \pm 1.4^{a}$	1.3 ± 0.1^{cd}	2.9 ± 0.26^{c}	0.7 ± 0.05^{d}	5.5 ± 0.18^{b}
	Chipped	$^{C}1.9 \pm 0.09^{a}$	0.3 ± 0.05^{d}	1.0 ± 0.02^{b}	0.5 ± 0.02^{c}	0.5 ± 0.02^{c}

Results are presented as mean ± SE of three biological replicates and two technical replicates for each biological replicates. Each value is a mean of six determinations for each time point. Means with different letters indicate significant differences in rows ($p \leq 0.05$) from the initial time point within the cultivar/selection. Left uppercase letters (A-E) on the mean of vitamin C indicate (processing effect) differences in column for each cultivar/selection.

Our vitamin C results were also consistent with published data by Kulen et al. (2013), who found that selection CO97215-2P/P had the highest decrease in vitamin C content after two months of storage and then increased slightly after four or seven months of storage at 4°C. The loss of vitamin C in fruits and vegetables is accelerated at higher temperatures, but some sensitive crops show more loss in vitamin C at low temperatures (Lee and Kader, 2000). However, in the case of CO97227-2P/PW, Atlantic, AC99-375-1RU, and All Red cultivars, the slight increase might be caused by genotype effect since the other cultivars did not show the same trend even though they were stored under the same conditions.

Storage temperature had a significant effect on vitamin C content in potato tubers. Potatoes stored at 10°C for three months lost less vitamin C than those stored for three months at 4°C except Atlantic, Lenape, and Mountain Rose. Our findings were in agreement with Karikka et al. (1944), who found that storage potato tubers at 10°C lost less in vitamin C content compared to storage at 4°C. However, storage potato tubers at 10°C for six months resulted in a significant decrease in vitamin C content in all tested potato cultivars. Yet storage for six months at either 4°C or 10°C resulted in slightly increased in vitamin C in Atlantic cultivar. Our data agreed also with published data by Dale et al. (2003), who studied the effect of storage for four months at 4°C on vitamin C content in potato tubers from 33 *Solanum tuberosum* genotypes. Significant losses were observed during storage in vitamin C content of all tested potato genotypes.

Potatoes are prepared using different cooking methods such as baking and frying. The highest decrease was observed in chipped potatoes, while the lowest one was in baked potatoes. The percentage loss of vitamin C was 39% in baked Russet Burbank and 62% in baked Atlantic from the original value of the raw tubers, while in the group of cultivars with color-fleshed

potatoes, the vitamin C content decrease ranged from 31 to 63% in (CO97227-2P/PW, Purple Majesty, respectively) of the original value of the raw tubers. Many authors have observed that vitamin C decrease in potato products during cooking depends on cultivar and cooking methods. Significant differences in vitamin C content were observed in chipped potatoes when compared to uncooked potatoes. The losses of vitamin C were maximized with chipping and frying. The percentage loss of vitamin C in chipped white-fleshed potatoes ranged from 84% to 95% in (AC99375-1RU and Rio Grande Russet, respectively) from the original value of the raw tubers. While in color-fleshed potatoes, the content of vitamin C decreased to 85% in the All Red cultivar and to 94% in Mountain Rose cultivar. The loss of potato vitamin C in chipped samples may be explained due to low water content and the presence of air during frying, which increased the rate of loss (Burg et al., 1995), but during baking the potato tubers were wrapped in food-grade aluminum foil. Our data agreed with published work by Han et al. (2004), who found that losses of vitamin C observed after oven-baking and frying in oil for four Korean potato cultivars was (33-51% and 55-79%, respectively). The percentage losses of vitamin in our baked potato samples (31%-63%) were higher than the reported values, which may be explained by differences in cooking methods. Their samples were baked for ten minutes at 200°C, while our potato samples were baked at 204°C for one hour. For our chips samples, the loss of vitamin C was high because of the difference in frying treatment. They parboiled potato slices in heated distilled water for seven minutes and then fried in oil for 30 s at 170°C followed by 30 s at the same frying temperature. We fried our potato slices for three-five minutes at 185°C. Navarre et al. (2010) studied the effect of baking on vitamin C in baby potatoes from Piccolo, Bintje, and Purple Majesty cultivar. Vitamin C content in baby potatoes did not significantly decrease after baking. This finding did not agree with our vitamin C data for baked potatoes because our

potatoes samples were matured while their samples were new potatoes. One report suggested that the loss of potato vitamin C during cooking was due to the enzymatic destruction and the oxidative degradation (Burg et al., 1995). Since potatoes are an important worldwide source of vitamin C, optimization of cooking methods can improve the vitamin C content of potato products, which have a beneficial impact on human diet.

4. Conclusions

Potatoes are one of the most nutrient-dense vegetables. Since potatoes are a cool-seasonal crop, potatoes are usually stored before being cooked and consumed. Potatoes are well known to have naturally high levels of antioxidant compounds such as vitamin C and anthocyanins. Those compounds are very sensitive to storage and cooking parameters. Thus, to retain health-benefiting compounds it is critical to adjust farm to fork operations for each cultivar/selection. As large amounts of potatoes are consumed after frying, it is important to develop novel processing methods that retain anti-oxidant and anti-inflammatory activity of potatoes. To retain the health-benefiting compounds, it is critical to consider adjusting the farm-to-fork operations for each cultivar/selection. As large amounts of potatoes are consumed after frying, it is critical to develop processing methods such as vacuum frying to retain the bioactive compounds.

CHAPTER SIX: CONCLUSION

Potatoes are a very popular food source for millions of people from different cultural backgrounds and are consumed mainly in the form of baked, chips or French fries. Besides having macro- and micro-nutrients, potatoes also contain a variety of health promoting compounds such as carotenoids, flavonoids, and caffeic acid. Consumption of color-fleshed potatoes has increased by 17% due to their putative health benefits. In contrast, potatoes also contain pre-processing toxicants, glycoalkaloids (GA: α-solanine and α-chaconine) and post-processing toxicant, acrylamide (AL). Potatoes are a cool-seasonal crop, and are generally stored up to 12 months before the potatoes reach the consumers. So, it is critical to understand the effect of genotype, storage conditions, and processing on the health-benefitting and toxic compounds (GA/AL), and sensory attributes of the final potato products. Our results, for the first time, demonstrate that three months of storage at 4ºC elevated the content of both GA and AL in potato chips, depending on the cultivar. However, storing potato tubers for six months either at 4ºC or 10ºC resulted in increase or decrease of GA in processed potatoes, depending on the cultivar. Content of GA/AL in potato chips positively correlated with the overall acceptability and negatively correlated with the ranking of panelist' preferences (lower ranking suggested greater consumer preference), even after storage. These results indicate that within the acceptable range, GA/AL content positively correlate with consumer preference. Storage of potato tubers for three months at 4°C resulted in an increase of the reducing sugars in the raw potato tubers, which led to a higher level of AL in potato chips. The interaction effect of storage time and storage temperature on AL content in potato chips and baked potatoes resulted in either increased, decreased or no change after six months of storage, depending on cultivar/selection.

AL content in potato chips positively strongly correlated with reducing sugars in raw potato tubers, but correlations were weak in baked potatoes.

For potato chips, appreciable storage-induced changes were observed in the sensory scores for all tested cultivars/selections. Moreover, storage had no effect on sensory attributes of all baked potato cultivars/selections except All Red, thus indicating that the tested cultivars/selections had different stability in term of storage temperature particularly for potato chips. Therefore, cultivars/selections and storage temperature should be considered to obtain a final product with high quality. Storage temperature either at 4°C or 10°C had no effect on the density for all tested cultivars except Russet Burbank tubers stored at 10°C. However, storage temperature had a significant effect on texture for potato tubers stored at 4°C, caused softening due to biochemical and physical changes in potato tubers. There was a strong correlation between anthocyanins in potato tubers stored at 4°C or 10°C and maximum force ($p \leq 0.05$). No other correlation coefficients were significant ($p \leq 0.05$).

Potatoes are also known as a good source of antioxidant compounds, including polyphenols, carotenoids, and vitamins. These compounds are determined by genotype and are very sensitive to storage and cooking parameters. To produce high quality potato products with high levels of health-benefiting compounds, it is important to consider genotype, storage, and processing parameters. Regarding the vitamin C content, Rio Grande Russet and Mountain Rose had the highest level of the vitamin C compared to the rest of cultivars/selections tested. Stored potato tubers either at 4°C or 10°C resulted in a rapid decline of potato vitamin C content irrespective of the genotype. Baking retained more vitamin C compared to chipping and frying. Color-fleshed potatoes had higher levels of bioactives compared to white-fleshed potatoes. There is a potential to adjust the farm-fork operations to maximize content of health-benefiting

compounds in color-fleshed potatoes. Besides having health benefiting compounds, potatoes are also known to contain undesirable compounds occurring either naturally (GA) or produced during processing of potatoes at high temperatures such as (AL). Thus, it is critical to develop a food systems approach that establishes the farm to fork operation to maximize the retention of bioactive compounds and minimize the GA and AL content, and to retain sensory attributes of potato products before releasing a new potato cultivar. These form to fork practices could be extended to other crops with some modifications with an aim to improve the health-profiles of foods to prevent disease and promote health.

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